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EVOLUÇÃO E BIOGEOGRAFIA DO GÊNERO DE RAIAS MARINHAS *HYPANUS* RAFINESQUE, 1818 (MYLIOBATIFORMES, DASYATIDAE)

FLÁVIA DE FIGUEIREDO PETEAN

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Evolução e biogeografia do gênero de raias marinhas *Hypanus* Rafinesque, 1818 (Myliobatiformes, Dasyatidae)

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“Quero dizer agora o oposto do que eu disse antes,
Eu prefiro ser essa metamorfose ambulante
Do que ter aquela velha opinião formada sobre tudo...”
(Raul Seixas, 1973)

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RESUMO

As raias do gênero *Hypanus* Rafinesque (1818) possuem ampla distribuição ao longo das costas Atlântica e Pacífica do continente americano, assim como uma espécie no Golfo da Guiné, no Atlântico Oriental. Como a maioria das espécies de *Hypanus* está classificada como "dados insuficientes" (DD) na Lista Vermelha de Espécies Ameaçadas da IUCN, há uma necessidade de se resolver problemas taxonômicos e de distribuição geográfica para futuras avaliações de seus estados de conservação. Com o estudo dos genomas mitocondriais de todas as espécies até o momento válidas do gênero (*H. americanus* (Hildebrand & Schroeder, 1928), *H. dipterurus* (Jordan & Gilbert, 1880), *H. guttatus* (Bloch & Schneider, 1801), *H. longus* (Garman, 1880), *H. marianae* (Gomes et al., 2000), *H. rudis* (Günther, 1870), *H. sabinus* (Lesueur, 1824) e *H. say* (Lesueur, 1817)) delimitamos 14 linhagens, sendo duas referentes às espécies *H. geijskesi* (Boeseman, 1948) e *H. colarensis* (Santos et al., 2004), que antes pertenciam ao gênero *Fontitrygon* (Last et al., 2016), e agora foram alocadas para o gênero *Hypanus*. A espécie com a maior distribuição geográfica, *H. americanus*, é um grupo não monofilético e a linhagem que ocorre desde o deságue do Rio Amazonas ao Sudeste do Brasil é uma espécie ainda não descrita e irmã de *H. rudis*, na costa Africana do Atlântico. Examinamos exemplares do clado *H. americanus* (*H. americanus*, *H. longus* e *H. rudis),* a fim de descrever uma nova espécie usando uma abordagem integrativa de dados de DNA *barcode*, morfologia e modelagem de nicho ecológico. Além disso, inferimos os tempos de divergência e identificamos as possíveis barreiras biogeográficas impostas pelo continente americano às linhagens de *Hypanus*: Istmo do Panamá, Península da Flórida, deságue do Rio Amazonas, distâncias entre os continentes separados pelos Oceanos Atlântico e Pacífico, e limitações de temperatura nos hemisférios norte e sul (águas temperadas e tropicais). Os resultados obtidos indicam que a distribuição geográfica das espécies de *Hypanus* é menor do que o que se definia, abrigando espécies ainda não descritas. Na costa brasileira ocorrem seis espécies do gênero, *H. colarensis, H. geijskesi, H. guttatus, H. marianae, H. say* e *H.* **sp. 1**, com pelo menos quatro espécies endêmicas, incluindo a nova espécie aqui descrita. Assim, com a delimitação morfológica, genética, de distribuição geográfica e hábitats adequados é possível fazer uma avaliação do status de conservação de algumas espécies que estão classificadas como "dados insuficientes" na IUCN, porém são amplamente pescadas e consumidas na costa brasileira.

Palavras-chave

Elasmobrânquios, biogeografia, taxonomia, genoma mitocondrial, modelagem de nicho ecológico, conservação

ABSTRACT

Stingrays of the genus *Hypanus* Rafinesque (1818) have distribution areas along Atlantic and Pacific coasts of the American continent, as well as a species at the Guinea Gulf, in Eastern Atlantic. Since most *Hypanus* species are classified under Data Deficiency (DD) at IUCN Red List of Threatened Species, there is a need to solve their taxonomic and geographic distribution issues for future evaluations of their conservation statuses. By studying mitochondrial genomes of all valid species *(H. americanus* (Hildebrand & Schroeder, 1928), *H. dipterurus* (Jordan & Gilbert, 1880)*, H. guttatus* (Bloch & Schneider, 1801)*, H. longus* (Garman, 1880)*, H. marianae* (Gomes et al., 2000)*, H. rudis* (Günther, 1870)*, H. sabinus* (Lesueur, 1824), and *H. say* (Lesueur, 1817))) we delimited 14 lineages, and two of them are the species *H. geijskesi* (Boeseman, 1948) and *H. colarensis* (Santos et al., 2004), that used to belong to the genus *Fontitrygon* (Last et al., 2016), and were now allocated to the genus *Hypanus*. The species with the widest distribution, *H. americanus,* is a non-monophyletic group and the lineage that occurs south of Amazon river discharge to Southeastern Brazil is a yet undescribed species and sister to *H. rudis*, in Atlantic African coast. We examined specimens of the clade *H. americanus* (*H. americanus, H. longus*, and *H. rudis)* to describe a new species using an integrative approach of data from DNA barcode, morphology, and ecological niche modeling. Besides, we inferred *Hypanus*' lineages divergence times and identified possible biogeographic barriers posed by the American continent: Isthmus of Panama, Florida Peninsula, Amazon River water discharge, distance between continents separated by the Atlantic and Pacific Oceans, and temperature limitations in Northern and Southern Hemispheres (temperate and tropical waters). The results indicate the geographic distribution of *Hypanus* species is smaller than previously defined, encompassing yet undescribed species. At the Brazilian coast there are six species of the genus, *H. colarensis, H. geijskesi, H. guttatus, H. marianae, H. say*, and *H.* **sp. 1**,with at least four endemic ones, including the newest herein described. Therefore, with the morphological, genetic, geographical distribution and identification of suitable habitats it is possible to accomplish an evaluation of the conservation status of some species que are classified under

Data Deficient in IUCN, even though they are widely fished and consumed in the Brazilian coast.

Keywords

Elasmobranchs, biogeography, taxonomy, mitochondrial genome, ecological niche modeling, conservation

INTRODUÇÃO GERAL

A Biologia Evolutiva é uma ciência baseada na teoria de que as espécies estão relacionadas pela história de uma ancestralidade comum (Darwin, 1859). É um conceito antigo, assim como associar relações filogenéticas às distribuições geográficas pretéritas (Ronquist & Sanmartín, 2011). Quando a Cladística foi proposta por Hennig (1966), este método de inferências de relações entre táxons foi aplicado à biogeografia, surgindo a biogeografia filogenética (Brundin, 1965). Táxons que compartilham histórias filogenéticas similares, assim como ocupam áreas parecidas, provavelmente compartilham uma história biogeográfica comum (Ronquist & Sanmartín, 2011), que pode ser inferida pela reconstrução de suas áreas ancestrais (Ronquist, 1994). A sistemática filogenética e a biogeografia ajudam a compreender as relações entre as espécies e suas distribuições através de uma abordagem histórica dos eventos biológicos e geológicos (Harvey & Pagel, 1991).

Um grupo que inclui as raias, Batoidea, teve sua relação filogenética avaliada de distintas maneiras dentro do clado Neoselachii (tubarões e raias viventes) além de alguns grupos fósseis. Alguns autores posicionam Batoidea como grupo irmão de tubarões (Garman, 1913; Bigelow & Schroeder, 1953; Naylor et al., 2012b), outros como um subgrupo dentro de um clado parafilético “tubarões” (Compagno, 1973; De Carvalho, 1996; Shirai, 1996). Apesar das divergências no posicionamento do grupo, todos os autores concordam que Batoidea é monofilético (McEachran & Aschliman, 2004).

Compagno (1973) propôs uma divisão de Batoidea em quatro ordens: Torpediniformes, Pristiformes, Rajiformes e Myliobatiformes. Nishida (1990) dividiu a ordem Myliobatiformes em seis clados, sendo a família Dasyatidae um deles. Rosenberger (2001) estudou as relações entre as espécies do gênero *Dasyatis* Rafinesque (1810) sensu lato utilizando dados morfológicos e concluiu que o gênero não forma um grupo natural, já que a topologia final da análise filogenética mostra três outras espécies de Myliobatiformes dentro do clado *Dasyatis* s.l. Análises de dados moleculares por Naylor *et al.* (2012) concordam com a hipótese de Rosenberger (2001) que *Dasyatis* s.l. não é um grupo monofilético.

Dessa forma, Last *et al.* (2016) fizeram uma revisão da família Dasyatidae com base em dados morfológicos e moleculares e a dividiram em quatro subfamílias (Dasyatinae, Hypolophinae, Neotrygoninae e Urogymninae). Além disso, o que era conhecido como gênero *Dasyatis* s.l., foi desmembrado em oito gêneros (*Dasyatis* sensu stricto, *Pteroplatytrygon, Taeniurops, Bathytoshia, Hemitrygon, Hypanus, Telatrygon* e *Megatrygon*) na subfamília Dasyatinae que não possui caracteres sinapomórficos, mas foi agrupada por combinações de caracteres morfológicos externos e morfometria, além da formação de clusters a partir de dados moleculares.

Assim, o gênero de Dasyatinae mais diverso ao redor do continente americano é *Hypanus* Rafinesque (1818), com oito espécies válidas: *H. americanus* (Hildebrand & Schroeder, 1928), *H. dipterurus* (Jordan & Gilbert, 1880), *H. guttatus* (Bloch & Schneider, 1801), *H. longus* (Garman, 1880), *H. marianae* (Gomes, Rosa & Gadig, 2000), *H. rudis* (Günther, 1870), *H. sabinus* e *H. say* (Lesueur, 1817), a maioria distribuída na costa Atlântica. Contudo, *H. dipterurus* e *H. longus* ocorrem na costa Pacífica, e *H. rudis* no Golfo da Guiné, no Atlântico Oriental. Seis dessas espécies formam um cluster com base em dados moleculares, os quais não foram obtidos para as espécies *H. marianae* e *H. rudis*; porém, esse gênero também foi definido a partir de dados morfológicos, sem a determinação de sinapomorfias (Last *et al.*, 2016).

Dessa forma, a diferenciação e identificação morfológica e molecular de *Hypanus* no Atlântico Ocidental é importante para saber se este gênero é realmente válido e qual é sua composição, distribuição e história evolutiva. Porém, para se levantar uma hipótese evolutiva para este grupo de espécies, é necessário estudar todo o gênero, incluindo as espécies da costa Pacífica da América e do Atlântico Oriental, e compará-lo com representantes de outros gêneros da subfamília Dasyatinae e família Dasyatidae. Na costa leste do continente americano há a ocorrência de nove espécies de Dasyatinae: *Bathytoshia centroura* (Mitchill, 1815), *Dasyatis hypostigma* Santos & Carvalho (2004), *Fontitrygon colarensis* (Santos, Gomes & Charvet-Almeida, 2004), *Fontitrygon geijskesi* (Boeseman, 1948), *H. americanus*, *H. guttatus*, *H. marianae*, *H. sabinus*, *H. say*, seguindo a proposição de Last *et al.* (2016) e o Catalog of Fishes (Fricke *et al.*, 2019), e as distribuições nos mapas do Chondrichthyan Tree of Life (Naylor, 2019).

Para a identificação molecular das espécies de Chondrichthyes, o marcador mitocondrial NADH2 (nicotinamida adenina dinucleotídeo desidrogenase subunidade 2) é amplamente usado por ser um gene codificante de rápida evolução, e possibilitar a distinção de espécies proximamente relacionadas (Naylor et al., 2012b). Assim, esse marcador pode ser considerado um DNA *barcode* para Chondrichthyes, e já há um grande banco de dados disponível para comparações (Naylor et al., 2012b). Com o avanço de tecnologias e a queda dos custos de sequenciamentos genéticos, pesquisadores têm caracterizado o DNA mitocondrial (mtDNA) de Chondrichthyes (Chen *et al.*, 2013; Yang *et al.*, 2013; Shen *et al.*, 2015; Yang & Naylor, 2016). Porém, poucos estudos usam esses dados para análises filogenéticas e biogeográficas para este grupo de animais (Doane *et al.*, 2018; Gaitán-Espitia *et al.*, 2016). O mtDNA tem uma alta taxa de evolução e é importante para o estudo de relações evolutivas, especialmente de linhagens com divergências recentes (5-10 Ma) (Brown et al., 1979).

Há vários métodos de delimitação de espécies usando a teoria de coalescência, os quais mostram estrutura genética de linhagens, não necessariamente espécies. Assim, é importante usar os métodos, mas olhar os resultados como possíveis hipóteses de espécies a serem testadas por análises subsequentes (Sukumaran & Knowles, 2017). Além disso, também pode-se utilizar as informações do mtDNA para se estudar cada espécie a nível populacional e identificar possíveis espécies crípticas e as unidades operacionais de manejo, importante para manutenção da diversidade genética de uma espécie (Fišer *et al.*, 2018). Após delimitar as linhagens evolutivas a partir de dados de mtDNA, pode-se testar essas hipóteses de espécies com dados morfológicos e morfométricos que auxiliem a diagnosticar cada linhagem independente evolutivamente.

Assim, com a delimitação molecular e morfológica de cada espécie é possível desenvolver mapas preditivos de distribuição geográfica a fim de caracterizar as linhagens também do ponto de vista ambiental e ecológico. A modelagem de nicho ecológico e distribuição espacial representa um método empírico de desenhar inferências estatísticas sobre o que direciona as variações das espécies sob diferentes processos ecológicos e evolutivos (Zimmermann et al., 2010) e, até o momento, foi pouco utilizada para estudos em Elasmobrânquios (Melo-Merino et al., 2020). Essa ferramenta também pode ser usada para resolver problemas de conservação, ou explorar conceitos evolutivos (Zimmermann et al., 2010) através da delimitação de hábitats preferenciais para determinada espécie e identificação de possíveis barreiras ambientais para sua dispersão.

Um modelo de hábitat é uma representação numérica das preferências de hábitat de uma espécie. Ele pode ser usado para fazer inferências sobre os requisitos de hábitat e as respostas mais prováveis a mudanças ambientais, ou para prever abundância, densidade, capacidade de carregamento, ou probabilidade de ocupação de um lugar com base em suas características ambientais (Wintle *et al.*, 2010). Assim, o mapeamento de hábitat e espécies é uma ferramenta muito importante já que fornece uma distribuição geográfica mais precisa, e possibilita o manejo (Young & Carr, 2015). Esses mapas preditivos gerados permitem identificar áreas mais sensíveis para as espécies estudadas e, consequentemente, facilitam sua conservação.

Como a maioria das espécies de *Hypanus* está classificada como “dados insuficientes” (DD) na Lista Vermelha de Espécies Ameaçadas da IUCN (International Union for

Conservation of Nature and Natural Resources), com exceção de *H. sabinus* e *H. say* que estão como “pouco preocupante” (LC) (IUCN., 2019), um dos estudos mais importantes para se iniciar uma investigação acerca de suas situações de ameaça é a taxonomia; ou seja, definir quais são as espécies, suas distribuições e o que as difere de outras similares. Juntas, as ferramentas moleculares, morfológicas e ecológicas irão auxiliar na delimitação de hipóteses de espécies do gênero *Hypanus* e inferir suas histórias evolutivas. Todas essas informações combinadas são a base para outros estudos gerarem mais conhecimento sobre as espécies e propor ações conservacionistas.

A conservação da biodiversidade tem algumas defasagens devido à falta de integração entre distintas áreas da Biologia (Diniz-Filho et al., 2013). Quatro delas são apontadas como desafios para a conservação: (1) o déficit Linneano (Brown & Lomolino, 1998), que corresponde à ausência de descrições das espécies, gerando um desconhecimento completo sobre a biodiversidade; (2) o Wallaceano (Lomolino, 2004) é aquele em que, dadas as descrições das espécies, não se sabe suas distribuições geográficas (3) Hutchinsoniano (Mokany & Ferrier, 2011), que considera que as espécies e suas respectivas áreas de ocorrência tenham sido descritas, porém, a relação da espécie com o ambiente em que ela vive é desconhecida; e (4) Darwiniano (Diniz-Filho et al., 2013), que corresponde à ausência de filogenias entre essas espécies e as incertezas nas estimativas de tempos de divergência (Whittaker et al., 2005; Cardoso et al., 2011; Diniz-Filho et al., 2013).

Esses quatro déficits serão abordados neste trabalho com o objetivo de diminuir os obstáculos que separam as áreas da Biologia Evolutiva e Conservação. O Déficit Linneano será eliminado pela descrição de possíveis espécies novas; Wallaceano pela atualização da distribuição geográfica das espécies de *Hypanus*; Hutchinsoniano pela modelagem de nicho ecológico identificando as características ambientais mais adequadas para a ocorrência de determinadas espécies. Finalmente, o Darwiniano será eliminado pela análise filogenética do gênero relacionando-o aos outros gêneros da mesma subfamília e de outras relacionadas. A integração dessas informações propiciará uma abordagem conservacionista da biogeografia (Whittaker et al., 2005).

Dificuldades

Raias não são um grupo fácil de se trabalhar dado que a coleta e transporte desses animais marinhos de grande porte exigem muito financiamento e equipamentos adequados. Assim, as maneiras mais efetivas de se trabalhar com dados genéticos das raias é através da coleta de pequenas amostras diretamente com pescadores, feiras e mercados, ou através de parcerias com colaboradores que possam fornecer amostras. No entanto, os animais estão muitas vezes fragmentados e a identificação só pode ser confirmada *a posteriori* com as análises moleculares. Ainda, a coleta ativa não-letal de exemplares exige ferramentas adequadas e uma equipe que possa auxiliar no manuseio dos exemplares e armazenamento das amostras.

Além disso, para se estudar a morfologia das raias é imprescindível trabalhar em coleções científicas que possuem exemplares em seus acervos. A maioria dessas coleções está em instituições estrangeiras e, por serem animais de grande porte, o envio de material é inviável; fazendo com que seja necessário ir até os museus. Além disso, grande parte das coletas e descrições dos materiais-tipo foram feitas na primeira metade do século XIX; dessa forma, muitos exemplares não estão em boas condições, o que dificulta as análises morfológicas.

Para tentar solucionar a dificuldade de conseguir um grande número de amostras de tecido para análises genéticas, sequenciamos não apenas o genoma mitocondrial, mas também os exomas nucleares dos mesmos exemplares (Li et al., 2015). Assim, aumentamos o número de loci a serem estudados por indivíduo, população, ou espécie. Com o intuito de superar a baixa qualidade dos espécimes preservados em coleções e tornar o estudo anatômico mais detalhado em menor tempo, fizemos tomografias computadorizadas de quase todas as espécies válidas do gênero. Porém, devido à limitação do tempo necessário para se concluir uma tese de doutorado, esses dados genéticos nucleares e morfológicos não foram incluídos neste trabalho, mas serão acrescidos nos manuscritos advindos deste estudo.

OBJETIVO GERAL

Reconstruir a história evolutiva das raias do gênero *Hypanus* para levantar hipóteses sobre os processos que embasaram os padrões biogeográficos atuais observados e identificar possíveis espécies crípticas.

OBJETIVOS ESPECÍFICOS

- Inferir as relações filogenéticas entre as espécies de *Hypanus*, estimar datas de divergência e discutir possíveis eventos biogeográficos para os padrões de distribuição encontrados atualmente;

- Revisar taxonomicamente de forma integrativa o clado *Hypanus americanus* e descrever uma nova espécie;
- Mapear a distribuição geográfica e identificar possíveis fatores ambientais e geológicos que influenciam a distribuição geográfica da espécie nova;
- Proporcionar informações taxonômicas e de distribuição geográfica para a avaliação do estado de conservação de algumas espécies *Hypanus* na Lista Vermelha de Espécies Ameaçadas da IUCN.

REFERÊNCIAS BIBLIOGRÁFICAS

Bigelow, H. B.; Schroeder, W. C. 1953. **Fishes of the western North Atlantic Part II. Sawfishes, guitarfishes, skates and rays**. 588 p.

Bloch, M. E.; Schneider, J. G. 1801. **Systema Ichthyologiae Iconibus cx Ilustratum. Post obitum auctoris opus inchoatum absolvit, correxit, interpolavit Jo. Gottlob Schneider, Saxo.** Berolini: Sumtibus Auctoris Impressum et Bibliopolio Sanderiano Commissum. i-lx + 1-584, Pls. 1-110. p.

Boeseman, M. 1948. Some preliminary notes on Surinam sting rays, including the description of a new species. **Zoologische Mededelingen 30, n. 2**: 31–47.

Brown, J. H.; Lomolino, M. V. 1998. **Biogeography**. 2nd. ed. Sunderland, Massachusetts: Sinauer Press. 560 p.

Brown, W. M.; George, M.; Wilson, A. C. 1979. Rapid evolution of animal mitochondrial DNA. **Proceedings of the National Academy of Sciences of the United States of America 76, n. 4**: 1967–1971.

Brundin, L. 1965. On the Real Nature of Transantarctic Relationships. **Evolution 19, n. 4**: 496–505.

Cardoso, P.; Erwin, T. L.; Borges, P. A. V.; New, T. R. 2011. The seven impediments in invertebrate conservation and how to overcome them. **Biological Conservation 144, n. 11**: 2647–2655.

Chen, X.; Ai, W.; Ye, L.; Wang, X.; Lin, C.; Yang, S. 2013. The complete mitochondrial genome of the grey bamboo shark (*Chiloscyllium griseum*) (Orectolobiformes: Hemiscylliidae): Genomic characterization and phylogenetic application. **Acta Oceanologica Sinica 32, n. 4**: 59–65.

Compagno, L. 1973. Interrelationships of living elasmobranchs. **Zoological Journal of the Linnean Society 53, n. Suppl. 1**: 15–61.

Darwin, C. 1859. **On the origin of species by means of natural selection, or preservation of favoured races in the struggle for life**. London: John Murray.

De Carvalho, M. R. 1996. Higher-Level Elasmobranch Phylogeny, Basal Squaleans, and Paraphyly. *In*: Stiassny, M.; Parenti, L.; Johnson, G. (Ed.). **Interrelationships of Fishes**. California: Academic Press. p. 35–62.

Diniz-Filho, J. A. F.; Loyola, R. D.; Raia, P.; Mooers, A. O.; Bini, L. M. 2013. Darwinian shortfalls in biodiversity conservation. **Trends in Ecology and Evolution 28, n. 12**: 689–695.

Doane, M. P.; Kacev, D.; Harrington, S.; Levi, K.; Pande, D.; Vega, A.; Dinsdale, E. A. 2018. Mitochondrial recovery from shotgun metagenome sequencing enabling phylogenetic analysis of the common thresher shark (*Alopias vulpinus*). **Meta Gene 15, n. October 2017**: 10–15.

Fišer, C.; Robinson, C. T.; Malard, F. 2018. Cryptic species as a window into the paradigm shift of the species concept. **Molecular Ecology 27, n. 3**: 613–635.

Fricke, R.; Eschmeyer, W. N.; Van der Laan, R. 2019. **Eschmeyer's Catalog of Fishes: genera, species, references**. Disponível em: <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp>.

Gaitán-Espitia, J. D.; Solano-Iguaran, J. J.; Tejada-Martinez, D.; Quintero-Galvis, J. F. 2016. Mitogenomics of electric rays: evolutionary considerations within Torpediniformes (Batoidea; Chondrichthyes). **Zoological Journal of the Linnean Society 178, n. 2**: 257–266.

Garman, S. 1880. New species of selachians in the museum collection. **Bulletin of the Museum of Comparative Zoology 6, n. 11**: 167–172.

Garman, S. 1913. **The Plagiostomia. (Sharks, Skates, and Rays)**. Cambridge, USA: Memoirs of the Museum of Comparative Zoology at Harvard College XXXVI. 528 p.

Gomes, U. L.; Rosa, R. S.; Gadig, O. B. F. 2000. *Dasyatis marianae* sp. n.: A new species of stingray (Chondrichthyes: Dasyatidae) from the Southwestern Atlantic. **Copeia 2000, n. 2**: 510–515.

Günther, A. 1870. **Catalogue of the fishes in the British Museum. Catalogue of the Physostomi, containing the families Gymnotidae, Symbranchidae, Muraenidae, Pegasidae, and of the Lophobranchii, Plectognathi, Dipnoi, ...[thru] ... Leptocardii, in the British Museum**. V. 8 ed. 549 p.

Harvey, P. H.; Pagel, M. D. 1991. **The comparative method in evolutionary biology**. Oxford: Oxford University Press. 239 p.

Hennig, W. 1966. **Phylogenetic Systematics**. University of Illinois Press. 263 p.

Hildebrand, S. F.; Schroeder, W. C. 1928. **Fishes of Chesapeake Bay**. US Government Printing Office. .

IUCN. 2019. **The IUCN Red List of Threatened Species**. Disponível em: <https://www.iucnredlist.org/>.

Jordan, D. S.; Gilbert, C. R. 1880. Notes on a collection of fishes from San Diego, California. **Proceedings of the United States National Museum 3, n. 106**: 23–34.

Last, P. R.; Naylor, G. J. P.; Manjaji-Matsumoto, B. M. 2016. A revised classification of the family Dasyatidae (Chondrichthyes: Myliobatiformes) based on new morphological and molecular insights. **Zootaxa 4139, n. 3**: 345–368.

Lesueur, C. A. 1824. Description of several species of the Linnaean genus *Raia*, of North

America. **Journal of the Academy of Natural Sciences 4, n. 1**: 100–121, Pls. 4-6.

Lesueur, C. A. 1817. Description of three new species of the genus *Raja*. **Journal of the Academy of Natural Sciences 1, n. pt 1, 3**: 41–45.

Li, C.; Corrigan, S.; Yang, L.; Straube, N.; Harris, M.; Hofreiter, M.; White, W. T.; Naylor, G. J. P. 2015. DNA capture reveals transoceanic gene flow in endangered river sharks. **Proceedings of the National Academy of Sciences 112, n. 43**: 13302–13307.

Lomolino, M. V. 2004. Conservation biogeography. *In*: Lomolino, M. V.; Heaney, L. R. (Ed.). **Frontiers of Biogeography: new directions in the geography of nature**. Sunderland, Massachusetts: Sinauer Associates. p. 293–296.

McEachran, J. D.; Aschliman, N. 2004. Phylogeny of Batoidea. **Sharks and Their Relatives** : 79–113.

Melo-Merino, S. M.; Reyes-Bonilla, H.; Lira-Noriega, A. 2020. Ecological niche models and species distribution models in marine environments: A literature review and spatial analysis of evidence. **Ecological Modelling 415**: 35.

Mokany, K.; Ferrier, S. 2011. Predicting impacts of climate change on biodiversity: A role for semi-mechanistic community-level modelling. **Diversity and Distributions 17**: 374–380.

Naylor, G. J.; Caira, J. N.; Jensen, K.; Rosana, K. a.; Straube, N.; Lakner, C. 2012a. Elasmobranch Phylogeny: A Mitochondrial Estimate Based on 595 Species. Historical Zoogeography of the Selachii. *In*: Carrier, J.; Musick, J.; Heithaus, M. (Ed.). **Biology of Sharks and their Relatives**. 2nd. ed. Boca Raton: CRC Press. p. 31–56.

Naylor, G. J. P.; Caira, J. N.; Jensen, K.; Rosana, K. A. M.; White, W. T.; Last, P. R. 2012b. A DNA Sequence-based approach to the identification of shark and ray species and its implications for global elasmobranch diversity and parasitology. **Bulletin of the American Museum of Natural History 367**: 262.

Naylor, G. J. P. 2019. **Chondrichthyan Tree of Life**. Disponível em: <https://sharksrays.org/>.

Nishida, K. 1990. Phylogeny of the suborder Myliobatoidei. **Memoirs of the Faculty of Fisheries Hokkaido University 37**: 1–108.

Rafinesque, C. S. 1810. **Caratteri di alcuni nuovi generi e nuove specie di animali e piante della Sicilia, con varie osservazioni sopra i medisimi**. Per le stampe di Sanfilippo. 105 p.

Ronquist, F. 1994. **Ancestral Areas and Parsimony**. 267–274 p.

Ronquist, F.; Sanmartín, I. 2011. Phylogenetic Methods in Biogeography. **Annual Review of Ecology, Evolution, and Systematics 42, n. 1**: 441–464.

Rosenberger, L. 2001. Phylogenetic Relationships within the Stingray Genus *Dasyatis* (Chondrichthyes: Dasyatidae). **Copeia 2001, n. 3**: 615–627.

Santos, H. R. S.; Gomes, U. L.; Charvet-Almeida, P. 2004. A new species of whiptail stingray of the genus *Dasyatis* Rafinesque, 1810 from the Southwestern Atlantic Ocean (Chondrichthyes: Myliobatiformes: Dasyatidae). **Zootaxa 492**: 1–12.

Shen, K.-N.; Chang, C.-W.; Tsai, S.-Y.; Wu, S.-C.; Lin, Z.-H.; Chan, Y.-F.; Chen, C.-H.;

Hsiao, C.-D.; Borsa, P. 2015. Next generation sequencing yields complete mitogenomes of Leopard whipray (*Himantura leoparda*) and Blue-spotted stingray (*Neotrygon kuhlii)* (Chondrichthyes: Dasyatidae). **Mitochondrial DNA** : 1–2.

Shirai, S. 1996. Phylogenetic Interrelationships of Neoselachians (Chondrichthyes: Euselachii). *In*: Stiassny, M.; Parenti, L.; Johnson, G. (Ed.). **Interrelationships of Fishes**. New York: Academic Press. p. 9–34.

Sukumaran, J.; Knowles, L. L. 2017. Multispecies coalescent delimits structure, not species. **Proceedings of the National Academy of Sciences 2016**: 201607921.

Whittaker, R. J.; Araújo, M. B.; Jepson, P.; Ladle, R. J.; James, E. M. W.; Willis, K. J. 2005. Conservation Biogeography: Assessment and Prospect. **Diversity and Distributions 11, n. 1**: 3–23.

Wintle, B. A.; Runge, M. C.; Bekessy, S. A. 2010. Allocating monitoring effort in the face of unknown unknowns. **Ecology Letters 13, n. 11**: 1325–1337.

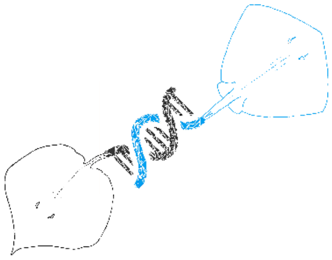
Yang, B.; Zhang, J.; Yamaguchi, A.; Zhang, B. 2013. Mitochondrial genome of *Dasyatis bennettii* (Chondrichthyes: Dasyatidae). **Mitochondrial DNA 24, n. 4**: 344–346.

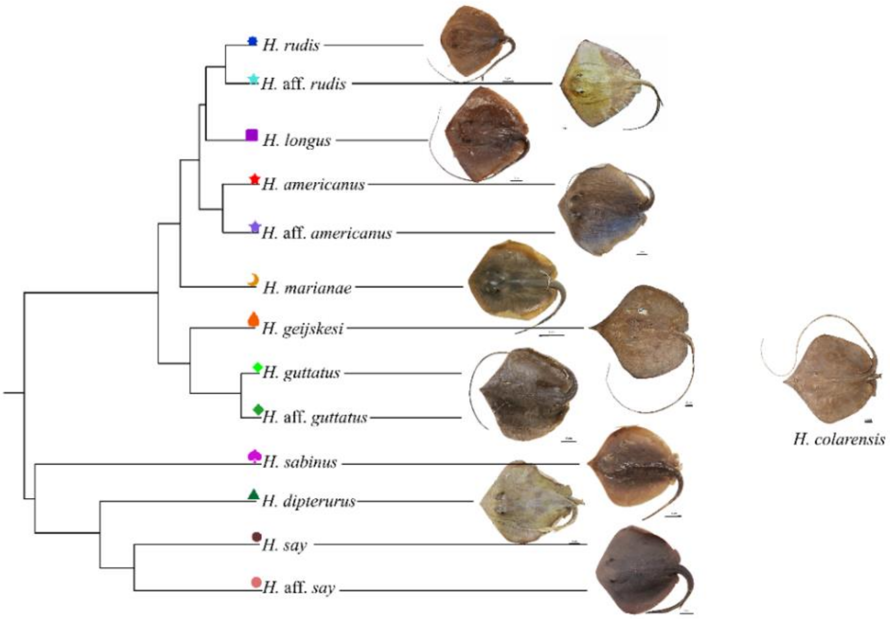
Yang, L.; Naylor, G. J. P. 2016. Long-PCR based next generation sequencing of the whole mitochondrial genome of the peacock skate *Pavoraja nitida* (Elasmobranchii: Arhynchobatidae). **Mitochondrial DNA Part A 27, n. 2**: 943–944.

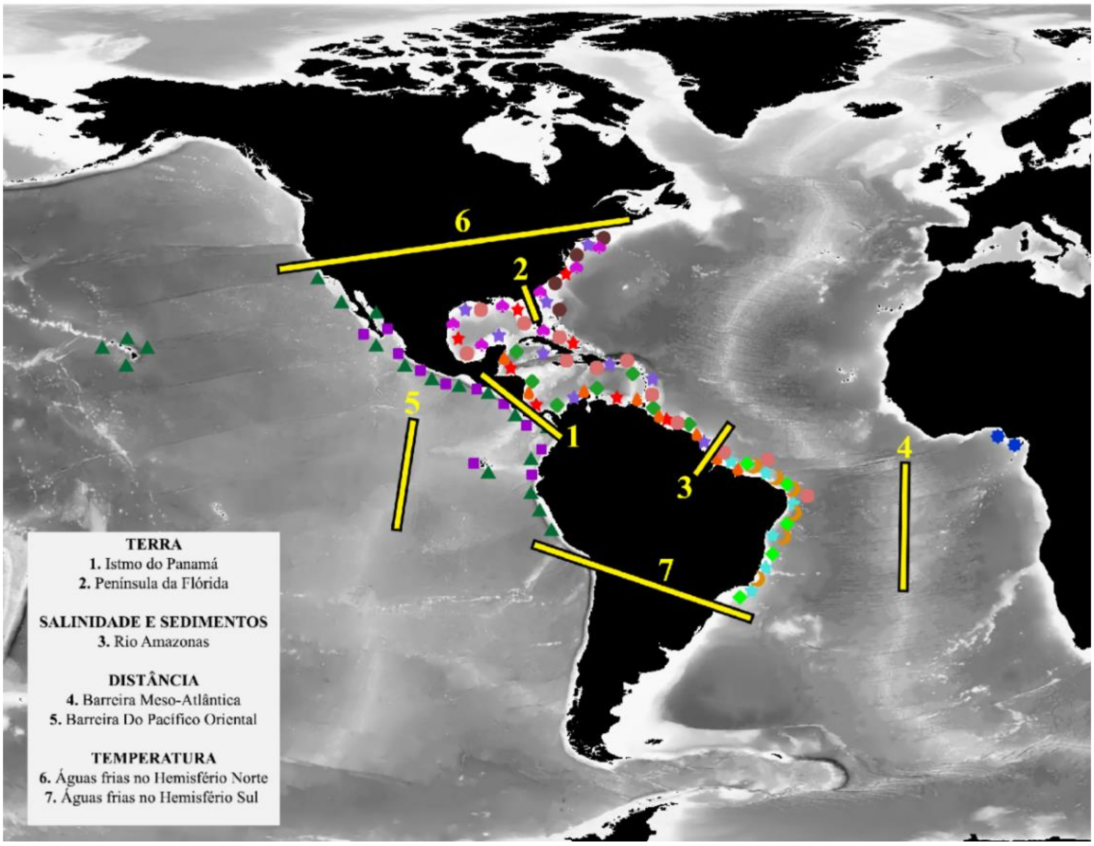
Young, M.; Carr, M. H. 2015. Application of species distribution models to explain and predict the distribution, abundance and assemblage structure of nearshore temperate reef fishes. **Diversity and Distributions 21, n. 12**: 1428–1440.

Zimmermann, N. E.; Edwards, T. C.; Graham, C. H.; Pearman, P. B.; Svenning, J. C. 2010. New trends in species distribution modelling. **Ecography 33, n. 6**: 985–989.

CAPÍTULO I. O papel do continente americano na diversificação das raias do gênero *Hypanus* Rafinesque, 1818 (Myliobatiformes: Dasyatidae)







The role of the American continent on the diversification of the stingrays' genus *Hypanus* Rafinesque, 1818 (Myliobatiformes: Dasyatidae)

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ABSTRACT

The stingrays of the resurrected genus *Hypanus* Rafinesque, 1818 encompass eight valid species (previously considered as *Dasyatis*) distributed both in the Atlantic and Pacific oceans. However, the phylogenetic relationships amongst some of them were based on a single mitochondrial gene and did not involve all putative *Hypanus* species, lacking *H. marianae* and *H. rudis*. To address the monophyly of the genus and evaluate their divergence times and possible routes of dispersion we sequenced the whole mitochondrial genomes of all valid species using the gene capture methodology and performed a Bayesian Inference phylogeny. In addition to the monophyly of the genus, *H. americanus* (Hildebrand & Schroeder, 1928) is a species-complex and the lineage from South America is closely related to the African *H. rudis* (Günther, 1870). These species are the sister group of *H. longus* (Garman, 1880) from the Pacific coast of the American continent, a cladogenetic event derived from the vicariant closure of Isthmus of Panama. *H. americanus* sensu stricto (from Northwestern Atlantic and Caribbean) seems to be composed by two lineages. A third clade regards the species-complex *H. guttatus* (Bloch & Schneider, 1801), with one lineage in Central and another in South America, a speciation event probably related to the Amazon barrier. Another species-complex concerns *H. say* (Lesueur 1817), with one lineage at the Eastern coast of the United States and another in the Gulf of Mexico, separated by the Florida Peninsula. *Hypanus marianae* (Gomes, Rosa & Gadig, 2000), endemic to Northeastern Brazil, whose phylogenetic relationships within the genus were unknown, is a sister-group to the clade composed by *H. americanus* species-complex, *H. longus*, and *H. rudis*. We also identified the species *Fontitrygon geijskesi* as more closely related to *H. guttatus* than to other *Fontitrygon* species, thus it should be allocated to *Hypanus* as *H. geijskesi*. The genus originated 33.97 Ma [26.13-36.09 HPD] and its subsequent diversification was probably related to a world cooling event in which these stingrays were restricted to tropical waters in Western Atlantic. Oceanic geophysical changes are consistent with the divergence times of lineages within this group of stingrays, which harbors a hidden diversity that might affect the conservation status of some species.

KEYWORDS

Biogeography, America, phylogenetic relationships, speciation.

INTRODUCTION

Marine speciation and the American continent

Since Darwin (1859), one of the most interesting questions in biology has been speciation (Rocha and Bowen 2008). Four types of cladogenesis explain species' diversification: allopatric, peripatric, parapatric, and sympatric, all of which must deal with different levels of isolation and gene flow (Coyne and Orr 2004; Lawson et al. 2015). However, in marine habitats, there are not as many land barriers for speciation (Rocha et al. 2007) as there are in freshwater environments that could isolate populations and constrain gene flow, favoring speciation (Rocha and Bowen 2008).

Is geographic isolation a necessary milieu for speciation in marine systems? To comprehend the current distribution of species we need to study their geographic histories, which encompass their origin, dispersal, and extinction in space and time (Ree et al. 2005). Speciation is a complex biological event to be explained simply by geographic isolation in marine environment. Barriers to gene flow can be hard (land areas), soft (water masses, marine currents), or even the combination of them throughout periods of time, which linked with ecological modifications, can provide some ingredients for diversification (Bowen et al. 2013). The diversification capacity of biogeographical barriers and, eventually, speciation events are uncertain and depend on the environment and the organisms' biology (Craig et al. 2006).

The Theory of American Degeneracy by George Louis Leclerc (Buffon 1766) claimed that American fauna was less diverse, smaller, and weaker than the European due to the climate and humidity supplied by the vast vegetation that provided a melancholic environment. It gave the idea that America was a land of "degraded" species (Sloan 2019). This perception continued for more than 100 years when Alexander van Humboldt (Von Humboldt 1849) said this Theory was against the already known physical laws (Dugatkin 2019). Besides, Darwin also pointed out the great diversity of South American mammals (Dugatkin 2019). The thought overturn came with discoveries of the heterogeneity of forms in America, which are still in process with studies showing cryptic species where widely distributed ones were thought to exist (Havill et al. 2018). Therefore, the American continent poses many barriers not only to land but also to marine taxa (Hurtado et al. 2016).

The final closure of the Isthmus of Panama 2.8 Ma (O'Dea et al. 2018) is a well-known hard barrier and a vicariant cause of speciation by separating Eastern Pacific from Western Atlantic faunas (Floeter et al. 2008). Its formation began with the tectonic collision of Central and South America 23-25 Ma (Farris et al. 2011). This event continues nowadays but it was not until 10 Ma that deep-water exchange between the Atlantic and Pacific stopped (Newkirk and Martin 2009). However, until a narrowing of this passage 4 Ma, shallow water kept flowing (O'Dea et al. 2016). The final closure of this event dates to 2.8 Ma (O'Dea et al. 2018), when ceased the gene flow between populations on both sides. It also agrees with a period of world glaciations from 2.8 to 2 Ma, when there was a decline in sea levels (Tiedemann et al. 1994). The combination of the landmass uplift with low sea-levels increased the separation of marine lineages at the Atlantic and Pacific Oceans (O'Dea et al. 2016).

With surface water exchange restriction between Atlantic and Pacific, part of the Atlantic North Equatorial current that used to flow towards the Pacific was redirected to the northeast, boosting the Gulf Stream (Keigwin 1982; Haug and Tiedemann 1998). A great global cooling followed by oceanographic changes occurred during the late Neogene and approximately 3 Ma a shoaling thermocline prompted high fluctuations in ice volume, sea level, and sea surface temperatures. (Johnson et al. 2007).

Currently, the coast of the Peninsula of Florida and its surrounding islands are at the Tropical Northwestern Atlantic Province form the Floridian ecoregion (Spalding et al. 2007). It has distinct geographical, biological, and ecological features from the adjacent east and west province Warm Temperate Northwestern Atlantic, as well as the Bahamian ecoregion (Spalding et al. 2007). During the Pleistocene, shallow water areas in Caribbean was reduced by 89%, with change in mean slope from 1.4 to 10.1 m/Km (Bellwood and Wainwright 2002;

Ludt and Rocha 2015), forming a land barrier for marine organisms (Bowen and Avise 1990; Reeb and Avise 1990).

Soft barriers in marine environment are those that might permit some organisms' flow not completely interrupting populations' connections (Rocha and Bowen 2008). Within the Atlantic Ocean basin, eastern and western organisms are isolated by the Mid-Atlantic Barrier (MAB) (Floeter et al. 2008). Even though there could be some dispersal and founder events (Mayr 1942), they are unusual and populations on both sides follow independent evolutionary paths (Muss et al. 2001). The Atlantic Ocean is considered a soft barrier since it is more than 4,500 km wide but transposable, and the final land disconnection between Africa and South America dates to ~90 Ma (Berggren and Hollister 1974); however, some organisms can still span through it, as the lemon shark (Schultz et al. 2008).

The commonest fishes' and invertebrates' route in migrating across the Atlantic is from West (America) to East (Africa) (Briggs 1995a). Western Atlantic could be considered a region of species radiation, even though the direction towards Eastern Atlantic could be counterintuitive given that North and South Equatorial Currents flow westwards. The subsurface Equatorial Undercurrent possibly plays an important role in helping the transportation eastwards despite being weaker and smaller than westwards Equatorial ones (Briggs 1974; Muss et al. 2001).

On the other side of America, the East Pacific Barrier (EPB) separates Central and Western Pacific from the Tropical Eastern Pacific, which is a singular biogeographic region (Ekman 1953; Lessios and Robertson 2006; Giddens et al. 2019). This 5000 km barrier isolates eastern and western faunas, even though there might some gene flow in both directions (Lessios and Robertson 2006). The closer relationship of Eastern Pacific fauna to Western Atlantic reveals an ancestral connectivity of these areas previously to the closure of the Isthmus of Panama (Coates and Obando 1996; Allen 2008).

Mid-Oceanic Barriers, as the MAB and EPB, have depths of 800 to 3500 m (Watling et al. 2013) and isolate land areas by great distances, which are barrier impediments for coastal species to cross (Riehl et al. 2018). A possibility to span through them is via planktonic larval dispersal by many organisms (Muss et al. 2001). However, these barriers are large enough to isolate most fishes of the tropical realm, especially species with short larval periods or those who lack a larval phase have limited dispersal capacity and are restricted to smaller geographical areas, such as reef fishes *Elacatinus* (Taylor and Hellberg 2006) and *Malacoctenus* (Dias et al. 2019). Stochastic processes could happen in which individuals might colonize isolated areas (islands, for example) and go through a divergent speciation (Pinheiro et al. 2017).

A widely known soft barrier is the Orinoco and Amazon Rivers outflow of freshwater and nutrients to the Atlantic (Rocha 2003), which started at the late Miocene (~10 Ma) when the Andes reached their final configuration (Hoorn et al. 1995). Environmental barriers, such as water temperature, salinity, and sedimentation, are also relevant for parapatric speciation events (Rocha and Bowen 2008), once adaptation to distinct situations leads to reproductive isolation of diverging populations (Cutter and Gray 2016).

At the end of the Eocene (~37 Ma) , the circum-Antarctic marine current was organized, and permanent ice sheets were already covering North and South poles. This current was boosted throughout the continuing continents' split, cooling the water, and probably restricting the movement of tropical organisms, inhibiting their migration to high latitudes (Bellwood and Wainwright 2002). Therefore, the dispersal of organisms between Atlantic and Pacific by the Southern tip of South America was possible only for those who could stand low temperatures, and usually towards the east due to strong eastward currents around the tip (Riemann-Zürneck, 1986 *apud* Häussermann & Försterra, 2005).

One of the hypotheses concerning the geographic origin of diversity is the Center of Speciation, from which organisms migrate to peripheral areas followed by modifications and do not return to where they came (Bowen et al. 2013). The Caribbean is widely known for its species diversity in a high rate of radiation as it can be considered a Center of Speciation (Briggs & Bowen, 2013).

Species' dispersal ability contribute to colonization through soft barriers and expose populations to gene flow, hindering the allopatric speciation process (Avise 2000). However, if during periods of geographic isolation by some biogeographic barrier the populations achieve a reproductive isolation, even with sporadic rencounter they will keep on divergent evolutionary routes (Rocha 2003), as many reef fishes examples shown by Floeter *et al.*, (2008), Pinheiro *et al.* (2018).

***Hypanus* stingrays**

The American continent presents a high variety of hard and soft barriers that allow scientists to test several hypotheses about speciation and diversification. Some of the barriers include: closure of Isthmus of Panama, Peninsula of Florida, freshwater outflow to Atlantic Ocean, mid-Atlantic Barrier, East-Pacific Barrier, besides variations in temperature due to its great latitudinal extension, salinity, sedimentation, depth, and distance from other continents.

Beyond having a suitable area for assessing these biogeographical hypotheses, an interesting group are the stingrays of the genus *Hypanus* Rafinesque, 1818, which occur on both Atlantic and Pacific coasts of America, besides the Atlantic African coast. These stingrays are coastal, viviparous, live in marine and estuarine habitats, and do not tolerate low-temperature waters (Last et al. 2016a).

The world widely distributed genus *Dasyatis* Rafinesque, 1810 was indicated as a non-monophyletic group of stingrays based on morphological data (Rosenberger 2001), and subsequent studies on the Nicotinamide adenine dinucleotide dehydrogenase 2 (ND2) mitochondrial gene corroborated this hypothesis (Naylor *et al.* 2012). Recently, Last et al. (2016b) revised Dasyatidae based on morphological and molecular data and divided it into four subfamilies (Dasyatinae, Hypolophinae, Neotrygoninae, and Urogymninae). Besides, what was previously known as *Dasyatis* was separated into eight genera (*Dasyatis*, *Pteroplatytrygon* Fowler, 1910, *Taeniurops* Garman, 1913, *Bathytoshia* Whitley, 1933, *Hemitrygon* Müller and Henle, 1838, *Hypanus*, *Telatrygon* Last et al., 2016b, and *Megatrygon* Last et al., 2016b in the subfamily Dasyatinae, but grouped by morphological similarities and molecular clusters.

The richest Dasyatinae genus in the American continent is *Hypanus* with eight valid species: *H. americanus* (Hildebrand and Schroeder 1928), *H. dipterurus* (Jordan and Gilbert 1880), *H. guttatus* (Bloch and Schneider 1801), *H. longus* (Garman 1880), *H. marianae* (Gomes et al. 2000), *H. rudis* (Günther 1870), *H. sabinus* (Lesueur 1824), and *H. say* (Lesueur 1817). Except *H. rudis* from Guinea Gulf, at the western coast of the African continent, and *H. dipterurus* and *H. longus* from the Pacific Ocean, all other five species occur in the Atlantic coast of America (Figure 1), suggesting the probable Center of Origin of the genus at Western Central Atlantic due to its richness at the Atlantic and the knowledge of the Caribbean as a center of species' diversification (Bowen et al. 2013). The monophyly of this group was based on an ND2 mitochondrial DNA maximum likelihood phylogenetic analyses. However the placement of *H. marianae* and *H. rudis* was not tested, and they were considered as belonging to the same genus due to morphologic data (Last et al. 2016b).

Another Dasyatidae genus has a similar pattern of species diversification in the Atlantic Ocean: *Fontitrygon* Last et al., 2016b, in which *F. margarita* (Günther 1870), *F. margaritella* (Compagno and Roberts 1984), *F. ukpam* (Smith 1863), and *F. garouaensis* (Stauch and Blanc 1963) occur in the African continent and *F. colarensis* (Santos et al. 2004) and *F. geijskesi* (Boeseman 1948) in the Northern coast of South America. However, these American species were not included in Last *et al.* (2016) revision of Dasyatidae due to lack of samples, remaining provisionally in the genus *Fontitrygon*.

In marine environments it is not uncommon to identify early stages of speciation in para- or sympatric lineages with an incipient genetic differentiation (Avise 2000; Potkamp and Fransen 2019). This event has already been observed in some sharks (Corrigan and Beheregaray 2009) and rays (Kashiwagi et al. 2012) with great geographic dispersal but genetically restrained by environmental ecological features (Bowen et al. 2013).

River outflow, salinity, temperature, depth, marine currents, and land and oceanic barriers, which are environmental characteristics that delimit the habitat occupied by marine organisms in provinces and realms (Spalding et al. 2007), are probably the most important biogeographic barriers for these stingrays.

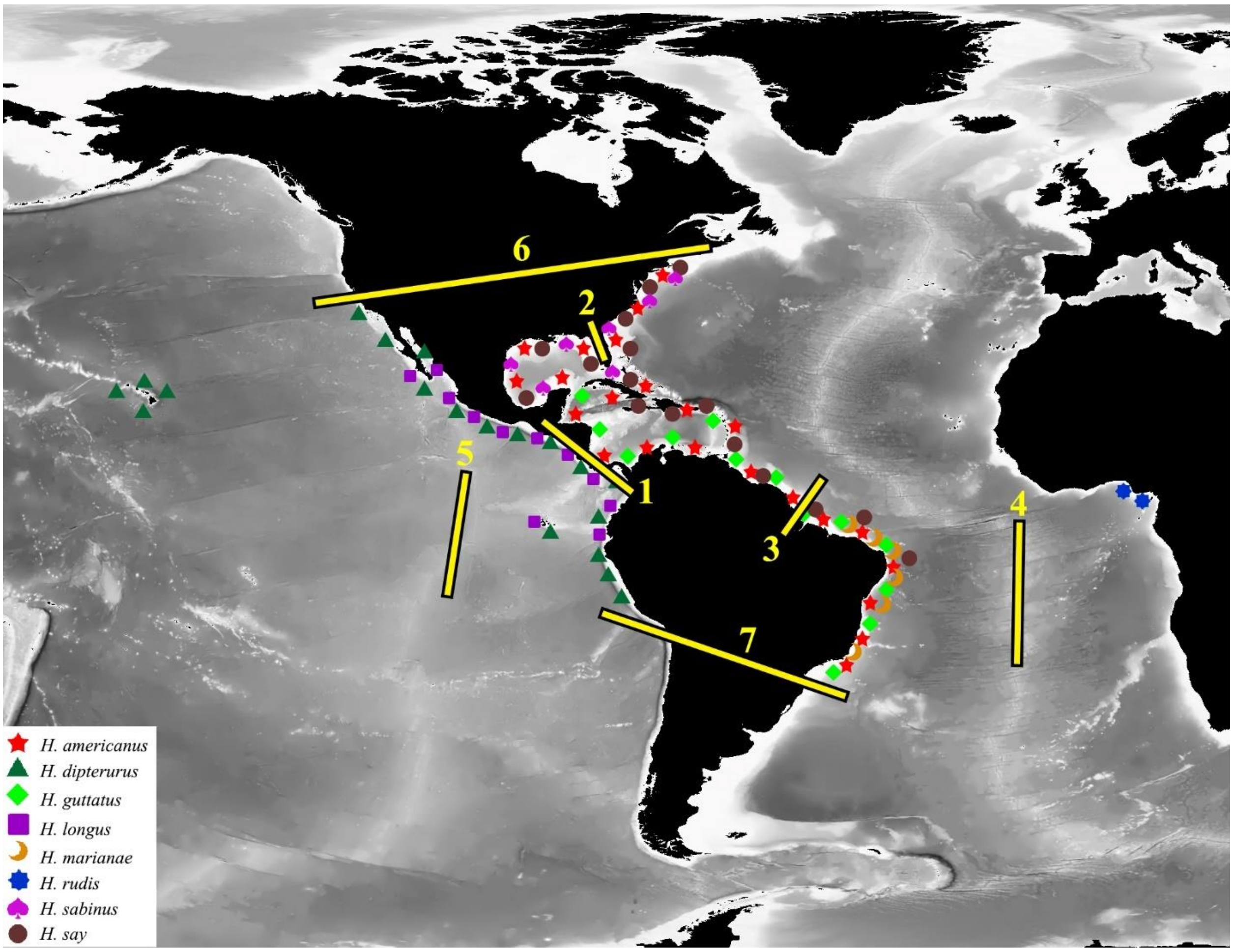


Figure 1. Geographic distributions of eight valid species of *Hypanus* and biogeographical barriers posed by the American continent: 1 and 2, land (Isthmus of Panama and Peninsula of Florida); 3, salinity and sediments (Amazon river outflow); 4 and 5, distance (Mid-Atlantic Barrier and East Pacific Barrier); 6 and 7, temperature (colder waters in Northern and Southern hemispheres).

DNA usage

With the advances of technologies and decreasing costs of sequencing, researchers have been characterizing mitochondrial genomes (mtDNA) of Chondrichthyans (Chen et al. 2013; Yang et al. 2013, 2016; Shen et al. 2015b; Si et al. 2016; Yang and Naylor 2016). However, only a few studies have used these data for phylogenetic and biogeographic analyses within this group of animals (Gaitán-Espitia *et al.*, 2016; Doane *et al.*, 2018; Yang *et al.*, in prep.). MtDNA has a rate of evolution that is valuable for studying relationships, especially of recently (5-10 Ma) diverged lineages (Brown et al. 1979); however, this rate is slower in Chondrichthyans when compared to mammals (Martin et al. 1992).

Genomic studies of DNA variation are becoming more common and may reduce the uncertainty that arises from coalescence variation among distinct markers (Ballard & Whitlock, 2004; Maddison, 1997). This great amount of data of the genomic era generated an appreciation for potential conflicting patterns of differentiation among mitochondrial and nuclear genomes that are not a consequence of incomplete lineage sorting but caused by factors such as differential selection, demographic asymmetries or hybridizations (Hudson and Turelli 2003). Corrigan *et al.* (2017) found that the shark species *Carcharhinus galapagensis* and *C. obscurus* could be the same species by using the mitochondrial marker ND2; however, by using nuclear loci their specific-level separation became clear. The authors suggested that a combination of selection and demography created the observed patterns and that it is also necessary to study phenotypic and environmental data to understand the evolutionary processes that could have been responsible for their patterns. Exclusive mitochondrial, with limited nuclear, DNA introgression allows the independent evolution of lineages with current gene flow among populations (Bernal et al. 2017).

The inference of phylogenetic relationships through the Bayesian statistics using mitochondrial genome data and the molecular clock calibration to estimate divergence dates among clades is still a novelty in Chondrichthyan studies, but there are already some interesting results to compare to (Poortvliet et al. 2015). Besides biogeographic implications that could arise from the divergence times, tree topologies can show cryptic species.

Conservation

Even though benthic stingrays are known to have high connectivity, without population structure, throughout a large continuous coastal area (Le Port and Lavery 2012), given the many barriers around the American continent where *Hypanus* stingrays live, it is expected to be more cryptic lineages, which still have not been noticed within the group. The importance of refining their delimitation lies in aiding conservation actions. By defining unique evolutionary groups and recognizing their area of distribution, one can provide adequate data for the correct evaluation of threatened species status. Currently, six *Hypanus* species are Data Deficient at the Red List of Threatened Species (IUCN, 2019), except *H. sabinus* and *H. say* that are under the Least Concern (LC) status. Therefore, there is an urgency in correctly identifying the evolutionary units to change their evaluations assessment by IUCN.

Given that, we aim to define the genus and the lineages within *Hypanus*, its relationship to other Dasyatidae genera, and the relationships of its species to investigate the geographical origin of the genus. Besides, we will analyze the effects of biogeographical barriers in the diversification of *Hypanus* species' and identify possible cryptic ones and, consequently, proposing conservation measures.

MATERIAL AND METHODS

Sampling, DNA isolation, and sequencing

There were sampled all eight valid species belonging to the genus *Hypanus*, six representatives of almost all Dasyatinae genera (except *Megatrygon*), two of both Neotrygoninae genera, and four out of six of the genus *Fontitrygon* (subfamily Urogymninae), except *F. colarensis* and *F. ukpam*, adding up to 130 specimens. Sampled species locations and their distributions in Table 1 (details in Supp. Mat. 1). Valid names were obtained from Last *et al.* (2016a) and Catalog of Fishes (Fricke, R. et al. 2019), as well as distributions. Most tissues were collected in fish markets, making it unfeasible to preserve most of the specimens. Some were collected by authors and some were kindly provided by collaborators. Brazilian samples were collected under the permit SISBIO 54254-3 and registered at SISGEN.

Table 1. Sampled species of the genera *Hypanus*, *Telatrygon*, *Hemitrygon*, *Taeniurops*, *Pteroplatytrygon*, *Bathytoshia*, *Dasyatis*, *Neotrygon*, *Taeniura*, and *Fontitrygon*, their location and geographic distributions. *non-sampled species by Last *et al.* (2016b). EA: Eastern Atlantic, WA: Western Atlantic, EP: Eastern Pacific, WP: Western Pacific.

Species	**N**	**Sampled Locations**	**Distributions**
***Hypanus americanus* (Hildebrand & Schroeder, 1828)**	29	Virginia (USA), to Nicaragua, to Bahia (Brazil)	WA: Massachusetts (USA) to São Paulo (Brazil)

***Hypanus dipterurus* (Jordan & Gilbert, 1880)**	4	Baja California (Mexico)	EP: Hawaii (USA), California (USA) to northern Chile, including Galápagos Islands
***Hypanus guttatus* (Bloch & Schneider, 1801)**	31	From Belize to Bahia (Brazil)	EA: Gulf of Mexico to Paraná (Brazil)
***Hypanus longus* (Garman, 1880)**	4	Baja California (Mexico)	EP: Baja California (Mexico) to Ecuador, including Galápagos Islands
****Hypanus marianae* (Gomes, Rosa & Gadig, 2000)**	29	From Ceará to Bahia (Brazil)	WA: Northeastern Brazil
****Hypanus rudis* (Günther, 1870)**	4	Senegal and Ghana	EA: Gulf of Guinea
***Hypanus sabinus* (Lesueur, 1824)**	4	South Carolina and Mississippi (USA)	WA: Delaware (USA) to Gulf of Mexico
***Hypanus say* (Lesueur, 1817)**	8	South Carolina and Mississippi (USA)	WA: Massachusetts (USA) to Brazil
***Telatrygon acutirostra* (Nishida & Nakaya, 1988)**	1	Ariake Bay (Japan)	WP: China and southern Japan
***Hemitrygon akajei* (Müller & Henle, 1841)**	1	Ariake Bay (Japan)	WP: China and Japan to Malasia
***Taeniurops grabata* (Geoffroy St. Hilaire, 1817)**	1	Senegal	EA: Mediterranean Sea, Madeira and Canary Islands to Angola
***Pteroplatytrygon violacea* (Bonaparte, 1832)**	1	California (USA)	Cosmopolitan in tropical and warm temperate seas
***Bathytoshia lata* (Garman 1880)**	1	Hawaii (USA)	Indo-West Pacific, Hawaii (USA), Eastern Atlantic and Mediterranean Sea
***Dasyatis hypostigma* Santos & Carvalho, 2004**	1	Uruguay	WA: Southwestern Atlantic

***Neotrygon kuhlii* (Müller & Henle, 1841)**	1	Malasia	WP: Solomon Islands, Red Sea, Indo-West Pacific: East Africa, east to Philippines and Mariana Islands, north to Japan, Australia, and New Caledonia
***Taeniura lymma* (Forsskål, 1775)**	1	Indonesia	Red Sea, Indo-West Pacific: East and South Africa, east to Philippines and Papua New Guinea, north to Philippines, south to northern Australia
****Fontitrygon garouaensis* (Stauch & Blanc, 1962)**	1	Nigeria	EA: Nigeria and Cameroon
***Fontitrygon margarita* (Günther, 1870)**	1	Senegal	EA: Senegal to Congo
***Fontitrygon margaritella* (Compagno & Roberts, 1984)**	1	Senegal	EA: Mauritania to Angola
****Fontitrygon geijskesi* (Boeseman, 1948)**	6	North of Brazil	WA: Venezuela and Suriname to northern Brazil
Total	**130**		

Genomic DNA was extracted from muscular tissue samples using the E.Z.N.A Tissue DNA Kit (Omega Bio-Tek), then the amount of DNA of each sample was quantified using the Quibit Fluorometric to get from 0.5 to 3 ug of genomic DNA. For the first step of library preparation, the DNA was sheared to 500 bp using Covaris M220 Focused-ultrasonicator. Sheared DNA went through a size-selection process, SPRI (Solid Phase Reversible Immobilization) beads, to select fragments with more than 250 bp. Then, samples went through a series of reactions: blunt-end repair, adapter-ligation, fill-in, and pre-hybridization PCR with indexing primers for each sample, where all steps are carefully described by Li *et al.* (2015). Afterward, the method of Mitochondrial Gene Capture proceeded following Li *et al.* (2015), and an Illumina MiSeq Next Generation Sequencer was used for mitochondrial genome sequencing.

Assembly and mitogenome annotation

Adaptors were trimmed from the MiSeq reads using Trim Galore! (Krueger) script with the tool Cutadapt (Martin 2011) based on a Phred quality score of 30. Then, reads from an individual were assembled by mapping it to a known mitochondrial genome reference using GENEIOUS 7.9.1. Sequences were annotated using a MitoAnnotator pipeline at MitoFish website (Iwasaki et al. 2013), and RNA regions were excluded, leaving only protein-coding genes.

Alignments and phylogenetic reconstructions

Mitogenomes of all 135 specimens were aligned in GENEIOUS 7.9.1 using the MUSCLE algorithm. Mitochondrial control regions were excluded from all sequences since this region is highly variable among individuals and the coverage after mitochondrial gene capture was too low. The final alignment had 11,471 base pairs in 13 protein-coding genes. The analysis was performed to study both the relationships within the genus *Hypanus,* as well as its relations within the sub-family Dasyatinae and to Neotrygoninae and Urogymninae.

To select the molecular evolution model, we used the jModelTest2 (Guindon and Gascuel 2003; Darriba et al. 2012) in CIPRES Science Gateway (Miller et al. 2010) and, under Bayesian Inference Criteria, the best scheme for the protein-coding genes alignment was GTR+gamma+invariant sites. Phylogenetic analysis was conducted doing Maximum Likelihood (RAxML) (Stamatakis 2014) in CIPRES Science Gateway and Bayesian Inferences in BEAST 2.5 (Bouckaert et al. 2019). Then, we selected the species group *H. americanus* (comprising the species *H. americanus* from North to South America, *H. longus* from the Pacific, and *H. rudis,* from Africa), and one specimen of *H. marianae* as previously identified as a sister-group to the *H. americanus* group to do a calibration adjustment. Within the clade, we chose representatives of each independent lineage, in a total of eight sequences as the ingroup and one as the outgroup. The molecular evolution model was selected under the Bayesian Inference Criteria and the best scheme was TIM3+gamma+invariable sites. We did a Maximum Likelihood analysis of this subset of specimens and got a hypothesis of relationship among them, which was used as an initial topology for a Bayesian Inference analysis to get the divergence times.

Since this clade has species on both sides of the Isthmus of Panama, a possible speciation event might have occurred due to this closure. Therefore, we accepted the premise of this biogeographic event and a vicariant one for this clade and we calibrated the analysis using an average rate of 3.93×10^{-3} substitutions per site per million years. We took the average mitochondrial genomes' distance between geminate species separated by the Isthmus of Panama (*Rhizopriodon lalandii* and *R. longurio* (0.02) and *Rajella* spp. (0.007), which is 0.011 and divided by the most accepted date for its final closure: 2.8 My (O'Dea et al. 2018). We set the molecular clock at a relaxed lognormal distribution to allow the rate to vary among branches.

The Markov chain Monte Carlo (MCMC) analysis was conducted with 3×10^{8} generations and trees were sampled every 1,000 generations. The output .log file of BEAST was opened in TRACER to verify if the chains had reached a state of equilibrium and the effective sample size of the posterior was higher than 4,000. All the trees generated by BEAST were combined through the TreeAnnotator program with a burn-in of 10%, which presented a single topology with the posterior probabilities and dates of nodes. We obtained 4.5818 Ma (95% HPD interval from 2.8054 to 4.6399 Ma) for the clade containing species on both sides of the Isthmus of Panama, which was then used to calibrate the analysis of the whole dataset.

We created a subset of *Hypanus* independent lineages and one species as an outgroup, *Dasyatis hypostigma*. The Bayesian Inference analysis of this alignment was calibrated by suggesting the abovementioned date for the clade and setting the molecular clock to a relaxed lognormal rate distribution of 3.54×10^{-3} corrected from the previous analysis. The MCMC analysis was conducted with 5×10^{8} generations and trees were sampled every 5,000 generations. The following steps were the same as those used above.

Two calibration analyses were developed because the molecular rate of evolution is probably not the same throughout the whole genus, so we used it for the *H. americanus* species group analysis, as it is related to the biogeographic event of the closure of Isthmus of Panama. Then we used the date of divergence to calibrate the dataset instead of using one fixed molecular clock rate, which allowed the rate to vary among the branches.

Biogeographic analysis

To infer the biogeographic history of extant lineages of *Hypanus*, we performed several reconstructions of ancestral ranges using the R package BioGeoBEARS (Matzke 2013a). It implements the three most-used historical biogeography models in a likelihood framework, specifically the LAGRANGE dispersal-extinction-cladogenesis (DEC) model (Ree et al. 2005; Ree and Smith 2008), the likelihood version of DIVA (DIVALIKE) (Ronquist 1997), and a likelihood version of the range evolution model used by BayArea (BAYAREALIKE) (Landis et al. 2013) and Bayesian Binary Method (BBM) (Ronquist and Huelsenbeck 2003; Ali et al. 2012) of RASP (Yu et al. 2015). Each method considers distinct premises on anagenesis and cladogenesis (Ronquist and Sanmartín 2011), which considerably impact the results (Matzke 2014).

BioGeoBEARS allows the implementation of multiple models for the evaluation of diverse hypotheses by comparing the fit to observed data (Matzke 2013b, 2014). We executed a likelihood ratio test (LRT) for the nested models and the Akaike information criterion (AIC) and corrected for sample size (AICc) for both nested and non-nested models.

We performed a set of time-stratified analyses using the time-scaled tree of the genus *Hypanus* with *Dasyatis hypostigma* as an outgroup, accounting for nine models: the standard models (hereby called M0) DEC, DIVALIKE, and BAYAREALIKE (Matzke 2013a) an additional analysis of each including *x* (Van Dam and Matzke 2016) as a free parameter (M1), and another analyses including both *x* and *j* (Matzke 2014) as free parameters (M2). The *x* parameter modifies the dispersal rates estimating the probability of dispersal as a function of distance. By setting it as a free parameter, each branch is differently influenced by the distance between areas. To add a founder-event speciation to the model, we included the free *j* parameter which allows a descendent jump to a new range from an ancestor without prior range expansion (Matzke 2014). We considered seven time-ranges as they represent intervals with marine-species diversifications potential: 36-33, 33-23, 23-10.4, 10.4-9.5, 9.5-2.8, 2.8-2, 2-0 Ma. We considered seven possible areas in a combination of biogeographic realms and provinces of Spalding *et al.* (2007). Areas-allowed matrices between areas were defined for each of seven time slices.

The Biogeographical Stochastic Mapping (BSM) (Dupin et al. 2016; Matzke 2016) was also implemented in BioGeoBEARS using DEC+X+J model, with the highest likelihood, to evaluate its impact on range changes' frequencies. This analysis creates histories using both the times and geographical locations provided. These simulations are constrained to build the data given the phylogeny, which was averaged over 72 replicas to result in means and standard deviations of vicariance, dispersal, and within-area speciation events (Dupin et al. 2016).

Lineages' delimitation methods

By analyzing the relationships among species and their divergence dates, we noticed some valid species could be either paraphyletic or have long branches within them, suggesting possible distinct lineages. Therefore, we decided to do six species' delimitation analyses within the clades of *H. americanus* complex, *H. guttatus*, and *H. say* independently: multiple- and single-threshold Generalized Mixed Yule Coalescent (m-GMYC and s-GMYC, Fujisawa & Barraclough, 2013), multi-rate Poisson Tree Process (mPTP, Kapli et al., 2017), Bayesian Poisson Tree Process (bPTP, Zhang *et al.*, 2013), Automatic Barcode Gap Discovery (ABGD, Puillandre et al., 2012), and Bayesian Phylogenetics and Phylogeography (BPP, Flouri *et al.*, 2018). For each subset of data, we used the jModelTest2 to select the molecular evolution model and conducted a Maximum Likelihood analysis to get the relationships and nodes' bootstrap values, besides a Bayesian Inference analysis to get a topology and the posterior probabilities. These trees were used in all the following methods.

GMYC calculates the maximum likelihood for a model that merges diversification among species and cladogenesis within species on an ultrametric time-tree. m-GMYC and s-GMYC were performed in R software using the package *splits* (Ezard et al. 2017). PTP uses nucleotides' substitutions to estimate intra and interspecific processes. It identifies the shift among intra and interspecific processes by using one parameter for coalescence and another for speciation, and mPTP uses distinct branch rates for coalescence and speciation. For mPTP and bPTP analyses we used the webserver (https://species.h-its.org/ptp/). ABGD separates lineages into possible species based on a barcode gap (between intra and interspecific pairwise genetic distances). These analyses were also performed through a web server (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) using three distinct distance options (Jukes-Cantor, Kimura 2-parameter, and Simple), intraspecific prior ranges from 0.001 to 0.25 in ten steps, and relative gap width of 1.5.

BPP uses genomic datasets to adapt ancestral genetic polymorphisms and coalescent processes by using MCMC to calculate distinct species trees posterior probabilities (Flouri et al. 2018). For BPP analyses we evaluated four scenarios combining large and small ancestral populations and deep and recent divergences which were performed three times each, 5×10^5 generation samples and a burnin of 5,000: (a) large ancestral population with deep divergences, $\theta \sim$ invgamma(2, 0.1) and $\tau \sim$ invgamma(2, 0.1); (b) large ancestral population with recent divergences, $\theta \sim$ invgamma(2, 0.1) and $\tau \sim$ invgamma(3, 0.002); (c) small ancestral population with deep divergences, $\theta \sim$ invgamma(3, 0.002) and $\tau \sim$ invgamma(2, 0.1); and (d) small ancestral population with recent divergences, $\theta \sim$ invgamma(3, 0.002) and $\tau \sim$ invgamma(3, 0.002).

RESULTS

Phylogenetic inferences

Mitochondrial genomes of all currently valid species of the genus *Hypanus* are described in Supp. Mat. 2. The genus *Hypanus* sensu Last et al. (2016b) was recovered as monophyletic and sister to all other Dasyatid genera (except *Megatryon*, not sampled for this study) within the subfamily Dasyatinae (Figure 2). Dasyatinae is a sister-group to the subfamily Neotrygoninae, and these are sister to Urogymninae within the Dasyatidae family, as already suggested by Last *et al.* (2016b).

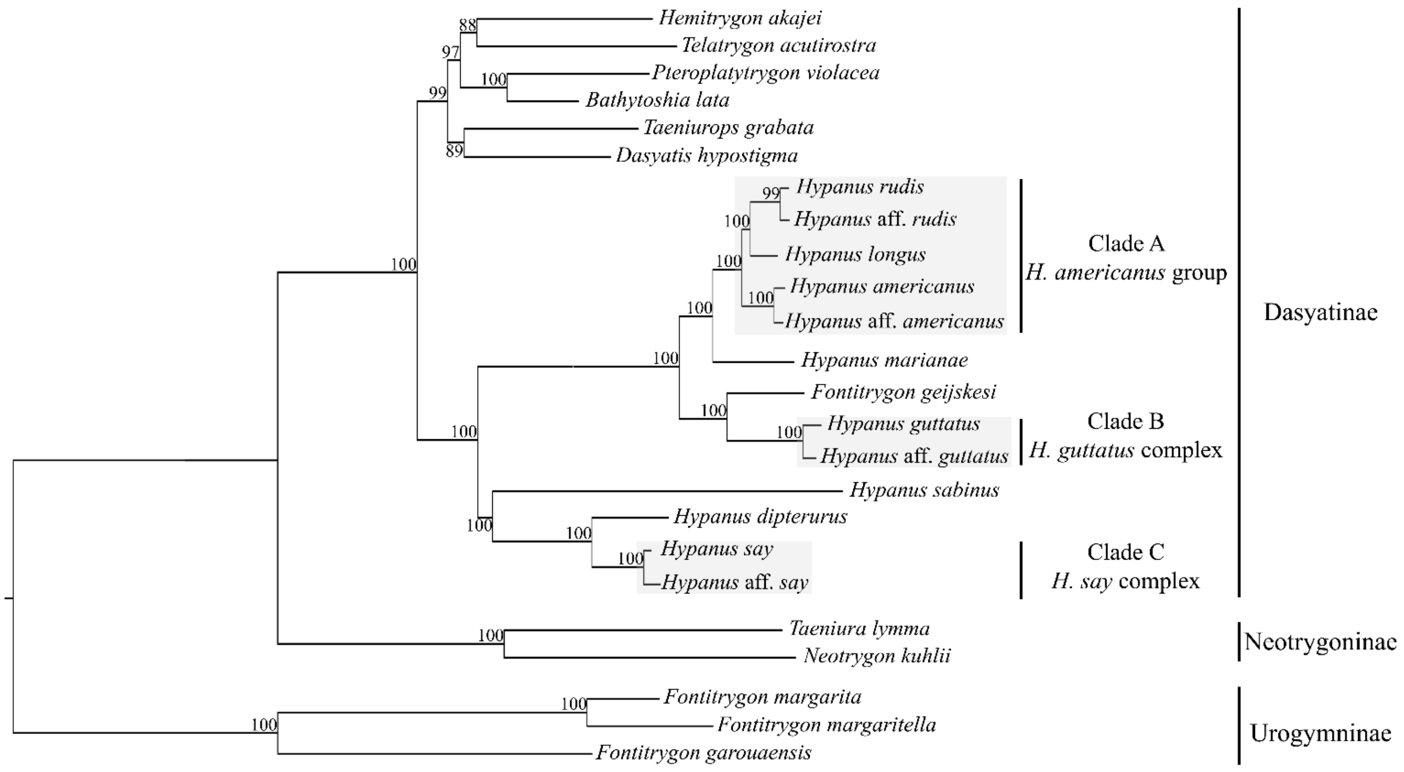


Figure 2. Maximum Likelihood tree of mtDNA with bootstrap values above each node with representatives of *Hypanus* species, Dasyatinae genera, Neotrygoninae genera, and Urogymninae. genus *Fontitrygon*.

There are clear lineages that correspond to valid species names: *H. dipterurus*, *H. longus*, *H. marianae*, *H. rudis*, and *H. sabinus*. However, the previously known *H. americanus*, Southern stingray, turned out to be a non-natural group (Clade A in Figure 2). This species has representatives from Massachusetts (USA) to São Paulo (Brazil), along the Atlantic coast of the American continent. It was thought to be a marine coastal species with a wide distribution.

The species *H. rudis*, which now has its relationship to other *Hypanus* lineages evaluated, is closely related to the supposedly *H. americanus* from South America, from now on called *Hypanus* aff. *rudis*. These two clades (*H. rudis* and *H.* aff. *rudis*) diverged approximately 1.28 Ma [0.86-1.76 HPD] from each other, and 4.18 Ma [3.18-5.24 HPD] from *H. longus* occurring in the Pacific coast of the American continent. The three clades together diverged from the Central/North American lineage of *H. americanus* 5.29 Ma [0.87-1.87 HPD]. What remained as *H. americanus* is, in fact, two independent lineages that diverged 1.36 Ma [0.87-1.87 HPD].]

The species *H. marianae*, that was not included in the previous molecular study (Last *et al.* 2016), is a monophyletic lineage and sister to the above-mentioned clade containing *H. americanus* sensu lato, *H. longus*, and *H. rudis*, and the time of divergence of these lineages is approximately 9.37 Ma [7.4-11.57 HPD]. Approximately 13.76 Ma [11.43-16.19 HPD] the lineage containing the species *H. guttatus* diverged from the clade above This species is distributed from Mexico to the south of Brazil and it harbors an independent lineage of specimens from Belize (Clade B in Figure 2). This is the same pattern observed for *H. americanus* (distinct Central/North American and South American lineages) and the divergence time within lineages of *H. guttatus* is older than *H. americanus* (2.12 Ma [1.35-2.95 HPD]).

Approximately 27.79 Ma [22.98-33.03 HPD], a lineage containing the species *H. dipterurus*, *H. sabinus*, and *H. say* diverged from the other *Hypanus* species. *H. sabinus*, which occurs from New York (USA) to Belize, is a monophyletic group. *H. dipterurus*, the second species of *Hypanus* on the Pacific coast, is sister to *H. say* in the Atlantic. Both *H. longus* and *H. dipterurus* are sympatric, however, their mitochondrial evolution has been distinct for more than 30.97 My [26.13-36.09 HPD], from a lineage related to the origin of the genus *Hypanus*. Nevertheless, their evolutionary histories are similar, since both are sister-groups to Atlantic species. The sister-clade to *H. dipterurus* is *H. say* and, even though they are separated by the American continent, as *H. longus* and *H.* aff. *rudis* + *H. rudis*, their divergence dates 9.33 Ma [6.39-33.03 HPD], some millions of years before the final closure of the Isthmus of Panama (2.8 Ma). Besides, within the clade of *H. say* it was clear the divergence of two lineages 1.56 Ma [0.98-2.18 HPD] (Clade C in Figure 2).

Also, six specimens of one species of the genus *Fontitrygon*, which is currently known as *F. geijskesi*, but not included in the Dasyatidae revision by Last *et al.* (2016), formed a sister-clade to *H. guttatus*, within the genus *Hypanus*, not *Fontitrygon*. So, it seems to be a valid species, but should be considered as *H. geijskesi*, and it diverged from *H. guttatus* 8.83 Ma [6.77-11.06 HPD]. The other three African species of the genus formed a cluster sister to all other Dasyatinae and Neotrygoninae.

Biogeography

For the biogeographic study, we used the time-tree inferred through a Bayesian analysis of the genus *Hypanus*, with *Dasyatis hypostigma* as the outgroup, in which nodes' posterior probability are all equal to one. Diversification events within the genus occurred during the Paleogene, Neogene, and Quaternary, given that four occurred at the Pleistocene.

The Likelihood-ratio test p (LRT) showed that models including *x* and *j* (dispersal relative to distance and founder events), were significantly best fitted in null models (Table 2) suggesting that distance and founder-event speciation are important to account for range changes in *Hypanus* stingrays. The highest likelihood of all nine models belongs to Dispersal-Extinction-Cladogenesis model with dispersal and founder-events (DEC+X+J), which also has the lowest AIC and AICc values.

Table 2. Pairwise comparison of all nine models tested by BioGeoBEARS. Alternative (alt) fields regard those with x as a free parameter and with x and j as free parameters; Null, without *x*, and without *x* and *j*. Bold Log of Likelihood (LnL) values are the highest ones, and bold AIC and AICc the smallest ones. * significative value of Likelihood-ratio test p (LTR).

Alternative model	**LnLalt**	**AICalt**	**AICcalt**	**Null model**	**LnLnull**	**AICnull**	**AICcnull**	**LRT**
DEC+X	-47.52	101	103.44	DEC	-53.59	111.2	112.27	0.0005*
DEC+X+J	**-42.39**	**92.79**	**97.23**	DEC+X	**-47.52**	**101**	**103.44**	0.0014*
DIVALIKE+X	-47.85	101.7	104.11	DIVALIKE	-53.29	110.6	111.68	0.001*
DIVALIKE+X+J	-45.65	99.31	103.75	DIVALIKE+X	-47.85	101.7	104.11	0.036
BAYAREALIKE+X	-57.27	120.5	122.93	BAYAREALIKE	-62.05	128.1	129.18	0.002*
BAYAREALIKE+X+J	-43.58	95.16	99.60	BAYAREALIKE+X	-57.27	120.5	122.93	1.7 x 10^{-7}*

The summary of 72 BSM counts (Table 3) shows that range expansion is the commonest type of event within *Hypanus*, accounting for 56.29% of all events, followed by founder-event, subset-speciation, and vicariance. There was a mean of 7.4 founder events from, approximately, 10-5 Ma, and means of 3.64 subset-speciation and 1.62 vicariant events which occurred, approximately, from 5-2 Ma and 4 Ma-present, respectively.

Table 3. Summary of biogeographical stochastic mapping counts for *Hypanus* using DEC+X+J model with mean, standard deviations (s.d.), and percentage of each event type estimated by the model.

		DEC+X+J	
Mode	**Type**	**Mean (s.d.)**	**%**
dispersal	range expansion	16.74 (1.35)	56.29
	range contraction	0	0.00
	founder event	7.4 (1.35)	24.88
within-area speciation	sympatry	0.33 (0.56)	1.11
	subset speciation	3.64 (1.2)	12.24
vicariance	vicariance	1.62 (1.09)	5.45

Through the reconstruction of ancestral states within the genus *Hypanus* we noticed its probable origin was at the Tropical Western Atlantic Ocean with further dispersal north-, south-, east-, and westwards followed by diversifications (Figure 3). There were two independent cladogenetic events in which specimens from an Atlantic lineage dispersed to the Pacific with further speciation: 9.33 Ma *H. dipterurus* and *H. say* + *H.* aff. *say*, and 4.18 Ma *H. longus* and *H. rudis* + *H.* aff. *rudis*. We can also observe the pattern of close relationships among Tropical Western Atlantic lineages, and a recent dispersal (1.28 Ma) to Eastern Atlantic.

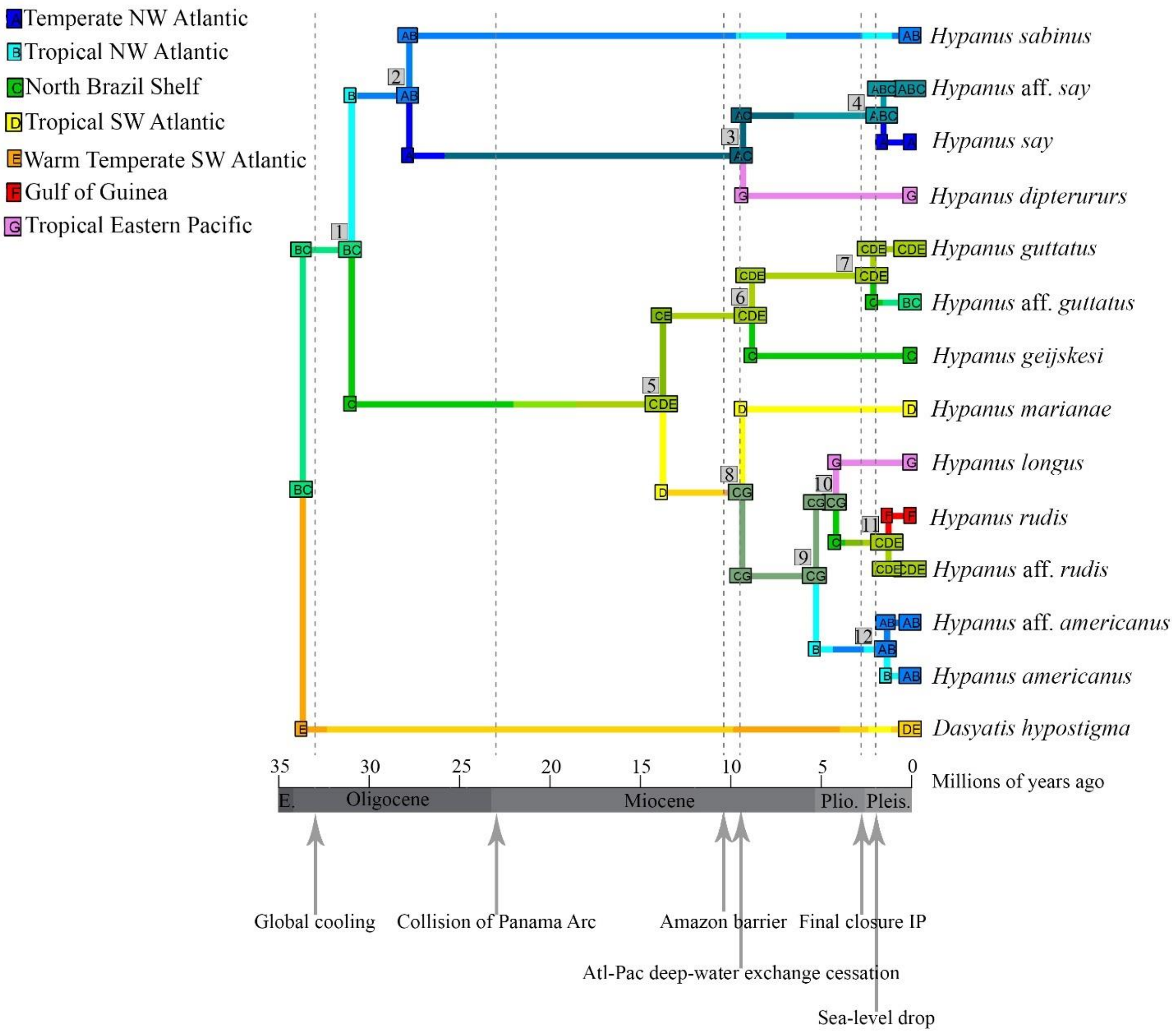
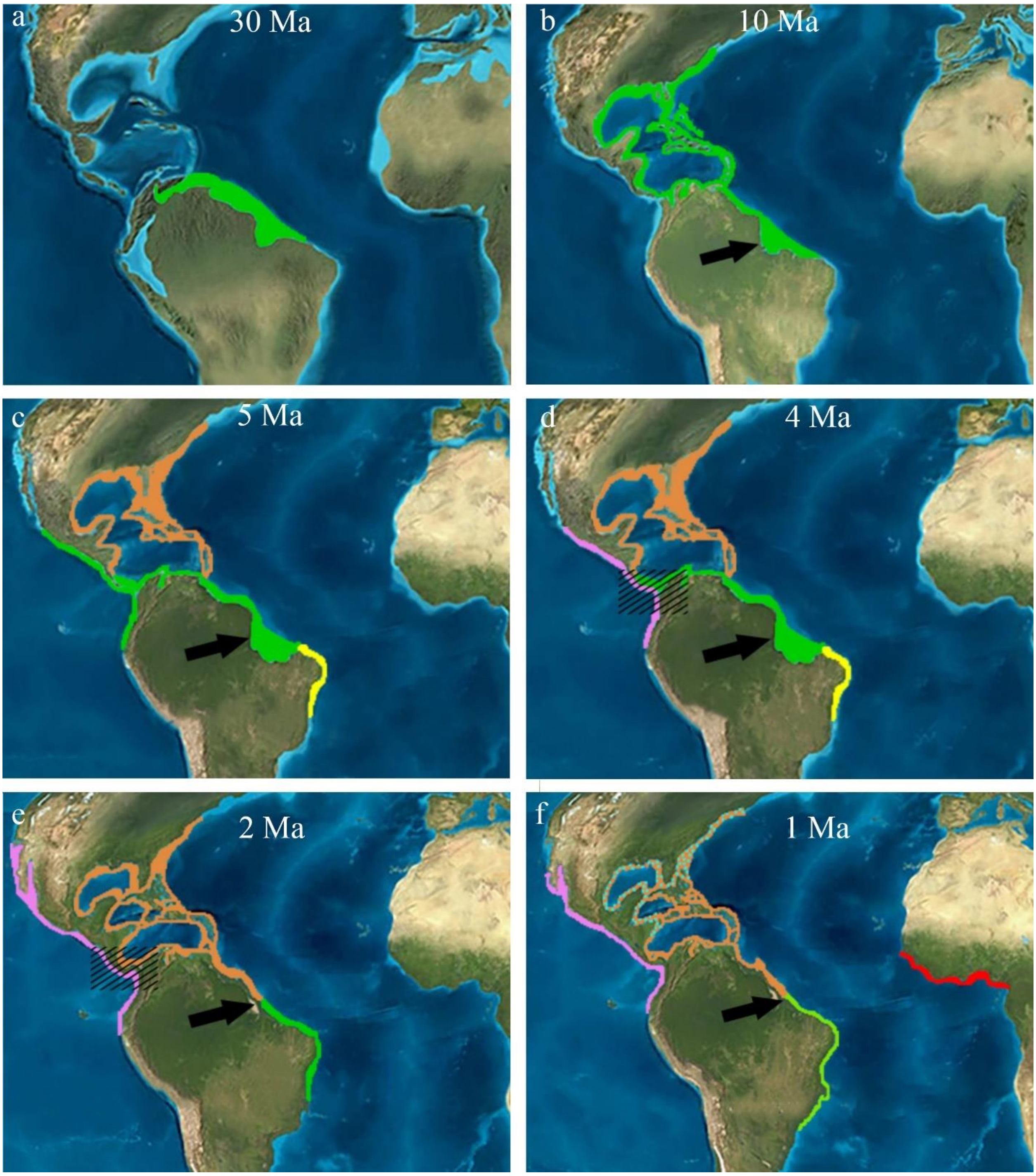


Figure 3 Biogeographic inference based on ancestral states reconstruction of *Hypanus* lineages using the stochastic analysis of the model DEC+X+J (LnL = -42.39). Nodes' colors and letters are hypothetical ancestral states according to the legend on top left corner; intermediate colors and combination of letters represent a rearrangement of up to three areas. Numbers in gray boxes on nodes are clade orders. *Dasyatis hypostigma* used as an outgroup. Biogeographic events represented by gray arrows: Global cooling (33 Ma), Collision of Panama Arc (23 Ma), Amazon barrier (10.4 Ma), Cessation of deep-water exchange between Atlantic and Pacific (9.5 Ma), Final closure of the Isthmus of Panama (2.8 Ma), and Sea-level drop (2.8-2.0 Ma).

Most diversification events within the genus culminating in extant lineages occurred no more than 10 Ma, showing a recent radiation of this clade. However, one currently valid species, *H. sabinus*, is probably the oldest lineage in the genus with 27.79 My of evolution, considering no extinction took place as there is still no known closely related fossil so far.

Summaries of geological, oceanographical, and climate events and *Hypanus* clades' divergences are in



Supp. Mat. 3 and Supp. Mat. 4, respectively. Evolutionary history reconstructions of Clades A, B, and C in Figure 4, Figure 5, and Figure 6, respectively.

Figure 4. Biogeographical reconstruction of *Hypanus* Clade A in six time periods. (a) 30 Ma, Clade 1 in green; (b) 10 Ma, Clade 8 in green, black arrow represents the Amazon river outflow;

(c) 5 Ma, *H. marianae* in yellow, Clade 10 in green, Clade 12 in orange; (d) 4 Ma, *H. marianae* in yellow, *H. longus* in pink, Clade 11 in green, Clade 12 in orange, progressive closure of the Isthmus of Panama; (e) 2 Ma, *H. longus* in pink, Clade 11 in green, Clade 12 in orange, *H. marianae* omitted; (f) 1 Ma, *H. longus* in pink, *H.* aff. *rudis* in green, *H. rudis* in red, *H. americanus* in orange, and *H.* aff *americanus* in light blue dots.

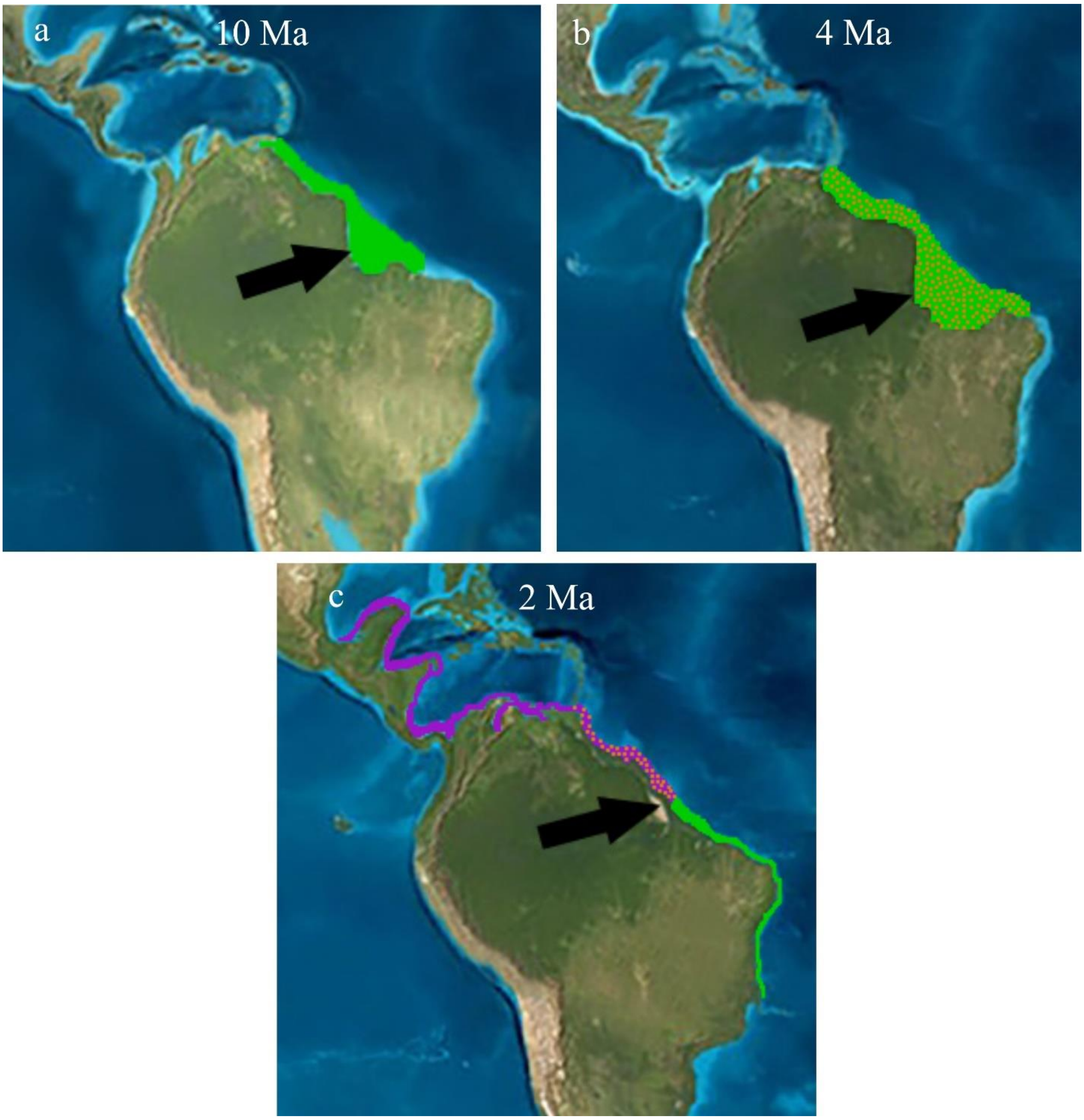


Figure 5. Biogeographical reconstruction of *Hypanus* Clade B in three time periods. (a) 10 Ma, Clade 6 in green, black arrow represents the Amazon river outflow; (b) 4 Ma, Clade 7 in green, *H. geijskesi* in orange dots; (c) 2 Ma, *H. guttatus* in green, *H.* aff. *guttatus* in purple, *H. geijskesi* in orange dots.

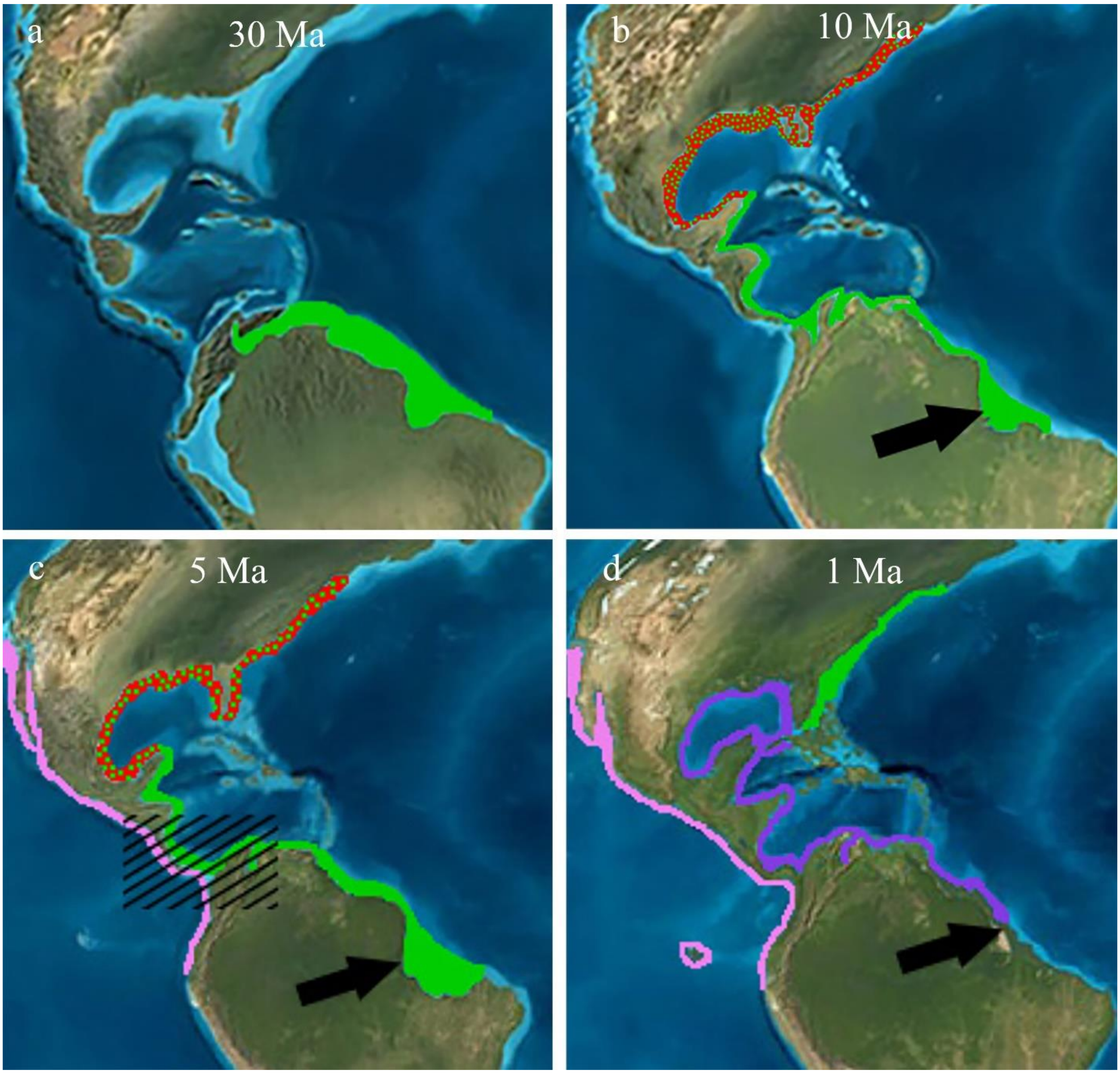


Figure 6. Biogeographical reconstruction of *Hypanus* Clade C in four time periods. (a) 30 Ma, Clade 1 in green; (b) 10 Ma, *H. sabinus* in red, Clade 3 in green, black arrow represents the Amazon river outflow; (c) 5 Ma, *H. sabinus* in red, *H. dipterurus* in pink, Clade 4 in green, progressive closure of the Isthmus of Panama; (d) 1 Ma, *H. dipterurus* in pink, *H. say* in green, *H.* aff. *say* in purple, *H. sabinus* omitted.

Delimitation of lineages

Candidate species of each species-group analyzed, *H. americanus*, *H. guttatus*, and *H. say*, by combining all six delimitation methods are in Figure 7, Figure *8*, and Figure *9*, respectively. In all three datasets, the GMYC suggested the highest number of subdivisions, while mPTP, ABGD, and BPP were the most conservative. We ran this last analysis 12 times in four models for distinct combinations of lineages' relationships and we got consistent results with previous analyses supported by a posterior probability of 1 for all of them, regardless of the model used. As a result, there should be a new sister-lineage to *H. rudis* in South America (that was previously identified as *H. americanus*), one to *H. americanus* from North and Central America, one to *H. guttatus* in Central America, and one to *H. say* in Gulf of Mexico.

The observed new lineages sister to known species are named as *affinis* to those they are closely related to. We kept the valid name according to the type locality of each species: type of *H. rudis* from Nigeria, so *H.* aff. *rudis* from South America; type of *H. americanus* from Chesapeake Bay (USA), but *H.* aff. *americanus* is sympatric; type of *H. guttatus* from Brazil, so *H.* aff. *guttatus* from Central America; and type of *H. say* from Egg Harbor (USA), so *H.* aff. *say* from Gulf of Mexico.

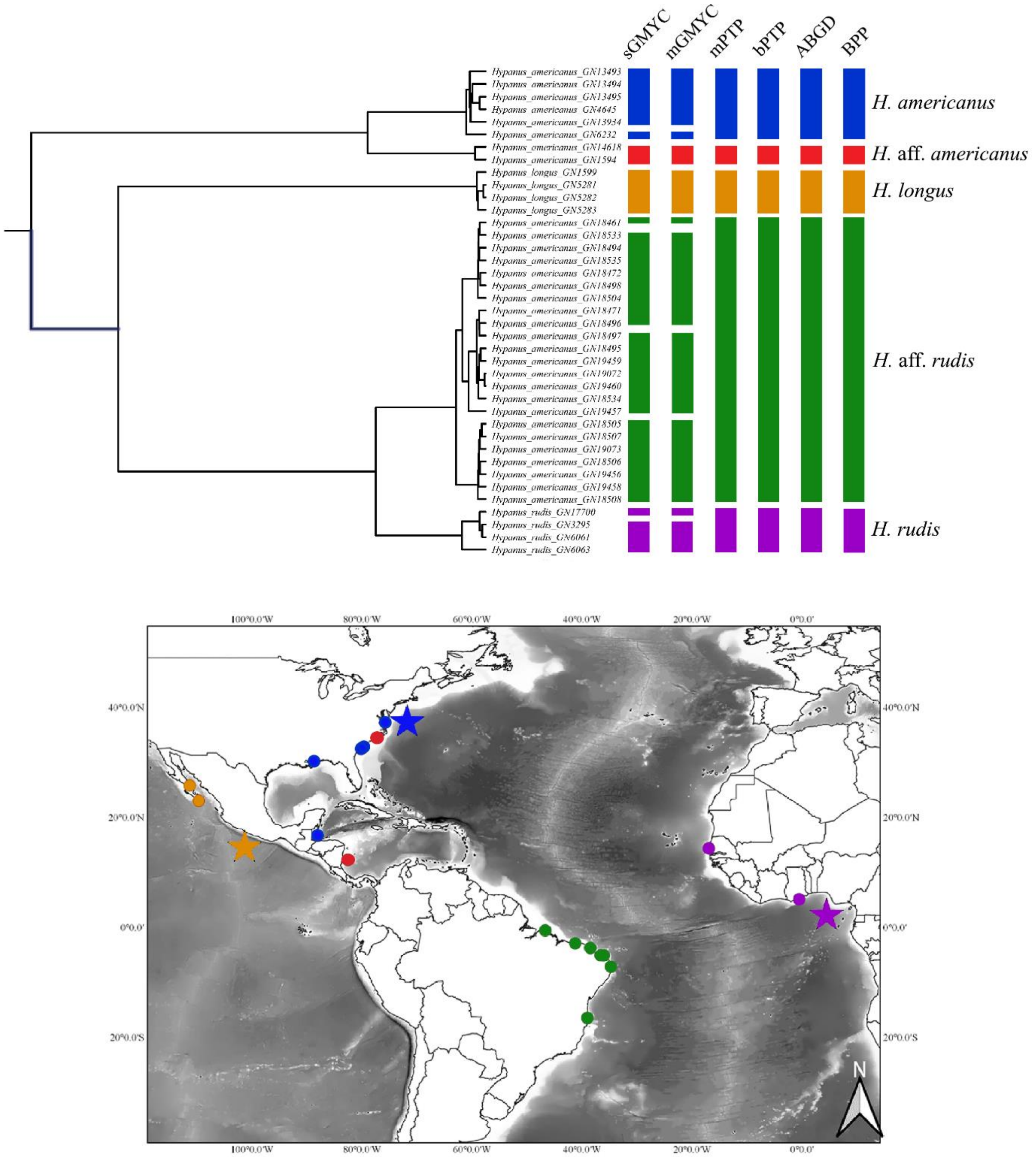


Figure 7. Candidate species of the clade *Hypanus americanus* species group (Clade A), according to six species delimitation analyses using the mtDNA. Possible species found in each analysis are portrayed as colored boxes in columns. In red, *H. americanus*; blue, *H.* aff. *americanus*; orange, *H. longus*; green, *H.* aff. *rudis*; purple, *H. rudis*. The same colors are used to represent sampled specimens in map below. Stars are the holotypes' locations of each valid species, which were not sampled.

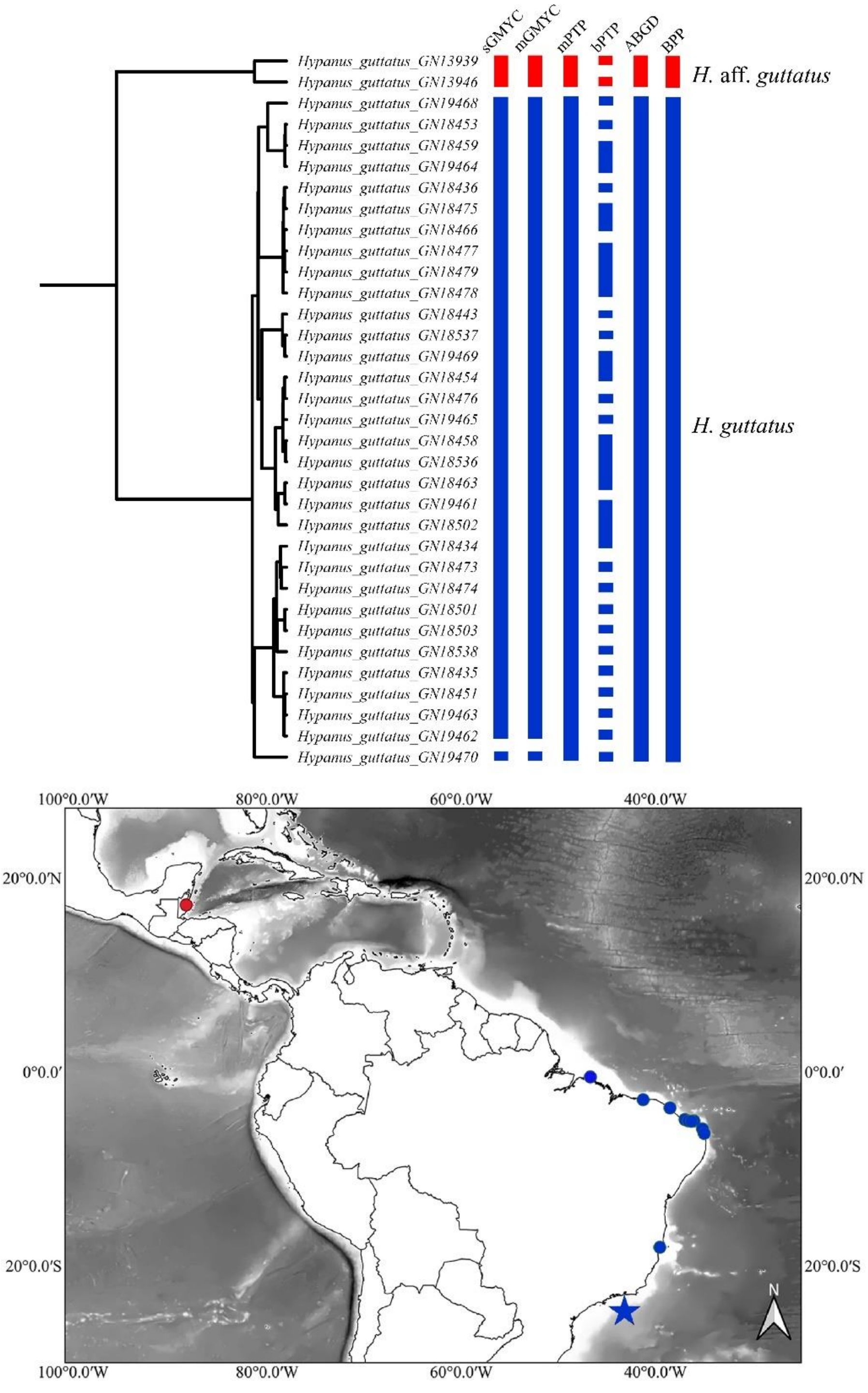


Figure 8. Candidate species of the clade *Hypanus guttatus* species-complex (Clade B), according to six species delimitation analyses using the mtDNA. Possible species found in each analysis are portrayed as colored boxes in columns. In blue, *H. guttatus*; red, *H.* aff. *guttatus*. The same colors are used to represent sampled specimens in map to the right. Blue star is the holotype location of the valid species, which was not sampled.

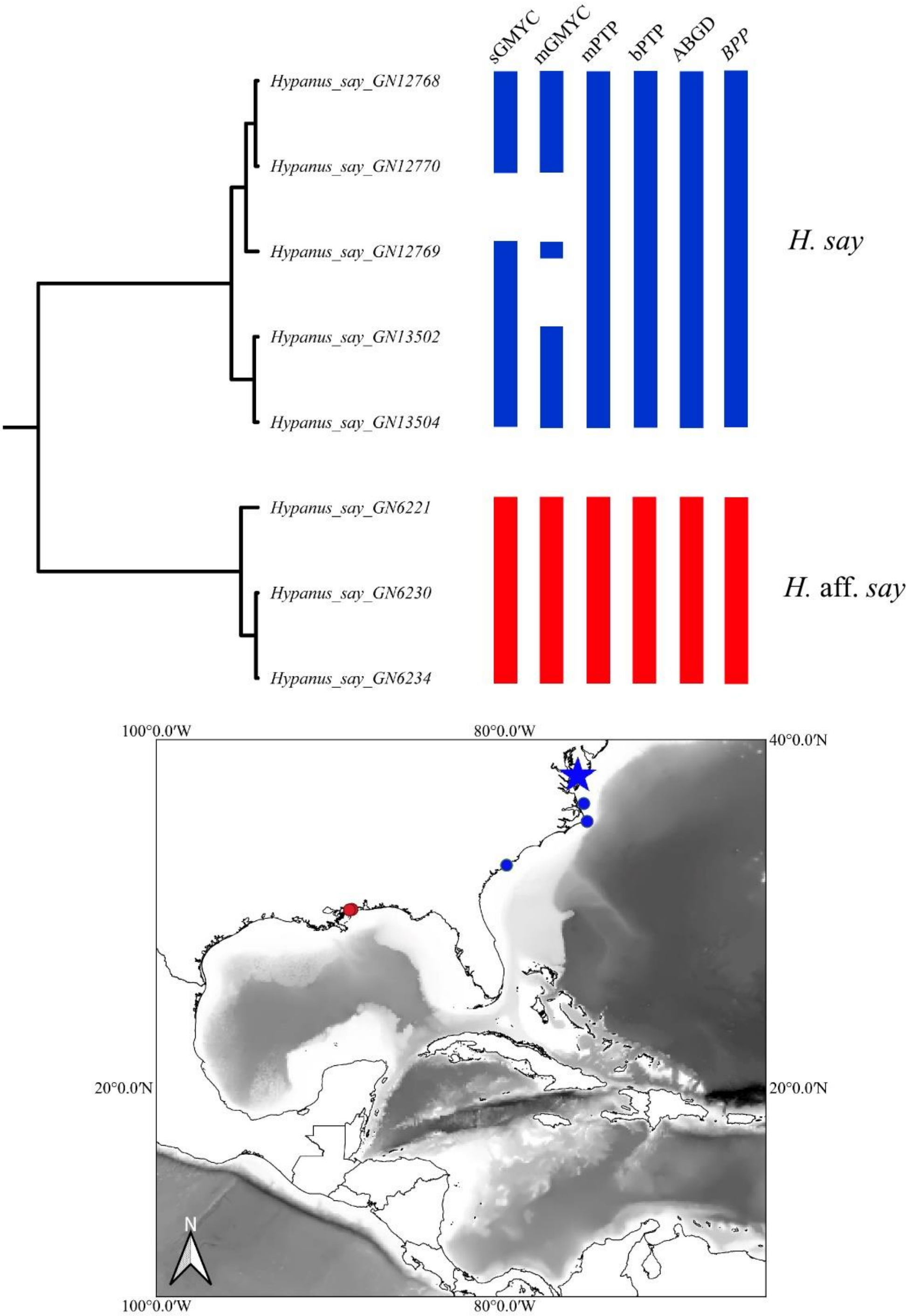


Figure 9. Candidate species of the clade *Hypanus say* species-complex (Clade C), according to six species delimitation analyses using the mtDNA. Possible species found in each analysis are portrayed as colored boxes in columns. In blue, *H. say*; red, *H.* aff. *say*. The same colors are used to represent sampled specimens in map to the right. Blue star is the holotype location of the valid species, which was not sampled.

Average pairwise distances within individuals of each *Hypanus* species vary from 0.036% in *H. longus* to 0.26% in *H.* aff. *guttatus* (Table 4). Regarding average pairwise distances between pairs of species, the smallest distances are 0.82% between *H. rudis* and *H.* aff. *rudis*, 0.83%

between *H. americanus* and *H.* aff *americanus*, and 0.95% between *H. say* and *H.* aff. *say* (Table 5). Interestingly, the distance between two geographically distant species as *H. longus*, from the Pacific, and *H. rudis*, from Africa is only 2.4%. This value may be due to a recent radiation within some clades, with possible four speciation events taking place in the Quaternary, four in the Neogene, and only one in the Paleogene. This last one is known as *H. sabinus* and has the highest distances to all other *Hypanus* lineages (11.74% from *H. dipterurus* is the smallest).

Table 4. Pairwise average distances of mitgen within *Hypanus* species in %.

Species	**% of average divergence within each species**
H. americanus	0.092
H. aff. *americanus*	0.070
H. longus	0.036
H. rudis	0.098
H. aff. *rudis*	0.121
H. marianae	0.068
H. guttatus	0.200
H. aff. *guttatus*	0.262
H. geijskesi	0.167
H. dipterurus	0.163
H. say	0.061
H. aff. *say*	0.047
H. sabinus	0.134

Table 5. Pairwise distances of mitgen between pairs of species in %. Hamer, *H. americanus*; Haffamer, *H.* aff. *americanus*; Hlon, *H. longus*; Hrud, *H. rudis*; Haffrud, *H.* aff. *rudis*; Hmari, *H. marianae*; Hgut, *H. guttatus*; Haffgut, *H.* aff. *guttatus*; Hgeij, *H. geijskesi*; Hdipt, *H. dipterurus*; Hsay, *H. say*; Haffsay, *H.* aff. *say*; Hsab, *H. sabinus*; Dhypo, *Dasyatis hypostigma*.

%	Hamer	Haffamer	Hlon	Hrud	Haffrud	Hmari	Hgut	Haffgut	Hgeij	Hdipt	Hsay	Haffsay	Hsab
Haffamer	0.83												
Hlon	2.69	2.69											
Hrud	3.11	3.12	2.40										
Haffrud	3.08	3.14	2.43	0.82									
Hmari	4.91	4.94	4.61	4.96	4.95								
Hgut	6.84	6.86	6.72	7.01	6.94	7.19							
Haffgut	7.02	7.04	6.88	7.21	7.11	7.24	1.37						
Hgeij	6.49	6.44	6.40	6.67	6.56	6.97	5.35	5.53					

Hdipt	11.24	11.20	11.02	11.16	11.15	11.14	11.57	11.75	11.71				
Hsay	10.89	10.89	10.60	10.80	10.85	10.66	11.13	11.28	11.08	4.57			
Haffsay	10.97	11.01	10.80	11.00	11.06	10.83	11.35	11.53	11.39	4.76	0.95		
Hsab	13.05	13.03	12.80	12.93	12.96	13.55	13.21	13.38	13.01	11.74	11.81	11.89	
Dhypo	14.06	14.09	13.85	14.09	14.07	13.91	14.20	14.32	14.02	13.50	13.06	13.19	14.77

DISCUSSION

Phylogenetic considerations

Hypanus was recovered as monophyletic by using whole mitochondrial genomes of all species previously attributed to it, corroborating the use of the ND2 gene as a *barcode* for Chondrichthyes (Naylor et al. 2012b). The authors of the work revising the family Dasyatidae did not have samples of some lineages, which were hereby included. The species *H. rudis* and *H. marianae* belong to the genus *Hypanus*, as implied by morphological similarities by Last *et al.* (2016a).

However, for the monophyly of the genus, representatives of the species *Fontitrygon geijskesi* that were expected to be related to African species *F. margarita*, *F. margaritella*, and *F. garouaensis*, should be considered as *Hypanus*, as it is nested within it as sister to *H. guttatus*. Therefore, we suggest the reallocation of *F. geijskesi* to the genus *Hypanus* with a new name combination as *Hypanus geijskesi* (Boeseman, 1948), as observed by Rodrigues Filho *et al.* (2020), even though the analysis lacks a phylogenetic inference of its relationship to other *Hypanus* lineages. We tried sampling *F. colarensis* with no success and its phylogenetic relationship to other Dasyatidae is yet to be tested, even though it is morphologically similar to *H. guttatus* and *H. geijskesi* (Santos et al. 2004; Last et al. 2016b), such as diamond-shaped body, long snout, and lack of dorsal caudal fold. Despite not having samples for assessing it, we also suggest the transition of *F. colarensis* to *Hypanus* as *Hypanus colarensis* (Santos et al. 2004) (Table 6). However, we also do not exclude the hypothesis of *F. colarensis* as a hybrid between female specimens of *H. guttatus* and male *H. geijskesi* which could be explained by some hereby identified *H. guttatus* as actually belonging to *H. colarensis*. It could be tested by sequencing nuclear markers of these species and analyzing their relationships comparatively to mitochondrial ones.

Table 6. New species' names combinations for *Hypanus*, their geographical distribution, and tissue accession number at the University of Florida. *name change

Suggested species ID	**Former species ID**	**Location**	**Tissue accession number**
Hypanus americanus	*Hypanus americanus*	WA: Massachusetts (USA) to Central America	13494
**Hypanus* aff. *americanus*	*Hypanus americanus*	WA: Massachusetts (USA) to Central America	14618
Hypanus longus	*Hypanus longus*	EP: Baja California (Mexico) to Ecuador, including Galápagos Islands	1599
Hypanus rudis	*Hypanus rudis*	EA: Gulf of Guinea	3295
**Hypanus* aff. *rudis*	*Hypanus americanus*	WA: Northern to Southeastern South America	18461
Hypanus marianae	*Hypanus marianae*	WA: Northeastern Brazil	18437
Hypanus guttatus	*Hypanus guttatus*	WA: Northern to Southeastern South America	18434
**Hypanus* aff. *guttatus*	*Hypanus guttatus*	WA: Gulf of Mexico to Northern South America	13939
**Hypanus geijskesi*	*Fontitrygon geijskesi*	WA: Venezuela and Suriname to Northern Brazil	19471
Hypanus dipterurus	*Hypanus dipterurus*	EP: Hawaii (USA), California (USA) to Northern Chile, including Galápagos Islands	1598
Hypanus say	*Hypanus say*	WA: Massachusetts (USA) to Peninsula of Florida	12768
**Hypanus* aff. *say*	*Hypanus say*	WA: Gulf of Mexico	6221

Hypanus sabinus	*Hypanus sabinus*	WA: Delaware (USA) to Gulf of Mexico	6226
**Hypanus colarensis*	*Fontitrygon colarensis*	EA: Northern Brazil	-

We also recognized a monophyletic Dasyatinae subfamily, with *Hypanus* as the sister-genus to all other, and sister to Neotrygoninae; and the family Urogymninae, represented by the genus *Fontitrygon*, supports the subfamilies rooting.

The *Hypanus* species with the largest geographic distribution, *H. americanus*, from Massachusetts (USA) to São Paulo (Brazil), was recognized to be a non-natural clade. Specimens from the Brazilian coast that used to be named as *H. americanus* are more closely related to *H. rudis* at Eastern Atlantic. Besides, what was left of *H. americanus* in Central and North America are also two lineages, leaving what was known as *H. americanus* as three distinct ones. Another species with a great geographic distribution from Mexico to Southeastern Brazil is *H. guttatus*, which also has shown to be two lineages. Therefore, we realize these marine coastal stingrays usually do need to occupy large areas and there are indeed more lineages within *Hypanus* as previously thought.

Origin of *Hypanus*

The final closure of the Isthmus of Panama (2.8 Ma by O'Dea *et al.*, 2018) is the commonest used paleogeographic event in marine organisms evolutionary studies (Dudgeon et al. 2012). By using this date to estimate the divergence date between a *Hypanus* species from Eastern Pacific, *H. longus*, and its sister-clade from Atlantic (*H. rudis* + *H.* aff. *rudis*), we got an average nucleotide substitution rate per year for *Hypanus* of 3.5356 x 10^{-9} (95% HPD: 3.5277 x 10^{-9} - 3.5434 x 10^{-9}), which falls within the average rates for elasmobranchs 2.15 x 10^{-9} - 12.0 x 10^{-9} (Dudgeon et al. 2012). These rates are much slower than those for mammals (5.4 10^{-8} in CytB and COI), probably due to slower metabolic rates in Elasmobranch (Martin et al. 1992), and also slower than Sarcopterygii even using mitochondrial genomes (Inoue et al. 2010). Poortvliet *et al.* (2015) found a rate of 8.82 x 10^{-9} for mobulids, which is higher than that for *Hypanus*.

During the Eocene-Oligocene transition 33.9 Ma, there was a cooling event not only in high latitudes (Liu et al. 2009) but also at the tropics, with ~2.5 °C decrease in sea-surface temperature (Lear et al. 2008). Considering that *Hypanus* stingrays are currently distributed mostly at the Tropical Atlantic and Tropical Eastern Pacific realms (Spalding et al. 2007), we infer they cannot stand low water temperatures, especially during this global cooling.

Therefore, the probable origin of the genus *Hypanus* approximately 30.97 Ma was the Tropical Western Atlantic Ocean, which could have been a refuge for these organisms. The origin of *Hypanus* (Clade 1 of Figure 3) was followed by a vicariant event in which a lineage became restricted to Tropical Northwestern Atlantic and another to North Brazil Shelf provinces of Spalding *et al.* (2007).

Briggs & Bowen (2013) points out that the Caribbean is widely known for its species diversity in a high rate radiation as it can be considered a center of speciation (Bowen et al. 2013). Even though we do not refute this hypothesis regarding the genus *Hypanus*, we can also extend their origin southwards to also include the Brazilian coast as an important refuge for the clade during the global cooling, and could also be a consequence of a parapatric divergence process from an ancestral lineage (Pinheiro et al. 2018).

Other three vicariant events, more recent than 5 Ma are in Clades 4, 7, and 10 (Figure *3*), gave origin to five extant lineages (*H. say*, *H.* aff. *say*, *H. guttatus*, *H.* aff. *guttatus*, and *H. longus*). Founder events might have been also very important for the diversification of *Hypanus* and can be observed in Clades 3, 8, 9, and 11 with the origin of three nominal species (*H. dipterurus*, *H. marianae*, *H. rudis*) and a lineage (*H.* aff. *rudis*). Other relevant cladogenetic events within the group were the subset of sympatric species that have been occurring throughout the genus evolution and were responsible for diversifications in Clades 2, 5, 6, and 12 with the origin of four extant lineages (*H. sabinus*, *H. geijskesi*, *H. americanus*, and *H.* aff. *americanus*).

Hypanus has two very distinct clades with deep divergence dates. The oldest (27.79 Ma) encompasses the lineages *H. sabinus*, *H. dipterurus*, *H. say*, and *H.* aff. *say* (Clade 2). This group dispersed northwards, reaching the Eastern coast of the USA, followed by a founder event in Tropical Eastern Pacific 9.33 Ma (*H. dipterurus*). The other *Hypanus* group (Clade 5) is more speciose and, according to the biogeographic analysis (DEC+X+J), its cladogenetic events originated at the Brazilian coast, encompassing the provinces of North Brazil Shelf, Tropical Southwestern Atlantic, and Warm Temperate Southwestern Atlantic.

Most speciation within Clade 5 occurred at the Brazilian coast with some probable dispersal and founder events to Eastern Atlantic (*H. rudis*), and a vicariant event due to the closure of the Isthmus of Panama (*H. longus*). There is also a speciation event that was probably related to ecological adaptations since *H. marianae* is the only reef-associated *Hypanus* (Gomes et al. 2000). Another interesting event is the cladogenesis within *H. americanus* sensu stricto, where we can observe two lineages with distinct evolutionary histories co-existing in the Gulf of Mexico and Eastern coast of the USA, as already observed by Richards, DeBiasse,

& Shivji (2018). It could have been a sympatric event or an allopatric during the Pleistocene exposing the Peninsula of Florida (Bowen and Avise 1990; Bellwood and Wainwright 2002), followed by a secondary contact after the sea-rise.

***Hypanus*' divergence times and biogeographic barriers**

The genus *Hypanus* is an interesting group to study the effect of well-known biogeographic barriers: (Figure 1: 1) hard (closure of the Isthmus of Panama 2.8 Ma): *H. longus* x (*H. rudis*+*H.* aff. *rudis*) 4.18 Ma, and *H. dipterurus* x (*H. say*+*H.* aff. *say*) 9.33 Ma; (Figure 1: 2) hard (Peninsula of Florida during the Pleistocene): *H. say* x *H.* aff. *say* 1.56 Ma, *H. americanus* x *H.* aff. *americanus* 1.36 Ma; (Figure 1: 3) soft (Amazon river outflow 10 Ma): *H. marianae* 9.37 Ma, *H. geijskesi* x (*H.* aff. *guttatus*+*H. guttatus*) 8.83 Ma *H. guttatus* x *H.* aff. *guttatus* is 2.12 Ma; and (Figure 1: 4) soft (mid-Atlantic Ocean): *H. rudis* x *H.* aff. *rudis* 1.28 Ma.

Isthmus of Panama

Even though we would expect some cladogenetic events to have occurred at the same time within the genus, as the separation of sister-species by the Isthmus of Panama in two independent lineages, they may not have been simultaneous: *H. longus* x (*H. rudis*+*H.* aff. *rudis*) 4.18 Ma, and *H. dipterurus* x (*H. say*+*H.* aff. *say*) 9.33 Ma. Both *Hypanus* clades with species on both Eastern Pacific and Western Atlantic date before the final closure of the Isthmus of Panama.

Based on distinct taxa and many genes, O'Dea *et al.* (2016) calculated that average divergence time between transisthmian species ranges between 27 to 3.2 Ma. Newkirk & Martin (2009) suggested that 9.5 Ma was the cessation of deep-water exchange between the Pacific and Atlantic, and Collins *et al.* (1996) implies that approximately 8 Ma there was already formed a biogeographic barrier. Before the final closure of the Isthmus of Panama (~9 Ma), the Pacific ocean used to supply marine currents towards the Atlantic; a feature that ceased around this date (Kirillova et al. 2019). So, it is possible that both *Hypanus* dispersions to the Pacific happened after the diminishment (or interruption) of water flow in the opposite direction (to the Atlantic), allowing some stingrays to span through the area. During the 12 My of closure of the Isthmus, most pairs of species were separated by distinct levels of the forming land barrier (Lessios 2008).

There are some other examples of this vicariant event in Elasmobranchs, as the sister-species of stingrays *Styracura schmardae* and *S. pacifica* on Atlantic and Pacific coasts of America, respectively, which are related to the origin of South American freshwater stingrays (Lovejoy 1996). Besides the geminate species *Squatina californica* and *S. dumeril* (angelsharks), from Pacific and Atlantic coasts respectively, diverged 6.11 ± 2.53 Ma (Stelbrink et al. 2010), in congruent dates to what we found for *Hypanus*. The currently valid species of eagle rays *Aetobatus narinari* has also shown a divergence between Atlantic and Pacific lineages dating 1.41 Ma (Sales et al. 2019).

Amazon River outflow to the Atlantic

The Amazon barrier can be considered as a soft one since its conditions vary often and its crossing by organisms depends on their plasticity to tolerate different environments (Pyron and Burbrink 2010). During low-sea levels the freshwater and sediments go towards the Atlantic Ocean deep-sea, causing a region of low salinity and high sedimentation (Luiz et al. 2012). Consequently, the normal salinity and clear water path organisms could take to cross the barrier is interrupted. When there was a large glacial period or if specimens could not cross through the path at high-level sea moments, organisms became isolated on both sides of the barrier, which could ultimately lead to isolation (Rocha 2003). The Pleistocene diversification of the clade *H. guttatus* and *H.* aff. *guttatus* 2.12 Ma is in accordance to the proposed biogeographic event since individuals of an ancestral population might have been separated during glacial periods. Ross Robertson, Karg, Leao de Moura, Victor, & Bernardi (2006) showed four diversification events within the parrotfish genus *Sparisoma* with trans-Amazonian splits during the Pleistocene.

The uplift of the Andes mountains reached its pinnacle twice throughout its development: 12 and 4.5 Ma, and it was around 10 Ma that sediments and water from the Andes reached the Atlantic Ocean, overlapping with a low-sea level and world climate cooling (Hoorn et al. 2010). Divergence dates of *H. geijskesi* (8.83 Ma) and *H. marianae* (9.37 Ma) are in agreement with this great freshwater outflow to the Atlantic, even though the establishment of the Amazon River carrying great amounts of sediment from the Andes started 6.8 Ma (Figueiredo et al. 2009). Both species have limited distributions and *H. marianae* tolerates only restricted environmental variables: high salinity, coastal closeness, and elevated temperatures (Costa et al. 2017). Therefore, it would be expected for this species to avoid a great freshwater mass and sediments as the Amazon river, which was observed at the population structure of Caribbean and Brazilian lineages of the saddled blenny *Malacoctenus triangulatus* (Dias et al. 2019).

Mid-Atlantic Ocean Barrier

The separation of the Brazilian *H.* aff. *rudis* and the African *H. rudis* 1.28 Ma falls within the range observed by Floeter *et al.* (2008) through analyzing many previous studies, in which trans-Atlantic divergence times vary from 5.5 Ma, to 3 Ma, to 1 Ma, and even some thousands of years. In a superficial thought, it would have been easier for trans-Atlantic lineages to have crossed waters between Africa and South America before the continents were completely separated (60 to 25 Ma) (Rosen 1975). However, the recent divergences exclude this hypothesis. According to Floeter *et al.* (2008), most trans-Atlantic species are pelagic-spawners in which larval phase lasts long, or they could be rafters crossing the Atlantic using other organisms or tools as “boats” (Luiz et al. 2012). Both cases do not apply for stingrays, so we could also eliminate these explanations.

Given that dispersal from America to Africa is the probable direction of colonization of many species, there are three possible ways of reaching Eastern Atlantic: through North or South Atlantic Gyres, or the Equatorial Undercurrent (Muss et al. 2001). Once these stingrays do not reach high latitudes and usually do not tolerate low temperatures (Menni and Stehmann 2000; Aguiar et al. 2009) North and South Atlantic Gyres could be excluded as possible routes. Therefore, we are left with the Equatorial Undercurrent, which helps us understand the very close relationship between the Brazilian *H.* aff. *rudis* and the African *H. rudis*. The Atlantic Equatorial Undercurrent (EUC) that flows eastwards has a velocity of 2.52 km/h, a depth of 150 m (Stalcup and Metcalf 1966), from 20 m to 200 m, and an average temperature of 20 °C (Muss et al. 2001). An approximate distance between Northeastern Brazil and Guinea Gulf in Africa is 3,500 km. Considering only the EUC velocity and the distance, nowadays it would take 74.4 days to cross the Atlantic ignoring all other variables.

Throughout the process of closure of the Isthmus of Panama, there were some deep modifications in marine currents (Haug and Tiedemann 1998; Schneider and Schmittner 2006) with some instability highlighted by sea-level oscillations during the Pliocene and Pleistocene: the worldwide average sea-level went from 25 m above (Grant et al. 2019) to 130 m below current (Clark et al. 2009). It was during this period that most organisms were able to cross from Western to Eastern Atlantic through a strong EUC (Muss et al. 2001).

These events were followed by a shoaling of the Atlantic thermocline, which then contributed to a restructuration and stabilization of the Atlantic Ocean basin (Philander and Fedorov 2003). Besides, at the Late Pleistocene interglacial periods, the ocean circulation was already very similar to the current pattern (Ravelo and Andreasen 2000), also demonstrating the reach of an equilibrium since the deep changes caused by the closure of the Isthmus of Panama. With this new scenario in the Atlantic Ocean basin, specimens could not keep dispersing eastwards on a regular basis.

Therefore, based on the arguments above-mentioned we are led to conclude that with the closure of the Isthmus of Panama 2.8 Ma (O'Dea et al. 2018), there was a high instability in the Atlantic Ocean with strong marine currents (Schneider and Schmittner 2006), one of which (EUC) helped to carry some specimens of a Brazilian population of stingrays to the Gulf of Guinea. Soon after, the Atlantic Ocean regained its stability (Philander and Fedorov 2003) and it became difficult for Brazilian stingrays to reach Africa and the other way around. Consequently, both populations became isolated 1.28 Ma with a possible lack of gene flow, not yet evaluated.

Many biogeographic studies have shown the species-sharing pattern between Western and Eastern Atlantic, mostly from America to Africa, that is, from the most speciose region to the least one (Vermeij and Rosenberg 1993; Briggs 1995b; Muss et al. 2001; Bernardi et al. 2004; Rocha et al. 2005). These studies focused in mollusks and reef fishes, both of which have usually have larval phases that could get caught in a marine current and cross the Atlantic Ocean. Hereby, we are discussing the dispersal event of a large vertebrate that is benthonic, mostly found in coastal and from shallow waters and with viviparous reproduction (Aguiar, Valentin, & Rosa, 2009; P. Last *et al.*, 2016). Therefore, we should not expect that these stingrays could easily disperse using marine currents through deep barriers as the mid-Atlantic Ocean and East Pacific Rise.

Many of these speciation events that happened less than 1.5 Ma might be consequences of the closure and related to the modifications and rearrangements of Atlantic Ocean circulation and sea-level oscillations (Ludt and Rocha 2015), besides the success of biogeographic barriers, as the Amazon or the mid-Atlantic (Floeter et al. 2008). As an example, a population of reef fishes colonized the Ascension Islands only 1.5 Ma (Joyeux et al. 2001), in a demonstration of a recent dispersal from Western Atlantic, as well as seven new lineages that diversified at the Vitória-Trindade Chain (Pinheiro et al. 2017). Besides, a trans-Atlantic species of coastal sharks, *Negaprion brevirostris*, has populations on Western and Eastern Atlantic that diverged 0.29 Ma with current lack of gene flow, using both the mitochondrial control region and microsatellites (Schultz et al. 2008). However, they are still considered the same species.

Islands

Nevertheless, some *Hypanus* species also occur in oceanic islands as *H.* aff. *rudis* in Atol das Rocas (Rosa and Moura 1997), and Fernando de Noronha (Aguiar et al. 2009); *H.* aff. *rudis, H. guttatus*, and *H. marianae* at the reefs of Parcel Manuel Luiz in Maranhão (Rocha and Rosa

2001); *H. guttatus* at the Vitória-Trindade Seamount Chain (Pinheiro et al. 2015); *H. rudis* in São Tomé and Príncipe Islands (identified as *Dasyatis* aff. *hastata* in Wirtz *et al.*, 2007); *H. longus* and *H. dipterurus* in the Galápagos Archipelago (Mccosker and Rosenblatt 2010; Hearn et al. 2014); and *H. dipterurus* in Hawaiian Islands (Randall et al. 1993).

As we have not sampled these species from the islands, we cannot test their dates of divergence from coastal lineages and if they should be considered new ones. So, we assume island populations belong to the same coastal species as previously identified by the above-mentioned authors. In almost all known cases, these stingrays do not need to span through long distances or very deep areas as the MAB and EPB, except for *H. dipterurus* from the Hawaiian Islands. It suggests that distance, temperature, and salinity may, in fact, influence their dispersal and distribution.

Since the Pacific North Equatorial Current connects the Galapagos Islands to South American coast, thus suggesting the flag cabrilla *Epinephelus labriformis* to be the same species on both regions (Craig et al. 2006), we will also assume it for *H. dipterurus* and *H. longus*. However, for *H. dipterurus* stingrays to reach the Hawaiian Islands, they had to cross the EPB in an analogous event to *H.* aff. *rudis* spanning to Eastern Atlantic. There are pieces of evidence of populations passing through this large biogeographic barrier (5000 km) both on east- and westward directions (Lessios and Robertson 2006). These authors gave an example of a reef fish, *Acanthurus triostegus*, in which the Hawaiian population was colonized by an Eastern Pacific one, approximately, 2 Ma. Even though we cannot test the biogeographical events that might have led to this pattern in *H. dipterurus*, we suggest a differential conservation management for Hawaiian population of stingrays, as it could be distinct from the coastal one and the archipelago is known for its high endemism (Cowman et al. 2017).

Pleistocene

The four recent diversification events in *Hypanus* during the Pleistocene are not surprising: *H. guttatus* and *H.* aff. *guttatus* (2.12 Ma), *H. say* and *H.* aff. *say* (1.56 Ma), *H. americanus* and *H.* aff. *americanus* 2 (1.36 Ma), and *H. rudis* and *H.* aff. *rudis* (1.28 Ma). Corrigan & Beheregaray (2009) found a similar pattern in wobbegong sharks in Indo-Pacific, and Kashiwagi *et al.* (2012) in devil rays. Even though Elasmobranchs have low nucleotides' substitution rates (Dudgeon et al. 2012), with new technologies and the acquirement of more data, we have been noticing more recent speciation events and a higher diversity of sharks and rays than previously known (White and Last 2012). Besides, the Pleistocene was remarkable by its many glacial-interglacial cycles (Lisiecki and Raymo 2005) that were followed by sea-level fluctuations. As we noticed for *Hypanus*, these fluctuations are directly related to diversifications, especially, in marine coastal organisms and a wide diversity of reef fishes (Pinheiro et al. 2018). As a comparison, populations of distinct species of the *Elacatinus* genus of reef fishes also diversified in the Caribbean during the Pleistocene (Taylor and Hellberg 2006). The biogeographic scenario observed for *H. say* and *H.* aff. *say* in Northwestern Atlantic and Gulf of Mexico is a common pattern in marine species as a consequence of water levels fluctuations during the Pleistocene and temporary passage closures at South Florida (Bowen and Avise 1990; Reeb and Avise 1990).

In the Indo-Australian Archipelago, coastal elasmobranchs' species also experienced the consequences of sea-level oscillations during the Plio-Pleistocene, where the widely distributed Dasyatid species *Neotrygon kuhlii* was found to be fragmented into many lineages (Puckridge et al. 2013; Borsa et al. 2018). Besides, this pattern is widely noticed amongst Australian Chondrichthyans' diversifications (Last and White 2011)

Deep divergence

There is a relationship between recent speciation events and extinctions in marine taxa, which is noticed by the absence of old lineages in these clades and consequent new niches availability derived from extinctions (Pinheiro et al. 2017). We observed many dispersal events throughout the evolution of *Hypanus*, which is a common scenario for marine stingrays, with lineages occupying wide areas and distinct environments hampering sympatric diversification. However, these cladogenetic events are quite common in this group and extinctions could be a diversification promoter by leaving opened niches (Pinheiro et al. 2017).

Hypanus sabinus is the oldest lineage within the genus which diverged 27.79 Ma and is distributed from New York (USA) to Mexico. Vermeij & Rosenberg (1993) affirmed that many mollusks' extinction events took place in Western Atlantic, where approximately 65% of Florida species became extinct around 3.5 Ma. We would expect to find more extant closely related species to *H. sabinus*; however, it may be possible that the clade went through some extinctions during the 10 My of closure of the Isthmus of Panama and its oceanic and climatic consequences. Therefore, throughout their evolutionary history, it is possible some *Hypanus* lineages went extinct, leaving extant species with ancient divergence dates whereas there could have been more lineages that did not leave traces or were not found yet.

Lineages' delimitations

Based on six distinct lineages' delimitation methods (sGMYC, mGMYC, mPTP, bPTP, ABGD, and BPP) we analyzed four clades within *Hypanus* that showed deeper divergences in phylogenetic analysis than expected for same-species evolution. The clade encompassed by *H. rudis* from Eastern Atlantic and *H.* aff. *rudis* from Brazilian coast appears as two distinct lineages for all applied methods. Thus, this population of stingrays from Southwestern Atlantic that used to be known as *Hypanus americanus* should be recognized as new lineage and distinct from the known *H. rudis*. The morphological similarity of *H. rudis* to *H.* aff. *rudis* was already known (Wirtz et al. 2007), and Compagno & Roberts (1984) suggested the co-occurrence of both in Eastern Atlantic.

Considering *Hypanus americanus* sensu stricto from Central and North America, there was not a concordant geographic and genetic structuration since one lineage includes stingrays from Eastern coast of the United States, Gulf of Mexico and Central America, and the other from Eastern coast of the United States and Central America. All species delimitation methods and haplotype network support a separation of these two sympatric lineages. These populations' relationships of the Southern Atlantic stingray, as *H. americanus* is known, between the Eastern coast of the USA and Central America was also noticed by Richards *et al.* (2018). These authors found a deeper divergence between this USA-Central America population and Bimini-West Indies, which are geographically separated by a small distance at South Florida. Even though we did not include samples from this latter area, we found two lineages.

The Southern Atlantic stingray is very common, especially in the Caribbean and Bahamas, and widely related to touristic activities. With these recent findings, we recommend more studies within this clade to clearly identify evolutionary unities and adequately protect these populations. In marine systems, it is not unlikely to find speciation events with gene flow (Potkamp and Fransen 2019), and diversification might be happening sym- or parapatrically. The co-existence of the two lineages of *H. americanus* and *H.* aff. *americanus*, which diverged 1.36 Ma illustrates this scenario. Further investigations towards the morphology and ecology are needed to confirm and describe these supposedly new species.

The third clade is composed by *H. guttatus*, a species that supposedly occurs from Mexico to Southeastern Brazil. All analyses suggested a deep divergence within the clade separating Central America's samples from Brazilian ones. Furthermore, there is a higher genetic similarity among stingrays at southern Amazon. We infer that, even though *H. guttatus* is a marine and estuarine species that stands low salinities environments, the great freshwater and nutrients influx of the Amazon system may be a barrier for these stingrays, which isolated north and south lineages (Rocha 2003; Hoorn et al. 2010).

With regards to *H. say*, the lineage from Eastern coast of USA has a different evolutionary history than that of Gulf of Mexico, with a support from all analyses hereby done. These results agree with the biogeographic proposals of Spalding *et al.* (2007) as both populations occur at the Warm Temperate North Atlantic province, but in distinct ecoregions and separated by the Floridian one. The southernmost portion of Florida Peninsula is known to have a detached ecosystem from the adjacent USA coast (Bowen and Avise 1990). This can also be seen in *H. americanus* in which samples from Bahamas (~50 Km from US coast) are more closely related to those US Virgin Islands than from Florida (Richards et al. 2018).

White *et al.* (2018) used mitochondrial protein-coding genes to infer phylogenetic relationships among devil rays and got genetic pairwise distances of 0.5% and 0.1% for pairs of species that were synonymized: *Mobula kuhlii* and *M. eregoodootenkee,* and *M. hypostoma* and *M. rochebrunei*, respectively. However, a distance of 0.4% between *M. birostris* and *M. alfredi* was not used as a threshold to synonymize these species since other analyses had already been done for this group by Kashiwagi *et al.* (2012), suggesting their divergence. The pairwise distances between the four newly proposed lineages of *Hypanus* and the valid species to which they currently belong are: *H. rudis* and *H.* aff. *rudis* 0.82%, *H. americanus* and *H.* aff. *americanus* 0.83%, *H. guttatus* and *H.* aff. *guttatus* 1.37%, and *H. say* and *H.* aff. *say* 0.95%.

Simultaneously, the largest distance between two *Hypanus* species is 13.55% in *H. sabinus* and *H. marianae*. Interspecific genetic distances within the genus vary from 0.82% to 13.55% which is high variation. However, intraspecific variation in *Hypanus* varies from 0.047% in *H.* aff. *say* to 0.26% in *H.* aff. *guttatus*, which are values at least four times smaller than interspecific distances. Therefore, we could suggest a threshold of 0.82% pairwise genetic distance using mitochondrial protein-coding genes as an indicator of species delimitations in *Hypanus*.

Even though we could consider these newly recognized lineages as cryptic species, it is important to take them into account as unique evolutionary units. To accomplish a precise management of species, we need their delimitation, and the species molecular one is a great start (Luo et al. 2018); however, the use of exclusively molecular tools might lead to over or under-estimation of species (Carstens et al. 2013) and hamper conservation efforts. Since species delimitation methods may have their flaws as they are unable to distinguish structure as a result of population-level processes or species boundaries, these results are only hypotheses of possible species as morphological and ecological data are recommended to describe a new species (Sukumaran and Knowles 2017). So, we are not describing any species until more data can be combined with molecular results to avoid failure (Carstens et al. 2013).

Our intention is to show distinct lineages with their own evolutionary histories regardless of naming each one of them.

CONCLUSIONS

Based on 13 protein-coding mitochondrial genes, we suggest the monophyly of the resurrected genus *Hypanus* by Last *et al.* (2016a) by corroborating that *H. marianae* and *H. rudis* are indeed *Hypanus*, besides incorporating two more species to the genus that were previously allocated in *Fontitrygon*. We could identify evolutionary lineages that represent currently known species, as well as suggest four new ones, not detected until now. These are sister-lineages to *H. americanus*, *H. guttatus*, *H. rudis*, and *H. say*, reducing the geographic distribution of these species, which might have an impact on their conservation status. Besides, we suggest a new name combination of *Fontitrygon geijskesi* since it is more closely related to *H. guttatus* than to other *Fontitrygon*, hence it should be *Hypanus geijskesi*. Even though *F. colarensis* was not sampled, we also suggested its closer relationship to *Hypanus* species due to its morphological and geographical similarities to *H. guttatus* and *H. geijskesi*, thus becoming *Hypanus colarensis*.

We traced back *Hypanus* evolutionary origin to the Tropical Western Atlantic Ocean. It is an interesting group to study speciation events, in which we can observe classic allopatric speciation due to vicariant event: closure of Isthmus of Panama (*H. longus* x (*H. rudis*+*H.* aff. *rudis*), and *H. dipterurus* x (*H. say*+*H.* aff. *say*)), and a dispersal through mid-Atlantic barrier with founder event at the Gulf of Guinea (*H. rudis* x *H.* aff. *rudis*). The outflow of the Amazon River, as a soft biogeographic barrier, also had a relevant role in ceasing gene flow and isolating lineages (*H. geijskesi*, *H. marianae*, *H. guttatus*, *H.* aff. *guttatus*). Besides, there is a putative example of speciation by ecological adaptation in *H. marianae*, as well as Pleistocene diversification at the Florida Peninsula (*H. americanus* x *H.* aff. *americanus* and *H. say* x *H.* aff. *say*). Finally, there is a deep divergence within the genus, in which the lineage currently known as *H. sabinus* has an independent evolution for 27.78 My. Even though we could delimit some evolutionary lineages within the genus, maybe there are even more if specimens from more locations are sampled. Besides, to formally describe species, morphological studies, the examination of type series, and ecological niche modeling should be performed to better define these stingrays.

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REFERENCES

Aguiar A.A., Valentin J.L., Rosa R.S. 2009. Habitat use by *Dasyatis americana* in a south-western Atlantic oceanic island. J. Mar. Biol. Assoc. United Kingdom. 89:1147–1152.

Ali S.S., Yu Y., Pfosser M., Wetschnig W. 2012. Inferences of biogeographical histories within subfamily Hyacinthoideae using S-DIVA and Bayesian binary MCMC analysis implemented in RASP (Reconstruct Ancestral State in Phylogenies). Ann. Bot. 109:95–107.

Allen G.R. 2008. Conservation hotspots of biodiversity and endemism for Indo-Pacific coral reef fishes. Aquat. Conserv. Mar. Freshw. Ecosyst. 18:541–556.

Avise J.C. 2000. Phylogeography: the history and formation of species. Cambridge, MA: Harvard University Press.

Ballard J.W.O., Whitlock M.C. 2004. The incomplete natural history of mitochondria. Mol. Ecol. 13:729–744.

Bellwood D.R., Wainwright P.C. 2002. The history and biogeography of fishes on coral reefs. In: Sale P.F., editor. Coral reef fishes: dynamics and diversity in a complex ecosystem. San Diego, CA: Academic Press. p. 5–32.

Berggren W.A., Hollister C.D. 1974. Paleogeography, Paleobiogeography and the History of Circulation in the Atlantic Ocean. Stud. Paleo-Oceanography. 20:126–186.

Bernal M.A., Gaither M.R., Simison W.B., Rocha L.A. 2017. Introgression and selection shaped the evolutionary history of sympatric sister-species of coral reef fishes (genus: *Haemulon*). Mol. Ecol. 26:639–652.

Bernardi G., Bucciarelli G., Costagliola D., Robertson D.R., Heiser J.B. 2004. Evolution of coral reef fish *Thalassoma* spp. (Labridae). 1. Molecular phylogeny and biogeography. Mar. Biol. 144:369–375.

Bigelow H.B., Schroeder W.C. 1953. Fishes of the western North Atlantic Part II. Sawfishes, guitarfishes, skates and rays. .

Bloch M.E., Schneider J.G. 1801. Systema Ichthyologiae Iconibus cx Ilustratum. Post obitum auctoris opus inchoatum absolvit, correxit, interpolavit Jo. Gottlob Schneider, Saxo. Berolini: Sumtibus Auctoris Impressum et Bibliopolio Sanderiano Commissum.

Boeseman M. 1948. Some preliminary notes on Surinam sting rays, including the description of a new species. Zool. Meded. 30:31–47.

Borsa P., Arlyza I.S., Hoareau T.B., Shen K. 2018. Diagnostic description and geographic distribution of four new cryptic species of the blue-spotted maskray species complex (Myliobatoidei: Dasyatidae; *Neotrygon* spp .) based on DNA sequences *. 36:827–841.

Bouckaert R., Vaughan T.G., Barido-Sottani J., Duchêne S., Fourment M., Gavryushkina A., Heled J., Jones G., Kühnert D., Maio N. De, Matschiner M., Mendes F.K., Müller N.F., Ogilvie H.A., Plessis L. du, Popinga A., Rambaut A., Rasmussen D., Siveroni I., Suchard M.A., Wu C.-H., Xie D., Zhang C., Stadler T., Drummond A.J. 2019. BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. PLoS Comput. Biol. 15:e1006650.

Bowen B.W., Avise J.C. 1990. Genetic structure of Atlantic and Gulf of Mexico populations of sea bass, menhaden, and sturgeon: Influence of zoogeographic factors and life-history patterns. Mar. Biol. 107:371–381.

Bowen B.W., Rocha L.A., Toonen R.J., Karl S.A. 2013. The origins of tropical marine biodiversity. Trends Ecol. Evol. 28:359–366.

Briggs J.C. 1974. Marine zoogeography. New York: McGraw Hill Book Company.

Briggs J.C. 1995a. Global Biogeography. Elsevier.

Briggs J.C. 1995b. Marine patterns, Part I. Global Biogeography. Developments in Paleontology and Stratigraphy. Elsevier. p. 207–250.

Briggs J.C., Bowen B.W. 2013. Marine shelf habitat: Biogeography and evolution. J. Biogeogr. 40:1023–1035.

Brown J.H., Lomolino M. V. 1998. Biogeography. Sunderland, Massachusetts: Sinauer Press.

Brown W.M., George M., Wilson A.C. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. U. S. A. 76:1967–1971.

Brundin L. 1965. On the Real Nature of Transantarctic Relationships. Evolution (N. Y). 19:496–505.

Buffon C. de. 1766. Histoire naturelle, générale et particulière. Paris: Imprimeries Royale.

Cardoso P., Erwin T.L., Borges P.A.V., New T.R. 2011. The seven impediments in invertebrate conservation and how to overcome them. Biol. Conserv. 144:2647–2655.

Carstens B.C., Pelletier T.A., Reid N.M., Satler J.D. 2013. How to fail at species delimitation. Mol. Ecol. 22:4369–4383.

de Carvalho M.R. 1996. Higher-Level Elasmobranch Phylogeny, Basal Squaleans, and Paraphyly. In: Stiassny M., Parenti L., Johnson G., editors. Interrelationships of Fishes. California: Academic Press. p. 35–62.

Chen X., Ai W., Ye L., Wang X., Lin C., Yang S. 2013. The complete mitochondrial genome of the grey bamboo shark (*Chiloscyllium griseum*) (Orectolobiformes: Hemiscylliidae): Genomic characterization and phylogenetic application. Acta Oceanol. Sin. 32:59–65.

Clark P.U., Dyke A.S., Shakun J.D., Carlson A.E., Clark J., Wohlfarth B., Mitrovica J.X., Hostetler S.W., McCabe A.M. 2009. The Last Glacial Maximum. Science (80-.). 325:710–714.

Coates A.G., Obando J.A. 1996. The geological evolution of the Central American Isthmus. In: Jackson J.B.C., Budd A.F., Coates A.G., editors. Evolution and Environment in Tropical America. Chicago: University of Chicago Press. p. 21–56.

Collins L.S., Coates A.G., Berggren W.A., Aubry M.P., Zhang J. 1996. The late Miocene Panama isthmian strait. Geology. 24:687–690.

Compagno L. 1973. Interrelationships of living elasmobranchs. Zool. J. Linn. Soc. 53:15–61.

Compagno L.J. V., Roberts T.R. 1984. Marine and freshwater stingrays (Dasyatidae) of West Africa, with description of a new species. Proceeding Calif. Acad. Sci. 43:283–300.

Corrigan S., Beheregaray L.B. 2009. A recent shark radiation: Molecular phylogeny, biogeography and speciation of wobbegong sharks (family: Orectolobidae). Mol. Phylogenet. Evol. 52:205–216.

Corrigan S., Maisano Delser P., Eddy C., Duffy C., Yang L., Li C., Bazinet A.L., Mona S., Naylor G.J.P. 2017. Historical introgression drives pervasive mitochondrial admixture between two species of pelagic sharks. Mol. Phylogenet. Evol. 110:122–126.

Costa T.L.A., Pennino M.G., Mendes L.F. 2017. Identifying ecological barriers in marine environment: The case study of *Dasyatis marianae*. Mar. Environ. Res. 125:1–9.

Cowman P.F., Parravicini V., Kulbicki M., Floeter S.R. 2017. The biogeography of tropical reef fishes: endemism and provinciality through time. Biol. Rev. 92:2112–2130.

Coyne J.A., Orr H.A. 2004. Speciation. Sunderland, Massachusetts: Sinauer Associates, Inc.

Craig M.T., Hastings P.A., Pondella D.J., Ross Robertson D., Rosales-Casián J.A. 2006. Phylogeography of the flag cabrilla *Epinephelus labriformis* (Serranidae): Implications for the biogeography of the Tropical Eastern Pacific and the early stages of speciation in a marine shore fish. J. Biogeogr. 33:969–979.

Cutter A.D., Gray J.C. 2016. Ephemeral ecological speciation and the latitudinal biodiversity gradient. Evolution (N. Y). 70:2171–2185.

Van Dam M.H., Matzke N.J. 2016. Evaluating the influence of connectivity and distance on biogeographical patterns in the south-western deserts of North America. J. Biogeogr. 43:1514–1532.

Darriba D., Taboada G.L., Doallo R., Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods. 9:772.

Darwin C. 1859. On the origin of species by means of natural selection, or preservation of favoured races in the struggle for life. London: John Murray.

Dias R.M., Lima S.M.Q., Mendes L.F., Almeida D.F., Paiva P.C., Britto M.R. 2019. Different speciation processes in a cryptobenthic reef fish from the Western Tropical Atlantic. Hydrobiologia. 837:133–147.

Diniz-Filho J.A.F., Loyola R.D., Raia P., Mooers A.O., Bini L.M. 2013. Darwinian shortfalls in biodiversity conservation. Trends Ecol. Evol. 28:689–695.

Doane M.P., Kacev D., Harrington S., Levi K., Pande D., Vega A., Dinsdale E.A. 2018. Mitochondrial recovery from shotgun metagenome sequencing enabling phylogenetic analysis of the common thresher shark (*Alopias vulpinus*). Meta Gene. 15:10–15.

Dudgeon C.L., Blower D.C., Broderick D., Giles J.L., Holmes B.J., Kashiwagi T., Kr??ck N.C., Morgan J.A.T., Tillett B.J., Ovenden J.R. 2012. A review of the application of molecular genetics for fisheries management and conservation of sharks and rays. J. Fish Biol. 80:1789–1843.

Dugatkin L.A. 2019. Buffon, Jefferson and the theory of New World degeneracy. Evol. Educ. Outreach. 12:1–8.

Dupin J., Matzke N.J., Särkinen T., Knapp S., Olmstead R.G., Bohs L., Smith S.D. 2016. Bayesian estimation of the global biogeographical history of the Solanaceae. J. Biogeogr. 44:887–899.

Ekman S. 1953. Zoogeography of the Sea. London: Sidgwick and Jackson.

Ezard T., Fujisawa T., Barraclough T. 2017. splits: SPecies' LImits by Threshold Statistics. .

Farris D.W., Jaramillo C., Bayona G., Restrepo-Moreno S.A., Montes C., Cardona A., Mora A., Speakman R.J., Glascock M.D., Valencia V. 2011. Fracturing of the Panamanian Isthmus during initial collision with: South America. Geology. 39:1007–1010.

Figueiredo J., Hoorn C., van der Ven P., Soares E. 2009. Late Miocene onset of the Amazon River and the Amazon deep-sea fan: Evidence from the Foz do Amazonas Basin. Geology. 37:619–622.

Fišer C., Robinson C.T., Malard F. 2018. Cryptic species as a window into the paradigm shift of the species concept. Mol. Ecol. 27:613–635.

Floeter S.R., Rocha L.A., Robertson D.R., Joyeux J.C., Smith-Vaniz W.F., Wirtz P., Edwards A.J., Barreiros J.P., Ferreira C.E.L., Gasparini J.L., Brito A., Falcón J.M., Bowen B.W., Bernardi G. 2008. Atlantic reef fish biogeography and evolution. J. Biogeogr. 35:22–47.

Flouri T., Jiao X., Rannala B., Yang Z. 2018. Species tree inference with BPP using genomic sequences and the multispecies coalescent. Mol. Biol. Evol. 35:2585–2593.

Fowler H.W. 1910. Notes on batoid fishes. Proc. Acad. Nat. Sci. Philadelphia. 62:468–475.

Fricke, R., Eschmeyer W.N., Van der Laan R. 2019. Eschmeyer's Catalog of Fishes: genera, species, references. Available from http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp.

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. Syst. Biol. 62:707–724.

Gaitán-Espitia J.D., Solano-Iguaran J.J., Tejada-Martinez D., Quintero-Galvis J.F. 2016. Mitogenomics of electric rays: evolutionary considerations within Torpediniformes (Batoidea; Chondrichthyes). Zool. J. Linn. Soc. 178:257–266.

Garman S. 1880. New species of selachians in the museum collection. Bull. Museum Comp. Zool. 6:167–172.

Garman S. 1913. The Plagiostomia. (Sharks, Skates, and Rays). Cambridge, USA: Memoirs of the Museum of Comparative Zoology at Harvard College XXXVI.

Giddens J., Goodell W., Friedlander A., Salinas-de-León P., Shepard C., Henning B., Berkenpas E., Sala E., Turchik A. 2019. Patterns in Bathyal Demersal Biodiversity and Community Composition Around Archipelagos in the Tropical Eastern Pacific. Front. Mar. Sci. 6:1–15.

Gomes U.L., Rosa R.S., Gadig O.B.F. 2000. *Dasyatis marianae* sp. n.: A new species of stingray (Chondrichthyes: Dasyatidae) from the Southwestern Atlantic. Copeia. 2000:510–515.

Grant G.R., Naish T.R., Dunbar G.B., Stocchi P., Kominz M.A., Kamp P.J.J., Tapia C.A., McKay R.M., Levy R.H., Patterson M.O. 2019. The amplitude and origin of sea-level variability during the Pliocene epoch. Nature.:1–5.

Guindon S., Gascuel O. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Syst. Biol. 52:696–704.

Günther A. 1870. Catalogue of the fishes in the British Museum. Catalogue of the

Physostomi, containing the families Gymnotidae, Symbranchidae, Muraenidae, Pegasidae, and of the Lophobranchii, Plectognathi, Dipnoi, ...[thru] ... Leptocardii, in the British Museum. .

Harvey P.H., Pagel M.D. 1991. The comparative method in evolutionary biology. Oxford: Oxford University Press.

Haug G.H., Tiedemann R. 1998. Effect of the formation of the Isthmus of Panama on Atlantic Ocean thermohaline circulation. Nature. 393:673–676.

Häussermann V., Försterra G. 2005. Distribution patterns of Chilean shallow-water sea anemones (Cnidaria: Anthozoa: Actiniaria, Corallimorpharia), with a discussion of the taxonomic and zoogeographic relationships between the actinofauna of the South East Pacific, the South West Atlantic a. Sci. Mar. 69:91–102.

Havill N.P., Gaimari S.D., Caccone A. 2018. Cryptic east-west divergence and molecular diagnostics for two species of silver flies (Diptera: Chamaemyiidae: *Leucopis*) from North America being evaluated for biological control of hemlock woolly adelgid. Biol. Control. 121:23–29.

Hearn A.R., Acuña A., Ketchum J.T., Peñaherrera C., Green J., Marshall A., Guerrero M., Schillinger G. 2014. Elasmobranchs of the Galapagos Marine Reserve. In: Denkinger J., Vinueza L., editors. The Galapagos Marine Reserve. New York: Springer Science+Business Media.

Hennig W. 1966. Phylogenetic Systematics. University of Illinois Press.

Hildebrand S.F., Schroeder W.C. 1928. Fishes of Chesapeake Bay. .

Hoorn C., Guerrero J., Sarmiento G.A., Lorente M. 1995. Andean tectonics as a cause for changing drainage patterns in Miocene northern South America. Geology. 23:237–240.

Hoorn C., Wesselingh F.P., Ter Steege H., Bermudez M.A., Mora A., Sevink J., Sanmartín I., Sanchez-Meseguer A., Anderson C.L., Figueiredo J.P., Jaramillo C., Riff D., Negri F.R., Hooghiemstra H., Lundberg J., Stadler T., Särkinen T., Antonelli A. 2010. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. Science (80-.). 330:927–931.

Hudson R.R., Turelli M. 2003. Stochasticity overrules the “three-times rule”: genetic drift, genetic draft, and coalescence times for nuclear loci versus mitochondrial DNA. Evolution (N. Y). 57:182–190.

Von Humboldt A. 1849. Aspects of nature, in different lands and different climates; with scientific elucidations. Lea and Blanchard.

Hurtado L.A., Mateos M., Mattos G., Liu S., Haye P.A., Paiva P.C. 2016. Multiple transisthmian divergences, extensive cryptic diversity, occasional long-distance dispersal, and biogeographic patterns in a marine coastal isopod with an amphi-American distribution. Ecol. Evol. 6:7794–7808.

Inoue J.G., Miya M., Lam K., Tay B.-H., Danks J. a, Bell J., Walker T.I., Venkatesh B. 2010. Evolutionary origin and phylogeny of the modern holocephalans (Chondrichthyes: Chimaeriformes): a mitogenomic perspective. Mol. Biol. Evol. 27:2576–86.

IUCN. 2019. The IUCN Red List of Threatened Species. Available from https://www.iucnredlist.org/.

Iwasaki W., Fukunaga T., Isagozawa R., Yamada K., Maeda Y., Satoh T.P., Sado T., Mabuchi K., Takeshima H., Miya M., Nishida M. 2013. MitoFish and MitoAnnotator: A Mitochondrial Genome Database of Fish with an Accurate and Automatic Annotation Pipeline. Mol. Biol. Evol. 30:2531–2540.

Johnson K.G., Todd J.A., Jackson J.B.C. 2007. Coral reef development drives molluscan diversity increase at local and regional scales in the late Neogene and Quaternary of the southwestern Caribbean. Paleobiology. 33:24–52.

Jordan D.S., Gilbert C.R. 1880. Notes on a collection of fishes from San Diego, California. Proc. United States Natl. Museum. 3:23–34.

Joyeux J.C., Floeter S.R., Ferreira C.E.L., Gasparini J.L. 2001. Biogeography of tropical reef fishes: The south Atlantic puzzle. J. Biogeogr. 28:831–841.

Kapli P., Lutteropp S., Zhang J., Kobert K., Pavlidis P., Stamatakis A., Flouri T. 2017. Multi-rate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo. Bioinformatics. 33:1630–1638.

Kashiwagi T., Marshall A.D., Bennett M.B., Ovenden J.R. 2012. The genetic signature of recent speciation in manta rays (*Manta alfredi* and *M. birostris*). Mol. Phylogenet. Evol. 64:212–218.

Keigwin L. 1982. Isotopic Paleoceanography of the Caribbean and East Pacific: Role of Panama Uplift in the Late Neogene Time. Science (80-.). 217:350–353.

Kirillova V., Osborne A.H., Frank M. 2019. Miocece restriction of the Pacific-North Atlantic throughflow strngthened Atlantic overturning circulation. Nat. Commun. 10:1–7.

Krueger T. Trim Galore! .

Landis M.J., Matzke N.J., Moore B.R., Huelsenbeck J.P. 2013. Bayesian Analysis of Biogeography when the Number of Areas is Large. Syst. Biol. 62:789–804.

Last P., Naylor G., Séret B., White W., de Carvalho M., Stehmann M. 2016a. Rays of the World. Csiro Publishing.

Last P.R., Naylor G.J.P., Manjaji-Matsumoto B.M. 2016b. A revised classification of the family Dasyatidae (Chondrichthyes: Myliobatiformes) based on new morphological and molecular insights. Zootaxa. 4139:345–368.

Last P.R., White W.T. 2011. Biogeographic patterns in the Australian chondrichthyan fauna. J. Fish Biol. 79:1193–213.

Lawson L.P., Bates J.M., Menegon M., Loader S.P. 2015. Divergence at the edges: Peripatric isolation in the montane spiny throated reed frog complex. BMC Evol. Biol. 15:1–15.

Lear C.H., Bailey T.R., Pearson P.N., Coxall H.K., Rosenthal Y. 2008. Cooling and ice growth across the Eocene-Oligocene transition. Geology. 36:251–254.

Lessios H.A. 2008. The Great American Schism: Divergence of Marine Organisms After the Rise of the Central American Isthmus. Annu. Rev. Ecol. Evol. Syst. 39:63–91.

Lessios H.A., Robertson D.R. 2006. Crossing the impassable: Genetic connections in 20 reef fishes across the eastern Pacific barrier. Proc. R. Soc. B Biol. Sci. 273:2201–2208.

Lesueur C.A. 1817. Description of three new species of the genus *Raja*. J. Acad. Nat. Sci.

1:41–45.

Lesueur C.A. 1824. Description of several species of the Linnaean genus *Raia*, of North America. J. Acad. Nat. Sci. 4:100–121, Pls. 4-6.

Li C., Corrigan S., Yang L., Straube N., Harris M., Hofreiter M., White W.T., Naylor G.J.P. 2015. DNA capture reveals transoceanic gene flow in endangered river sharks. Proc. Natl. Acad. Sci. 112:13302–13307.

Lisiecki L.E., Raymo M.E. 2005. A Pliocene-Pleistocene stack of 57 globally distributed benthic δ 18O records. Paleoceanography. 20:1–17.

Liu Z., Pagani M., Zinniker D., Deconto R.M., Huber M., Brinkhuis H., Shah S.R., Leckie R.M., Pearson A. 2009. Eocene-Oligocene Climate Transition. Science. 323:1187–1190.

Lomolino M. V. 2004. Conservation biogeography. In: Lomolino M. V., Heaney L.R., editors. Frontiers of Biogeography: new directions in the geography of nature. Sunderland, Massachusetts: Sinauer Associates. p. 293–296.

Lovejoy N.R. 1996. Systematics of myliobatoid elasrnobranchs: with emphasis on the phylogeny and historical biogeography of neotropical freshwater stingrays (Potamotrygonidae: Rajiformes). Zool. J. Linn. Soc. 117:207–257.

Ludt W.B., Rocha L.A. 2015. Shifting seas: The impacts of Pleistocene sea-level fluctuations on the evolution of tropical marine taxa. J. Biogeogr. 42:25–38.

Luiz O.J., Madin J.S., Robertson D.R., Rocha L.A., Wirtz P., Floeter S.R. 2012. Ecological traits influencing range expansion across large oceanic dispersal barriers: insights from tropical Atlantic reef fishes. Proc. R. Soc. B. 279:1033–1040.

Luo A., Ling C., Ho S.Y.W., Zhu C.D. 2018. Comparison of methods for molecular species delimitation across a range of speciation scenarios. Syst. Biol. 67:830–846.

Maddison W.P. 1997. Gene trees in species trees. Syst. Biol. 46:523–536.

Martin A.P., Naylor G.J.P., Palumbi S.R. 1992. Rates of mitochondrial DNA evolution in sharks are slow compared with mammals. Nature. 357:153–155.

Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencng reads. EMBnet.journal. 17:10–12.

Matzke N.J. 2013a. BioGeoBEARS: BioGeography with Bayesian (and Likelihood) Evolutionary Analysis in R Scripts. .

Matzke N.J. 2013b. Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. Front. Biogeogr. 5:242–248.

Matzke N.J. 2014. Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. Syst. Biol. 63:951–970.

Matzke N.J. 2016. Stochastic mapping under biogeographical models. .

Mayr E. 1942. Systematics and the Origin of Species. New York: Columbia University Press.

Mccosker J.E., Rosenblatt R.H. 2010. The Fishes of the Galápagos Archipelago: An Update. Proceeding Calif. Acad. Sci. 61:167–195.

McEachran J.D., Aschliman N. 2004. Phylogeny of Batoidea. Sharks Their Relat.:79–113.

Melo-Merino S.M., Reyes-Bonilla H., Lira-Noriega A. 2020. Ecological niche models and species distribution models in marine environments: A literature review and spatial analysis of evidence. Ecol. Modell. 415:35.

Menni R.C., Stehmann M.F.W. 2000. Distribution, environment and biology of batoid fishes off Argentina, Uruguay and Brazil. A review. Rev. del Mus. Argentino Ciencias Nat. Nueva Ser. 2:69–109.

Miller M.A., Pfeiffer W., Schwartz T. 2010. Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees. Proc. Gatew. Comput. Environ. Work.:1–8.

Mokany K., Ferrier S. 2011. Predicting impacts of climate change on biodiversity: A role for semi-mechanistic community-level modelling. Divers. Distrib. 17:374–380.

Müller J., Henle F.G.J. 1838. On the generic characters of cartilaginous fishes, with descriptions of new genera. Mag. Nat. Hist. 2:3–37; 88–91.

Muss A., Robertson D.R., Stepien C.A., Wirtz P., Bowen B.W. 2001. Phylogeography of *Ophioblennius*: the role of ocean currents and geography in reef fish evolution. Evolution (N. Y). 55:561–572.

Naylor G.J., Caira J.N., Jensen K., Rosana K. a., Straube N., Lakner C. 2012a. Elasmobranch Phylogeny: A Mitochondrial Estimate Based on 595 Species. Historical Zoogeography of the Selachii. In: Carrier J., Musick J., Heithaus M., editors. Biology of Sharks and their Relatives. Boca Raton: CRC Press. p. 31–56.

Naylor G.J.P. 2019. Chondrichthyan Tree of Life. Available from https://sharksrays.org/.

Naylor G.J.P., Caira J.N., Jensen K., Rosana K.A.M., White W.T., Last P.R. 2012b. A DNA Sequence-based approach to the identification of shark and ray species and its implications for global elasmobranch diversity and parasitology. Bull. Am. Museum Nat. Hist. 367:262.

Newkirk D.R., Martin E.E. 2009. Circulation through the Central American Seaway during the Miocene carbonate crash. Geology. 37:87–90.

Nishida K. 1990. Phylogeny of the suborder Myliobatoidei. Mem. Fac. Fish. Hokkaido Univ. 37:1–108.

O'Dea A., Lessios H.A., Coates A.G., Eytan R.I., Collins L.S., Cione A.L., Queiroz A. De, Farris D.W., Norris R.D., Restrepo-Moreno S.A., Stallard R.F., Woodburne M.O., Aguilera O.A., Aubry M.-P., Berggren W.A., Budd A.F., Cozzuol M.A., Coppard S.E., Finnegan S., Gasparini G.M., Grossman E.L., Johnson K.G., Keigwin L.D., Knowlton N., Leigh E.G., Leonard-Pingel J.S., Marko P.B., Pyenson N.D., Rachello-Dolmen P.G., Soibelzon E., Soibelzon L.H., Todd J.A., Vermeij G.J., Jackson J.B.C. 2018. Formation of the Isthmus of Panama: Response to Jaramillo et al. Sci. Adv.:1–5.

O'Dea A., Lessios H.A., Coates A.G., Eytan R.I., Restrepo-moreno S.A., Cione A.L., Collins L.S., Queiroz A. De, Farris D.W., Norris R.D., Stallard R.F., Woodburne M.O., Aguilera O., Aubry M., Berggren W.A., Budd A.F., Cozzuol M.A., Coppard S.E., Duque-caro H., Finnegan S., Gasparini G.M., Grossman E.L., Johnson K.G., Keigwin L.D., Knowlton N., Leigh E.G., Leonard-pingel J.S., Marko P.B., Pyenson N.D., Rachello-dolmen P.G., Soibelzon E., Soibelzon L., Todd J.A., Vermeij G.J., Jackson J.B.C. 2016. Formation of the Isthmus of Panama. Sci. Adv. 1:1–11.

Philander S.G., Fedorov A. V. 2003. Role of tropics in changing the response to Milankovich forcing some three million years ago. Paleoceanography. 18:1–11.

Pinheiro H.T., Bernardi G., Simon T., Joyeux J.-C., Macieira R.M., Gasparini J.L., Rocha C., Rocha L.A. 2017. Island biogeography of marine organisms. Nature. 549:82–85.

Pinheiro H.T., Mazzei E., Moura R.L., Amado-Filho G.M., Carvalho-Filho A., Braga A.C., Costa P.A.S., Ferreira B.P., Ferreira C.E.L., Floeter S.R., Francini-Filho R.B., Gasparini J.L., Macieira R.M., Martins A.S., Olavo G., Pimentel C.R., Rocha L.A., Sazima I., Simon T., Teixeira J.B., Xavier L.B., Joyeux J.C. 2015. Fish biodiversity of the Vitória-Trindade seamount chain, southwestern Atlantic: An updated database. PLoS One. 10:1–17.

Pinheiro H.T., Rocha L.A., Macieira R.M., Carvalho-Filho A., Anderson A.B., Bender M.G., Di Dario F., Ferreira C.E.L., Figueiredo-Filho J., Francini-Filho R., Gasparini J.L., Joyeux J.C., Luiz O.J., Mincarone M.M., Moura R.L., Nunes J. de A.C.C., Quimbayo J.P., Rosa R.S., Sampaio C.L.S., Sazima I., Simon T., Vila-Nova D.A., Floeter S.R. 2018. South-western Atlantic reef fishes: Zoogeographical patterns and ecological drivers reveal a secondary biodiversity centre in the Atlantic Ocean. Divers. Distrib.:1–15.

Poortvliet M., Olsen J.L., Croll D.A., Bernardi G., Newton K., Kollias S., O’Sullivan J., Fernando D., Stevens G., Galván Magaña F., Seret B., Wintner S., Hoarau G. 2015. A dated molecular phylogeny of manta and devil rays (Mobulidae) based on mitogenome and nuclear sequences. Mol. Phylogenet. Evol. 83:72–85.

Le Port A., Lavery S. 2012. Population structure and phylogeography of the short-tailed stingray, Dasyatis brevicaudata (Hutton 1875), in the southern hemisphere. J. Hered. 103:174–185.

Potkamp G., Fransen C.H.J.M. 2019. Speciation with gene flow in marine systems. Contrib. to Zool. 88:133–172.

Puckridge M., Last P.R., White W.T., Andreakis N. 2013. Phylogeography of the Indo-West Pacific maskrays (Dasyatidae, *Neotrygon*): A complex example of chondrichthyan radiation in the Cenozoic. Ecol. Evol. 3:217–232.

Puillandre N., Lambert A., Brouillet S., Achaz G. 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Mol. Ecol. 21:1864–1877.

Pyron R.A., Burbrink F.T. 2010. Hard and soft allopatry: Physically and ecologically mediated modes of geographic speciation. J. Biogeogr. 37:2005–2015.

Rafinesque C.S. 1810. Caratteri di alcuni nuovi generi e nuove specie di animali e piante della Sicilia, con varie osservazioni sopra i medisimi. Per le stampe di Sanfilippo.

Rafinesque C.S. 1818. [Review of] Journal of the Academy of Natural Sciences of Philadelphia. Vol. I. Part I. Am. Mon. Mag. Crit. Rev. 3:269–274.

Randall J., Earle J., Hayes T., Pittman C., Severns M., Smith R. 1993. Eleven New Records and Validations of Shore Fishes from the Hawaiian Islands. Pacific Sci. 47:222–239.

Ravelo A.C., Andreasen D.H. 2000. Enhanced circulation during a warm period. Geophys. Res. Lett. 27:1001–1004.

Ree R.H., Smith S.A. 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. Syst. Biol. 57:4–14.

Ree R.H., Smith S.A., Moore B.R., Webb C.O., Donoghue M.J. 2005. A Likelihood Framework for Inferring the Evolution of Geographic. Evolution (N. Y). 59:2299–2311.

Reeb C.A., Avise J.C. 1990. A genetic discontinuity in a continuously distributed species: Mitochondrial DNA in the American oyster, *Crassostrea virginica*. Genetics. 124:397–406.

Richards V.P., DeBiasse M.B., Shivji M. 2018. Deep mitochondrial lineage divergence among populations of the southern stingray (*Hypanus americanus* (Hildebrand & Schroeder, 1928)) throughout the Southeastern United States and Caribbean. Mar. Biodivers.

Riehl T., Lins L., Brandt A. 2018. The effects of depth, distance, and the Mid-Atlantic Ridge on genetic differentiation of abyssal and hadal isopods (Macrostylidae). Deep. Res. Part II Top. Stud. Oceanogr. 148:74–90.

Rocha L.A. 2003. Patterns of distribution and processes of speciation in Brazilian reef fishes. J. Biogeogr. 30:1161–1171.

Rocha L.A., Bowen B.W. 2008. Speciation in coral-reef fishes. J. Fish Biol. 72:1101–1121.

Rocha L.A., Craig M.T., Bowen B.W. 2007. Phylogeography and the conservation of coral reef fishes. Coral Reefs. 26:501–512.

Rocha L.A., Robertson D.R., Rocha C.R., Van Tassell J.L., Craig M.T., Bowen B.W. 2005. Recent invasion of the tropical Atlantic by an Indo-Pacific coral reef fish. Mol. Ecol. 14:3921–3928.

Rocha L.A., Rosa I.L. 2001. Baseline assessment of reef fish assemblages of Parcel Manuel Luiz Marine State Park, Maranhão, north-east Brazil. J. Fish Biol. 58:985–998.

Rodrigues Filho L.F. da S., Feitosa L.M., Silva Nunes J.L., Onodera Palmeira A.R., Martins A.P.B., Giarrizzo T., Carvalho-Costa L.F., Monteiro I.L.P., Gemaque R., Gomes F., Souza R.F.C., Sampaio I., Sales J.B. de L. 2020. Molecular identification of ray species traded along the Brazilian Amazon coast. Fish. Res. 223:105407.

Ronquist F. 1994. Ancestral Areas and Parsimony. .

Ronquist F. 1997. Dispersal-vicariance analysis: A new approach to the quantification of historical biogeography. Syst. Biol. 46:195–203.

Ronquist F., Huelsenbeck J.P. 2003. MrBayes3: Bayesian phylogenetic inference undermixed models. Bioinformatics. 19:1572–1574.

Ronquist F., Sanmartín I. 2011. Phylogenetic Methods in Biogeography. Annu. Rev. Ecol. Evol. Syst. 42:441–464.

Rosa R.S., Moura R.L. 1997. Visual assessment of reef fish community structure in the Atol das Rocas Biological Reserve, off northeastern Brazil. Proc. 8th Int. Coral Reef Symp.:983–986.

Rosen D.E. 1975. A Vicariance Model of Caribbean Biogeography. Syst. Biol. 24:431–464.

Rosenberger L. 2001. Phylogenetic Relationships within the Stingray Genus *Dasyatis* (Chondrichthyes: Dasyatidae). Copeia. 2001:615–627.

Ross Robertson D., Karg F., Leao de Moura R., Victor B.C., Bernardi G. 2006. Mechanisms of speciation and faunal enrichment in Atlantic parrotfishes. Mol. Phylogenet. Evol. 40:795–807.

Sales J.B.L., de Oliveira C.N., dos Santos W.C.R., Rotundo M.M., Ferreira Y., Ready J., Sampaio I., Oliveira C., Cruz V.P., Lara-Mendoza R.E., da Silva Rodrigues-Filho L.F. 2019. Phylogeography of eagle rays of the genus *Aetobatus*: *Aetobatus narinari* is restricted to the continental western Atlantic Ocean. Hydrobiologia. 836.

Santos H.R.S., Gomes U.L., Charvet-Almeida P. 2004. A new species of whiptail stingray of the genus *Dasyatis* Rafinesque, 1810 from the Southwestern Atlantic Ocean (Chondrichthyes: Myliobatiformes: Dasyatidae). Zootaxa. 492:1–12.

Schneider B., Schmittner A. 2006. Simulating the impact of the Panamanian seaway closure on ocean circulation, marine productivity and nutrient cycling. Earth Planet. Sci. Lett. 246:367–380.

Schultz J.K., Feldheim K.A., Gruber S.H., Ashley M. V., McGovern T.M., Bowen B.W. 2008. Global phylogeography and seascape genetics of the lemon sharks (genus *Negaprion*). Mol. Ecol. 17:5336–5348.

Shen K.-N., Chang C.-W., Tsai S.-Y., Wu S.-C., Lin Z.-H., Chan Y.-F., Chen C.-H., Hsiao C.-D., Borsa P. 2015a. Next generation sequencing yields complete mitogenomes of Leopard whipray (*Himantura leoparda*) and Blue-spotted stingray (*Neotrygon kuhlii)* (Chondrichthyes: Dasyatidae). Mitochondrial DNA.:1–2.

Shen K.-N., Tsai S.-Y., Chen C.-H., Hsiao C.-D., Durand J.-D. 2015b. Next generation sequencing yields the complete mitochondrial genome of the largescale mullet, Liza macrolepis (Teleostei: Mugilidae). Mitochondrial DNA. 00:1–2.

Shirai S. 1996. Phylogenetic Interrelationships of Neoselachians (Chondrichthyes: Euselachii). In: Stiassny M., Parenti L., Johnson G., editors. Interrelationships of Fishes. New York: Academic Press. p. 9–34.

Si R., Chen H., Ai W., Chen X., Chen S. 2016. Complete mitochondrial genome and the phylogenetic position of the Bowmouth guitarfish *Rhina ancylostoma* (Rajiformes, Rhinobatidae). Mitochondrial DNA Part B Resour. 1:270–272.

Sloan P. 2019. Evolutionary Thought Before Darwin. In: Zalta E.N., editor. The Stanford Encyclopedia of Philosophy. Metaphysics Research Lab, Stanford University.

Smith J.A. 1863. Notice of the Ukpam, a large species (probably new) of sting ray (*Trygon*, Cuv.), found in the Old Calabar River, Africa. Proc. R. Phys. Soc. Edinburgh. 2:64–69.

Spalding M.D., Fox H.E., Allen G.R., Davidson N., Ferdaña Z. a., Finlayson M., Halpern B.S., Jorge M. a., Lombana A., Lourie S. a., Martin K.D., Mcmanus E., Molnar J., Recchia C. a., Robertson J. 2007. Marine Ecoregions of the World: A Bioregionalization of Coastal and Shelf Areas. Bioscience. 57:573.

Stalcup M.C., Metcalf W.G. 1966. Direct Measurements of the Atlantic Equatorial Undercurrent. J. Mar. Res. 24:44–55.

Stamatakis A. 2014. RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. Bioinformatics. 30:1312–1313.

Stauch A., Blanc M. 1963. Description d’un sélacien rajiforme des eaux douces du Nord-

Cameroun: *Potamotrygon garouaensis* n.sp. Bull. du Muséum Natl. d'Histoire Nat. (Série 2). 34:166–171.

Stelbrink B., von Rintelen T., Cliff G., Kriwet J. 2010. Molecular systematics and global phylogeography of angel sharks (genus *Squatina*). Mol. Phylogenet. Evol. 54:395–404.

Sukumaran J., Knowles L.L. 2017. Multispecies coalescent delimits structure, not species. Proc. Natl. Acad. Sci. 2016:201607921.

Taylor M.S., Hellberg M.E. 2006. Comparative phylogeography in a genus of coral reef fishes: Biogeographic and genetic concordance in the Caribbean. Mol. Ecol. 15:695–707.

Tiedemann R., Kiel U., Shackleton J. 1994. Astronomic timescale for the Pliocene Atlantic δ18O and dust flux records of Ocean Drillin Program site 659. Paleoceanography. 9:619–638.

Vermeij G.J., Rosenberg G. 1993. Giving and receiving: the tropical Atlantic as donor and recipient region for invading species. Am. Malacol. Bull. 10:181–194.

Watling L., Guinotte J., Clark M.R., Smith C.R. 2013. A proposed biogeography of the deep ocean floor. Prog. Oceanogr. 111:91–112.

White W.T., Corrigan S., Yang L., Henderson A.C., Bazinet A.L., Swofford D.L., Naylor G.. J.P. 2018. Phylogeny of the manta and devilrays (Chondrichthyes : Mobulidae), with an updated taxonomic arrangement for the family. Zool. J. Linn. Soc. 182:50–75.

White W.T., Last P.R. 2012. A review of the taxonomy of chondrichthyan fishes: a modern perspective. J. Fish Biol. 80:901–17.

Whitley G.P. 1933. Studies in ichthyology. No. 7. Rec. Aust. Museum. 19:60–112.

Whittaker R.J., Araújo M.B., Jepson P., Ladle R.J., James E.M.W., Willis K.J. 2005. Conservation Biogeography: Assessment and Prospect. Divers. Distrib. 11:3–23.

Wintle B.A., Runge M.C., Bekessy S.A. 2010. Allocating monitoring effort in the face of unknown unknowns. Ecol. Lett. 13:1325–1337.

Wirtz P., Ferreira C.E.L., Floeter S.R., Fricke R., Gasparini J.L., Iwamoto T., Rocha L., Sampaio C.L.S., Schliewen U.K. 2007. Coastal fishes of São Tomé and Príncipe islands, Gulf of Guinea (Eastern Atlantic Ocean) - An update. Zootaxa.:1–48.

Yang B., Zhang J., Yamaguchi A., Zhang B. 2013. Mitochondrial genome of *Dasyatis bennettii* (Chondrichthyes: Dasyatidae). Mitochondrial DNA. 24:344–346.

Yang C.-P., Zhi T.-T., Zhang S., Yan S., Yang T. 2016. Complete mitochondrial genome of the pelagic stingray *Pteroplatytrygon violacea* (Myliobatiformes: Dasyatidae). Mitochondrial DNA Part A. 27:935–936.

Yang L., Naylor G.J.P. 2016. Long-PCR based next generation sequencing of the whole mitochondrial genome of the peacock skate *Pavoraja nitida* (Elasmobranchii: Arhynchobatidae). Mitochondrial DNA Part A. 27:943–944.

Young M., Carr M.H. 2015. Application of species distribution models to explain and predict the distribution, abundance and assemblage structure of nearshore temperate reef fishes. Divers. Distrib. 21:1428–1440.

Yu Y., Harris A.J., Blair C., He X.J. 2015. RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. Mol. Phylogenet. Evol. 87:46–49.

Zamudio K.R., Bell R.C., Mason N.A. 2016. Phenotypes in phylogeography: Species' traits, environmental variation, and vertebrate diversification. Proc. Natl. Acad. Sci. U. S. A. 113:8041–8048.

Zhang J., Kapli P., Pavlidis P., Stamatakis A. 2013. A general species delimitation method with applications to phylogenetic placements. Bioinformatics. 29:2869–2876.

Zimmermann N.E., Edwards T.C., Graham C.H., Pearman P.B., Svenning J.C. 2010. New trends in species distribution modelling. Ecography (Cop.). 33:985–989.

SUPPLEMENTARY MATERIAL

Supp. Mat. 1. Collected samples' locations and accession numbers at the University of Florida.

Nominal species ID	**Ocean Basin**	**Sampling location**	**Tissue accession number**
Hypanus americanus	Western North Atlantic	Sandshoal Inlet, Virginia, USA	4645
Hypanus americanus	Gulf of Mexico	Horn Island, Mississippi, USA	6232
Hypanus americanus	Western North Atlantic	Charleston Harbor, Morris Island, South Carolina, USA	13493
Hypanus americanus	Western North Atlantic	Edisto, South Carolina, USA	13494
Hypanus americanus	Western North Atlantic	Edisto, South Carolina, USA	13495
Hypanus americanus	Caribbean Sea	Inner Channel, Stann Creek, Belize	13934
Hypanus aff. *americanus*	Western North Atlantic	Florida to Massachusetts	1594
Hypanus aff. *americanus*	Caribbean Sea	Nicaragua	14618
Hypanus longus	Eastern North Pacific	Loreto, Baja California Sur, Mexico	1599
Hypanus longus	Eastern North Pacific	San Jose del Cabo, Baja California Sur, Mexico	5281

Hypanus longus	Eastern North Pacific	San Jose del Cabo, Baja California Sur, Mexico	5282
Hypanus longus	Eastern North Pacific	San Jose del Cabo, Baja California Sur, Mexico	5283
Hypanus rudis	Eastern Atlantic	Mbour, Senegal	3295
Hypanus rudis	Eastern Atlantic	Mbour, Senegal	6061
Hypanus rudis	Eastern Atlantic	Mbour, Senegal	6063
Hypanus rudis	Eastern Atlantic	Ghana	17700
Hypanus aff. *rudis*	Western South Atlantic	Macau, Rio Grande do Norte, Brazil	18461
Hypanus aff. *rudis*	Western South Atlantic	Caiçara do Norte, Rio Grande do Norte, Brazil	18471
Hypanus aff. *rudis*	Western South Atlantic	Caiçara do Norte, Rio Grande do Norte, Brazil	18472
Hypanus aff. *rudis*	Western South Atlantic	Macau, Rio Grande do Norte, Brazil	18494
Hypanus aff. *rudis*	Western South Atlantic	Macau, Rio Grande do Norte, Brazil	18495
Hypanus aff. *rudis*	Western South Atlantic	Porto Seguro, Bahia, Brazil	18496
Hypanus aff. *rudis*	Western South Atlantic	Porto Seguro, Bahia, Brazil	18497
Hypanus aff. *rudis*	Western South Atlantic	Porto Seguro, Bahia, Brazil	18498
Hypanus aff. *rudis*	Western South Atlantic	Pará, Brazil	18504
Hypanus aff. *rudis*	Western South Atlantic	Pará, Brazil	18505
Hypanus aff. *rudis*	Western South Atlantic	Pará, Brazil	18506
Hypanus aff. *rudis*	Western South Atlantic	Pará, Brazil	18507
Hypanus aff. *rudis*	Western South Atlantic	Pará, Brazil	18508
Hypanus aff. *rudis*	Western South Atlantic	Galinhos, Rio Grande do Norte, Brazil	18533
Hypanus aff. *rudis*	Western South Atlantic	Bitupitá, Ceará, Brazil	18534
Hypanus aff. *rudis*	Western South Atlantic	Bitupitá, Ceará, Brazil	18535
Hypanus aff. *rudis*	Western South Atlantic	João Pessoa, Paraíba, Brazil	19072
Hypanus aff. *rudis*	Western South Atlantic	João Pessoa, Paraíba, Brazil	19073
Hypanus aff. *rudis*	Western South Atlantic	Mucuripe, Ceará, Brazil	19456

Hypanus aff. r*udis*	Western South Atlantic	Mucuripe, Ceará, Brazil	19457
Hypanus aff. r*udis*	Western South Atlantic	Mucuripe, Ceará, Brazil	19458
Hypanus aff. r*udis*	Western South Atlantic	Mucuripe, Ceará, Brazil	19459
Hypanus aff. r*udis*	Western South Atlantic	Mucuripe, Ceará, Brazil	19460
Hypanus marianae	Western South Atlantic	Baía Formosa, Rio Grande do Norte, Brazil	18437
Hypanus marianae	Western South Atlantic	Macau, Rio Grande do Norte, Brazil	18465
Hypanus marianae	Western South Atlantic	Macau, Rio Grande do Norte, Brazil	18467
Hypanus marianae	Western South Atlantic	Abrolhos, Bahia, Brazil	18509
Hypanus marianae	Western South Atlantic	Abrolhos, Bahia, Brazil	18510
Hypanus marianae	Western South Atlantic	Abrolhos, Bahia, Brazil	18511
Hypanus marianae	Western South Atlantic	Abrolhos, Bahia, Brazil	18512
Hypanus marianae	Western South Atlantic	Abrolhos, Bahia, Brazil	18513
Hypanus marianae	Western South Atlantic	Abrolhos, Bahia, Brazil	18514
Hypanus marianae	Western South Atlantic	Ponta de Pedras, Pernambuco, Brazil	18515
Hypanus marianae	Western South Atlantic	Ponta de Pedras, Pernambuco, Brazil	18516
Hypanus marianae	Western South Atlantic	Ponta de Pedras, Pernambuco, Brazil	18517
Hypanus marianae	Western South Atlantic	Ponta de Pedras, Pernambuco, Brazil	18518
Hypanus marianae	Western South Atlantic	Ponta de Pedras, Pernambuco, Brazil	18519
Hypanus marianae	Western South Atlantic	Ponta de Pedras, Pernambuco, Brazil	18520
Hypanus marianae	Western South Atlantic	Caiçara do Norte, Rio Grande do Norte, Brazil	18521
Hypanus marianae	Western South Atlantic	Caiçara do Norte, Rio Grande do Norte, Brazil	18522

Hypanus marianae	Western South Atlantic	Caiçara do Norte, Rio Grande do Norte, Brazil	18523
Hypanus marianae	Western South Atlantic	Caiçara do Norte, Rio Grande do Norte, Brazil	18524
Hypanus marianae	Western South Atlantic	Caiçara do Norte, Rio Grande do Norte, Brazil	18525
Hypanus marianae	Western South Atlantic	Caiçara do Norte, Rio Grande do Norte, Brazil	18526
Hypanus marianae	Western South Atlantic	Salvador, Bahia, Brazil	18527
Hypanus marianae	Western South Atlantic	Salvador, Bahia, Brazil	18528
Hypanus marianae	Western South Atlantic	Salvador, Bahia, Brazil	18529
Hypanus marianae	Western South Atlantic	Salvador, Bahia, Brazil	18530
Hypanus marianae	Western South Atlantic	Salvador, Bahia, Brazil	18531
Hypanus marianae	Western South Atlantic	Salvador, Bahia, Brazil	18532
Hypanus marianae	Western South Atlantic	Mucuripe, Ceará, Brazil	19466
Hypanus marianae	Western South Atlantic	Mucuripe, Ceará, Brazil	19467
Hypanus aff. *guttatus*	Caribbean Sea	Inner Channel, Gales Point Manatee, Belize	13939
Hypanus aff. *guttatus*	Caribbean Sea	Inner Channel, Gales Point Manatee, Belize	13946
Hypanus guttatus	Western South Atlantic	Baía Formosa, Rio Grande do Norte, Brazil	18434
Hypanus guttatus	Western South Atlantic	São Cristóvão, Rio Grande do Norte, Brazil	18435
Hypanus guttatus	Western South Atlantic	Natal, Rio Grande do Norte, Brazil	18436
Hypanus guttatus	Western South Atlantic	São Cristóvão, Rio Grande do Norte, Brazil	18443
Hypanus guttatus	Western South Atlantic	São Cristóvão, Rio Grande do Norte, Brazil	18451
Hypanus guttatus	Western South Atlantic	São Cristóvão, Rio Grande do Norte, Brazil	18453

Hypanus guttatus	Western South Atlantic	São Cristóvão, Rio Grande do Norte, Brazil	18454
Hypanus guttatus	Western South Atlantic	São Cristóvão, Rio Grande do Norte, Brazil	18458
Hypanus guttatus	Western South Atlantic	São Cristóvão, Rio Grande do Norte, Brazil	18459
Hypanus guttatus	Western South Atlantic	Macau, Rio Grande do Norte, Brazil	18463
Hypanus guttatus	Western South Atlantic	Macau, Rio Grande do Norte, Brazil	18466
Hypanus guttatus	Western South Atlantic	Caiçara do Norte, Rio Grande do Norte, Brazil	18473
Hypanus guttatus	Western South Atlantic	Caiçara do Norte, Rio Grande do Norte, Brazil	18474
Hypanus guttatus	Western South Atlantic	Caiçara do Norte, Rio Grande do Norte, Brazil	18475
Hypanus guttatus	Western South Atlantic	Caiçara do Norte, Rio Grande do Norte, Brazil	18476
Hypanus guttatus	Western South Atlantic	Caiçara do Norte, Rio Grande do Norte, Brazil	18477
Hypanus guttatus	Western South Atlantic	Caiçara do Norte, Rio Grande do Norte, Brazil	18478
Hypanus guttatus	Western South Atlantic	Caiçara do Norte, Rio Grande do Norte, Brazil	18479
Hypanus guttatus	Western South Atlantic	Mucuri, Bahia, Brazil	18501
Hypanus guttatus	Western South Atlantic	Mucuri, Bahia, Brazil	18502
Hypanus guttatus	Western South Atlantic	Mucuri, Bahia, Brazil	18503
Hypanus guttatus	Western South Atlantic	Guamare, Rio Grande do Norte, Brazil	18536
Hypanus guttatus	Western South Atlantic	Bitupitá, Ceará, Brazil	18537
Hypanus guttatus	Western South Atlantic	Baía Formosa, Rio Grande do Norte, Brazil	18538
Hypanus guttatus	Western South Atlantic	Mucuripe, Ceará, Brazil	19461

Hypanus guttatus	Western South Atlantic	Mucuripe, Ceará, Brazil	19462
Hypanus guttatus	Western South Atlantic	Mucuripe, Ceará, Brazil	19463
Hypanus guttatus	Western South Atlantic	Mucuripe, Ceará, Brazil	19464
Hypanus guttatus	Western South Atlantic	Mucuripe, Ceará, Brazil	19465
Hypanus guttatus	Western South Atlantic	Pará, Brazil	19468
Hypanus guttatus	Western South Atlantic	Pará, Brazil	19469
Hypanus guttatus	Western South Atlantic	Pará, Brazil	19470
Hypanus geijskesi	Western South Atlantic	Pará, Brazil	19471
Hypanus geijskesi	Western South Atlantic	Pará, Brazil	19472
Hypanus geijskesi	Western South Atlantic	Pará, Brazil	19473
Hypanus geijskesi	Western South Atlantic	Pará, Brazil	19474
Hypanus geijskesi	Western South Atlantic	Pará, Brazil	19475
Hypanus geijskesi	Western South Atlantic	Pará, Brazil	19476
Hypanus dipterurus	Eastern North Pacific	Bahia de Los Angeles, Baja California, Mexico	1598
Hypanus dipterurus	Eastern North Pacific	Bahia de Los Angeles, Baja California, Mexico	5196
Hypanus dipterurus	Eastern North Pacific	Loreto, Baja California Sur, Mexico	5266
Hypanus dipterurus	Eastern North Pacific	Santa Rosalia, Baja California Sur, Mexico	5379
Hypanus aff. *say*	Gulf of Mexico	Horn Island, Mississippi, USA	6221
Hypanus aff. *say*	Gulf of Mexico	Horn Island, Mississippi, USA	6230
Hypanus aff. *say*	Gulf of Mexico	East Ship Island, Mississippi, USA	6234
Hypanus say	Western North Atlantic	North Carolina, USA	12768
Hypanus say	Western North Atlantic	North Carolina, USA	12769
Hypanus say	Western North Atlantic	North Carolina, USA	12770
Hypanus say	Western North Atlantic	South Carolina, USA	13502
Hypanus say	Western North Atlantic	South Carolina, USA	13504

Hypanus sabinus	Gulf of Mexico	Horn Island, Mississippi, USA	6226
Hypanus sabinus	Gulf of Mexico	Biloxi Ship Channel, Mississippi, USA	6229
Hypanus sabinus	Western North Atlantic	Charleston harbor, South Carolina, USA	13503
Hypanus sabinus	Western North Atlantic	Charleston harbor, South Carolina, USA	13516
Neotrygon orientale	South China Sea, Pacific Ocean	Tanjung Manis, Sarawak, Malaysia	3636
Taeniura lymma	Sulawesi Sea, Pacific Ocean	Tanjung Batu, East Kalimantan, Indonesia	4818
Telatrygon acutirostra	Western North Pacific	Ariake Bay, Japan	12930
Hemitrygon akajei	Western North Pacific	Ariake Bay, Japan	12904
Taeniurops grabatus	Eastern Atlantic	Mbour, Senegal	6065
Pteroplatytrygon violacea	Eastern North Pacific	California, USA	6498
Bathytoshia lata	North Pacific	Hawaii, USA	15451
Dasyatis hypostigma	Western South Atlantic	Uruguay	16151
Fontitrygon garouaensis	Eastern Atlantic	Kwara, Nigeria	19480
Fontitrygon margarita	Eastern Atlantic	Djifere, Senegal	6072
Fontitrygon margaritella	Eastern Atlantic	Mbour, Senegal	6066

Supp. Mat. 2. Mitogenomes’ content of each *Hypanus* valid species.

			***H. americanus* GN13934**					***H. dipterurus* GN5379**				
Name	**Strand**	**Direction**	**Length (bp)**	**Min**	**Max**	**Start codon**	**Stop codon**	**Length (bp)**	**Min**	**Max**	**Start codon**	**Stop codon**
tRNA-Phe	H	Forward	69	1	69			70	1	70		
rRNA-12S	H	Forward	963	70	1032			966	71	1036		
tRNA-Val	H	Forward	72	1033	1104			72	1037	1108		
rRNA-16S	H	Forward	1700	1105	2804			1700	1109	2808		
rRNA-Leu	H	Forward	75	2805	2879			75	2809	2993		
ND1	H	Forward	978	2881	3858	ATG	TAA	978	2885	3862	ATG	TAA
tRNA-Ile	H	Forward	69	3861	3929			70	3865	3934		
tRNA-Gln	L	Reverse	72	3935	4006			72	3940	4011		
tRNA-Met	H	Forward	71	4006	4076			71	4011	4081		
ND2	H	Forward	1046	4077	5122	ATG	TA-	1046	4082	5127	ATG	TA-
tRNA-Trp	H	Forward	70	5123	5192			70	5130	5197		
tRNA-Ala	L	Reverse	69	5193	5261			69	5198	5266		
tRNA-Asn	L	Reverse	73	5263	5335			73	5268	5340		
tRNA-Cys	L	Reverse	67	5372	5438			68	5377	5444		
tRNA-Tyr	L	Reverse	71	5445	5515			71	5451	5521		
COI	H	Forward	1557	5517	7073	GTG	TAA	1557	5523	7079	GTG	TAA
tRNA-Ser	L	Reverse	71	7083	7153			71	7089	7159		
tRNA-Asp	H	Forward	71	7154	7224			70	7160	7229		
COII	H	Forward	691	7228	7918	ATG	CTCT	691	7233	7923	ATG	CTCT
tRNA-Lys	H	Forward	74	7919	7992			74	7924	7997		
ATP8	H	Forward	168	7994	8161	ATG	TAA	168	7999	8166	ATG	TAA
ATP6	H	Forward	684	8152	8835	ATG	TAA	684	8157	8840	ATG	TAA
COIII	H	Forward	786	8835	9620	ATG	TAA	786	8840	9625	ATG	TAA
tRNA-Gly	H	Forward	71	9626	9696			71	9631	9701		
ND3	H	Forward	349	9697	10045	ATG	T	349	9702	10050	ATG	T
tRNA-Arg	H	Forward	71	10047	10117			71	10051	10121		
ND4L	H	Forward	297	10118	10414	ATG	TAA	297	10122	10418	ATG	TAA
ND4	H	Forward	1381	10408	11788	ATG	T	1381	10412	11792	ATG	T
tRNA-His	H	Forward	69	11789	11857			70	11793	11862		
tRNA-Ser	H	Forward	67	11859	11925			67	11864	11930		
tRNA-Leu	H	Forward	72	11928	11999			72	11933	12004		
ND5	H	Forward	1845	12000	13844	ATG	TAA	1845	12005	13849	ATG	TAA
ND6	L	Reverse	522	13841	14362	CTA	CAT	522	13846	14367	CTA	CAT
tRNA-Glu	L	Reverse	69	14364	14432			69	14369	14437		
CytB	H	Forward	1143	14440	15582	ATG	TAA	1143	14445	15587	ATG	TAA

tRNA-Thr	H	Forward	73	15584	15656			73	15590	15662		

			***H. geijskesi* GN19472**					***H. guttatus* GN18453**				
Name	**Strand**	**Direction**	**Length (bp)**	**Min**	**Max**	**Start codon**	**Stop codon**	**Length (bp)**	**Min**	**Max**	**Start codon**	**Stop codon**
tRNA-Phe	H	Forward	70	1	70			69	1	69		
rRNA-12S	H	Forward	964	71	1034			962	70	1031		
tRNA-Val	H	Forward	72	1035	1106			72	1032	1103		
rRNA-16S	H	Forward	1696	1107	2802			1699	1104	2802		
rRNA-Leu	H	Forward	75	2803	2877			75	2803	2877		
ND1	H	Forward	978	2878	3855	ATG	TAA	978	2879	3856	ATG	TAA
tRNA-Ile	H	Forward	69	3856	3924			69	3859	3927		
tRNA-Gln	L	Reverse	72	3925	3996			72	3933	4004		
tRNA-Met	H	Forward	71	3997	4067			71	4004	4074		
ND2	H	Forward	1049	4068	5116	ATG	TA-	1049	4075	5123	ATG	TA-
tRNA-Trp	H	Forward	70	5117	5186			70	5124	5193		
tRNA-Ala	L	Reverse	69	5187	5255			69	5194	5262		
tRNA-Asn	L	Reverse	73	5256	5328			73	5264	5336		
tRNA-Cys	L	Reverse	67	5329	5395			67	5373	5439		
tRNA-Tyr	L	Reverse	70	5396	5465			70	5446	5515		
COI	H	Forward	1557	5466	7022	GTG	TAA	1557	5517	7073	GTG	TAA
tRNA-Ser	L	Reverse	71	7023	7093			71	7083	7153		
tRNA-Asp	H	Forward	71	7094	7164			71	7154	7224		
COII	H	Forward	691	7165	7855	ATG	CTCT	691	7228	7918	ATG	CTCT
tRNA-Lys	H	Forward	74	7856	7929			74	7919	7992		
ATP8	H	Forward	168	7930	8097	ATG	TAA	168	7994	8161	ATG	TAA
ATP6	H	Forward	683	8098	8780	ATG	TTA	684	8152	8835	ATG	TAA
COIII	H	Forward	786	8781	9566	ATG	TAA	786	8835	9620	ATG	TAA
tRNA-Gly	H	Forward	71	9567	9637			71	9626	9696		
ND3	H	Forward	350	9638	9987	ATG	ATA	349	9697	10045	ATG	T
tRNA-Arg	H	Forward	71	9988	10058			71	10046	10116		
ND4L	H	Forward	297	10059	10355	ATG	TAA	297	10117	10413	ATG	TAA
ND4	H	Forward	1381	10356	11736	ATG	T	1381	10407	11787	ATG	T
tRNA-His	H	Forward	69	11737	11805			69	11788	11856		
tRNA-Ser	H	Forward	67	11806	11872			67	11858	11924		
tRNA-Leu	H	Forward	72	11873	11944			72	11926	11997		
ND5	H	Forward	1845	11945	13789	ATG	TAA	1845	11998	13842	ATG	TAA
ND6	L	Reverse	522	13790	14311	CTA	CAT	522	13839	14360	CTA	CAT
tRNA-Glu	L	Reverse	69	14312	14380			69	14362	14430		
CytB	H	Forward	1143	14381	15523	ATG	TAA	1143	14442	15584	ATG	TAA

tRNA-Thr	H	Forward	73	15524	15596			74	15586	15659		

			***H. longus* GN5282**					***H. marianae* GN18437**				
Name	**Strand**	**Direction**	**Length (bp)**	**Min**	**Max**	**Start codon**	**Stop codon**	**Length (bp)**	**Min**	**Max**	**Start codon**	**Stop codon**
tRNA-Phe	H	Forward	69	1	69			69	1	69		
rRNA-12S	H	Forward	963	70	1032			963	70	1032		
tRNA-Val	H	Forward	72	1033	1104			72	1033	1104		
rRNA-16S	H	Forward	1699	1105	2803			1703	1105	2807		
rRNA-Leu	H	Forward	75	2804	2878			75	2808	2882		
ND1	H	Forward	978	2880	3857	ATG	TAA	978	2884	3861	ATG	TAA
tRNA-Ile	H	Forward	69	3860	3928			70	3864	3933		
tRNA-Gln	L	Reverse	72	3934	4005			72	3939	4010		
tRNA-Met	H	Forward	71	4005	4075			71	4010	4080		
ND2	H	Forward	1046	4076	5121	ATG	TA-	1046	4081	5126	ATG	TA-
tRNA-Trp	H	Forward	70	5122	5191			70	5127	5196		
tRNA-Ala	L	Reverse	69	5192	5260			69	5197	5265		
tRNA-Asn	L	Reverse	73	5262	5334			73	5267	5339		
tRNA-Cys	L	Reverse	67	5371	5437			67	5376	5442		
tRNA-Tyr	L	Reverse	71	5444	5514			71	5449	5519		
COI	H	Forward	1557	5516	7072	GTG	TAA	1557	5521	7077	GTG	TAA
tRNA-Ser	L	Reverse	71	7082	7152			71	7087	7157		
tRNA-Asp	H	Forward	71	7153	7223			71	7158	7228		
COII	H	Forward	691	7227	7917	ATG	CTCT	691	7232	7922	ATG	CTCT
tRNA-Lys	H	Forward	74	7918	7991			75	7923	7997		
ATP8	H	Forward	168	7993	8160	ATG	TAA	168	7999	8166	ATG	TAA
ATP6	H	Forward	684	8151	8834	ATG	TAA	684	8157	8840	ATG	TAA
COIII	H	Forward	786	8834	9619	ATG	TAA	786	8840	9625	ATG	TAA
tRNA-Gly	H	Forward	71	9625	9695			71	9631	9701		
ND3	H	Forward	349	9696	10044	ATG	T	349	9702	10050	ATG	T
tRNA-Arg	H	Forward	71	10046	10116			71	10052	10122		
ND4L	H	Forward	297	10117	10413	ATG	TAA	297	10123	10419	ATG	TAA
ND4	H	Forward	1381	10407	11787	ATG	T	1381	10413	11793	ATG	T
tRNA-His	H	Forward	69	11788	11856			69	11794	11862		
tRNA-Ser	H	Forward	67	11858	11924			67	11864	11930		
tRNA-Leu	H	Forward	72	11927	11998			72	11933	12004		
ND5	H	Forward	1845	11999	13843	ATG	TAA	1845	12005	13849	ATG	TAA
ND6	L	Reverse	522	13840	14361	CTA	CAT	522	13846	14367	TTA	CAT
tRNA-Glu	L	Reverse	69	14363	14431			69	14369	14437		
CytB	H	Forward	1143	14441	15583	ATG	TAA	1143	14449	15591	ATG	TAA

tRNA-Thr	H	Forward	73	15585	15657			73	15593	15665		

			H. rudis GN6061					***H. sabinus* GN13516**				
Name	**Strand**	**Direction**	**Length (bp)**	**Min**	**Max**	**Start codon**	**Stop codon**	**Length (bp)**	**Min**	**Max**	**Start codon**	**Stop codon**
tRNA-Phe	H	Forward	69	1	69			70	1	70		
rRNA-12S	H	Forward	967	70	1036			968	71	1038		
tRNA-Val	H	Forward	72	1037	1108			72	1039	1100		
rRNA-16S	H	Forward	1698	1109	2806			1699	1111	2809		
rRNA-Leu	H	Forward	75	2807	2881			75	2810	2884		
ND1	H	Forward	978	2883	3860	ATG	TAA	978	2886	3863	ATG	TAA
tRNA-Ile	H	Forward	69	3863	3931			68	3867	3934		
tRNA-Gln	L	Reverse	73	3937	4009			72	3940	4011		
tRNA-Met	H	Forward	71	4009	4079			71	4011	4081		
ND2	H	Forward	1046	4080	5125	ATG	TA-	1047	4082	5128	ATG	TAA
tRNA-Trp	H	Forward	70	5126	5195			70	5129	5198		
tRNA-Ala	L	Reverse	69	5196	5264			69	5199	5267		
tRNA-Asn	L	Reverse	73	5266	5338			73	5269	5341		
tRNA-Cys	L	Reverse	67	5375	5441			67	5378	5444		
tRNA-Tyr	L	Reverse	71	5448	5518			70	5451	5520		
COI	H	Forward	1557	5520	7076	GTG	TAA	1557	5522	7078	GTG	TAA
tRNA-Ser	L	Reverse	71	7086	7156			71	7088	7158		
tRNA-Asp	H	Forward	71	7157	7227			70	7159	7228		
COII	H	Forward	691	7231	7921	ATG	CTCT	691	7232	7922	ATG	CTTT
tRNA-Lys	H	Forward	74	7922	7995			74	7923	7996		
ATP8	H	Forward	168	7997	8164	ATG	TAA	168	7998	8165	ATG	TAA
ATP6	H	Forward	684	8155	8838	ATG	TAA	684	8156	8839	ATG	TAA
COIII	H	Forward	786	8838	9623	ATG	TAA	786	8840	9625	ATG	TAA
tRNA-Gly	H	Forward	71	9629	9699			70	9631	9700		
ND3	H	Forward	349	9700	10048	ATG	T	349	9701	10049	ATG	T
tRNA-Arg	H	Forward	71	10050	10120			71	10051	10121		
ND4L	H	Forward	297	10121	10417	ATG	TAA	297	10122	10418	ATG	TAA
ND4	H	Forward	1381	10411	11791	ATG	T	1381	10412	11792	ATG	T
tRNA-His	H	Forward	69	11792	11860			69	11793	11861		
tRNA-Ser	H	Forward	67	11862	11928			67	11863	11929		
tRNA-Leu	H	Forward	72	11931	12002			72	11932	12003		
ND5	H	Forward	1852	12003	13854	ATG	TAA	1845	12004	13848	ATG	TAA
ND6	L	Reverse	522	13851	14372	CTA	CAT	522	13845	14366	CTA	CAT
tRNA-Glu	L	Reverse	69	14374	14442			69	14368	14436		
CytB	H	Forward	1143	14450	15592	ATG	TAA	1143	14448	15590	ATG	TAA

tRNA-Thr	H	Forward	73	15596	15668			72	15594	15665				

			***H. say* GN12770**				
Name	**Strand**	**Direction**	**Length (bp)**	**Min**	**Max**	**Start codon**	**Stop codon**
tRNA-Phe	H	Forward	71	1	71		
rRNA-12S	H	Forward	964	72	1035		
tRNA-Val	H	Forward	72	1036	1107		
rRNA-16S	H	Forward	1703	1108	2810		
rRNA-Leu	H	Forward	75	2811	2885		
ND1	H	Forward	978	2887	3864	ATG	TAA
tRNA-Ile	H	Forward	70	3867	3936		
tRNA-Gln	L	Reverse	72	3942	4013		
tRNA-Met	H	Forward	71	4013	4083		
ND2	H	Forward	1046	4084	5129	ATG	TA-
tRNA-Trp	H	Forward	70	5132	5199		
tRNA-Ala	L	Reverse	69	5200	5268		
tRNA-Asn	L	Reverse	73	5270	5342		
tRNA-Cys	L	Reverse	68	5379	5446		
tRNA-Tyr	L	Reverse	70	5423	5522		
COI	H	Forward	1557	5524	7080	GTG	TAA
tRNA-Ser	L	Reverse	71	7090	7160		
tRNA-Asp	H	Forward	70	7161	7230		
COII	H	Forward	691	7234	7924	ATG	CTCT
tRNA-Lys	H	Forward	74	7925	7998		
ATP8	H	Forward	168	8000	8167	ATG	TAA
ATP6	H	Forward	684	8158	8841	ATG	TAA
COIII	H	Forward	786	8841	9626	ATG	TAA
tRNA-Gly	H	Forward	71	9632	9702		
ND3	H	Forward	349	9703	10051	ATG	T
tRNA-Arg	H	Forward	71	10051	10122		
ND4L	H	Forward	297	10123	10419	ATG	TAA
ND4	H	Forward	1381	10413	11793	ATG	T
tRNA-His	H	Forward	69	11794	11862		
tRNA-Ser	H	Forward	67	11864	11930		
tRNA-Leu	H	Forward	72	11933	12004		
ND5	H	Forward	1845	12005	13849	ATG	TAA
ND6	L	Reverse	522	13846	14367	CTA	CAT
tRNA-Glu	L	Reverse	69	14369	14437		
CytB	H	Forward	1143	14445	15587	ATG	TAA

tRNA-Thr	H	Forward	73	15590	15662	

Supp. Mat. 3. Geological, oceanographical, and climate events related to evolutionary processes in *Hypanus*.

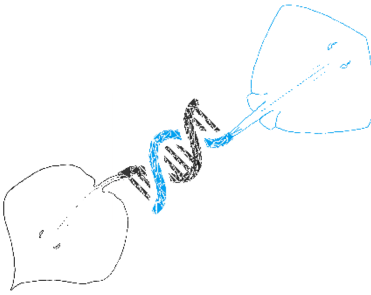
Date (in Ma)	**Event**	**Authors**
33	Global cooling	Lear *et al.* (2008;) Liu *et al.* (2009)
23	Collision of Panama Arc	Farris *et al.* (2011)
12	Pinnacle of Andes uplift	Hoorn *et al.* (2010)
10.4	Amazon River outflow	Hoorn *et al.* (1995)
9.5	Cessation of deep-water exchange between Atlantic and Pacific Oceans	Newkirk & Martin (2009)
2.8	Final closure of Isthmus of Panama	O’Dea *et al.* (2018)
2.8-2.0	Sea-level drop	Tiedemann *et al.* (1994)

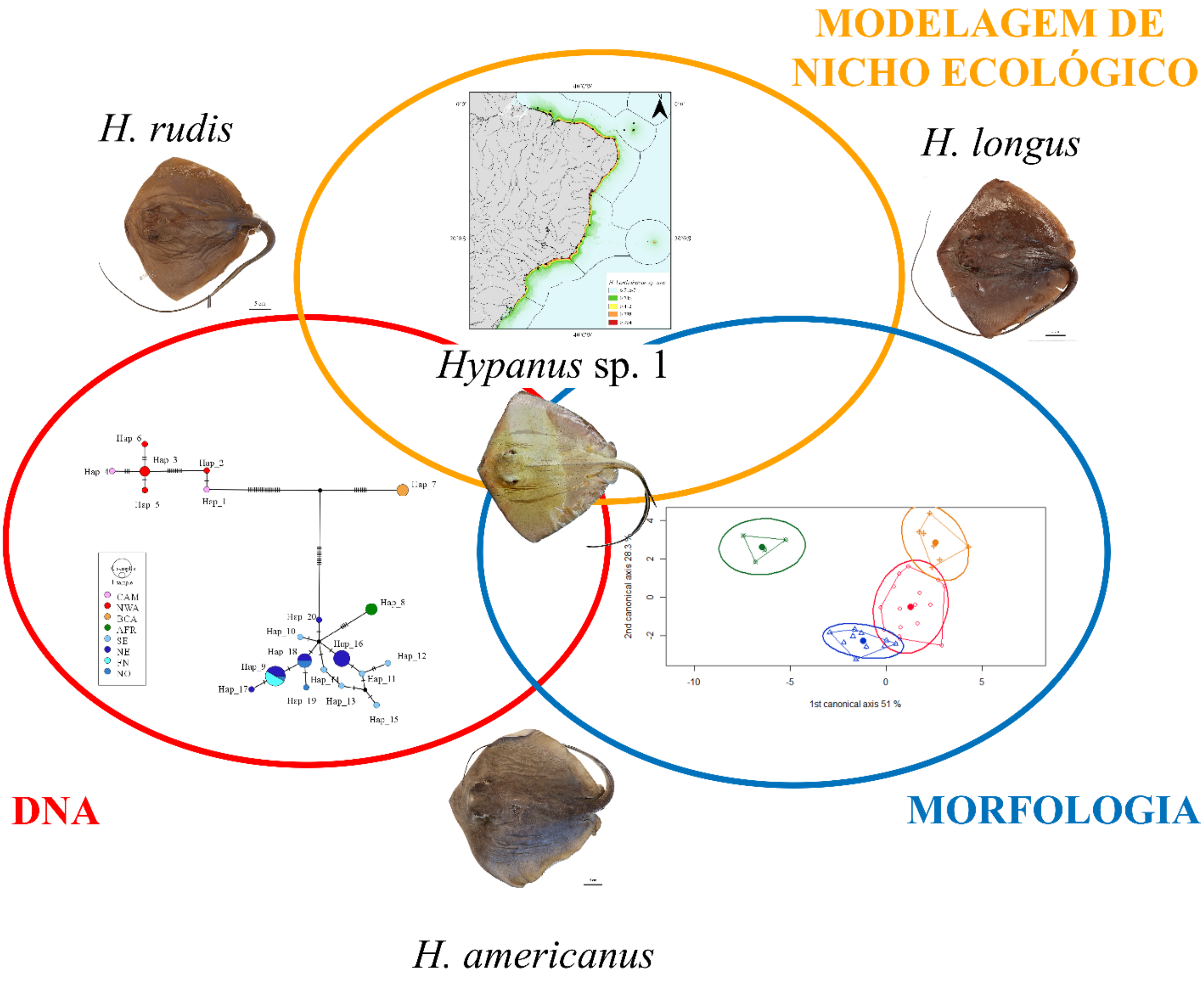
Supp. Mat. 4. Summary of *Hypanus* clades, their divergence dates and possible causes associated to events.

Date (in Ma)	**Clade**	**Processes**	**Oceanographic, Geological, and/or Hydrological possible causes**
30.97	**Clade 1** *Hypanus*	dispersal and vicariance	Refuge in Tropical Northwestern Atlantic due to Global cooling
27.79	**Clade 2** *H. sabinus*+*H. dipterurus*+H. aff. *say*+*H. say*	dispersal and subset sympatry	Ecological adaptations
9.33	**Clade 3** *H. dipterurus*+*H.* aff. *say*+*H. say*	dispersal and founder event	Dispersal towards Tropical waters and cessation of deep-water exchange between Atlantic and Pacific
1.56	**Clade 4** *H.* aff. *say*+*H. say*	dispersal and vicariance	Sea-level drop, interruption of water connection between East and West Florida
13.75	**Clade 5** *H. guttatus*+*H.* aff. *guttatus*+H. geijskesi+*H. marianae*+*H. longus*+*H. rudis*+*H.* aff. *rudis*+*H.* aff. *americanus*+*H. americanus*	dispersal and subset sympatry	Dispersal to Brazilian coast, and pinnacle of Andes uplift with some sediment outflow towards the Atlantic separating lineages
8.83	**Clade 6** *H. guttatus*+*H.* aff. *guttatus*+*H. geijskesi*	subset sympatry	Ecological adaptation to estuarine environment and Amazon river outflow
2.12	**Clade 7** *H. guttatus*+*H.* aff. *guttatus*	Vicariance	Sea-level drop and low-salinity and high-sediment environment at the Amazon river outflow

9.37	**Clade 8** *H. marianae*+*H. longus*+*H. rudis*+*H.* aff. *rudis*+*H.* aff. *americanus*+*H. americanus*	dispersal and founder event	Ecological adaptation to reef systems
5.29	**Clade 9** *H. longus*+*H. rudis*+*H.* aff. *rudis*+*H.* aff. *americanus*+*H. americanus*	Founder event	Reaching Gulf of Mexico and Temperate Northwestern Atlantic
4.18	**Clade 10** *H. longus*+*H. rudis*+*H.* aff. *rudis*	Vicariance	Closure of the Isthmus of Panama
1.28	**Clade 11** *H. rudis*+*H.* aff. *rudis*	dispersal and founder event	Dispersal through Mid-Atlantic barrier, and ocean circulation modifications due to final closure of Isthmus of Panama
1.36	**Clade 12** *H.* aff. *americanus*+*H. americanus*	wide sympatry	Sea-level drop, interruption of water connection between East and West Florida, and possible ongoing ecological adaptations

CAPÍTULO II. Descrição de uma nova espécie de raia marinha do gênero *Hypanus* Rafinesque, 1818 (Dasyatidae, Myliobatiformes) do Atlântico Sudoeste Tropical





Description of a new marine stingray species of the genus *Hypanus* Rafinesque, 1818 (Dasyatidae, Myliobatiformes) from the Tropical Southwestern Atlantic

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ABSTRACT

The recently resurrected genus of marine stingrays *Hypanus* Rafinesque, 1818 was shown to have many cryptic lineages, especially in the Western Atlantic where a species with a wide distribution from the Northeastern coast of USA to Southeastern Brazil proved to be paraphyletic. In an integrative approach combining genetics, morphology, and ecological niche modeling we delimited a new species of *Hypanus* occurring from the Amazon river mouth to São Paulo State coast in Brazil, including the northeastern oceanic islands. Based on the mitochondrial marker NADH dehydrogenase subunit 2 (ND2), the genetic distance between the new species *Hypanus* **sp. 1** and the other three valid closely related species (*H. americanus, H. longus,* and *H. rudis*) varied from 0.669% to 3.463%. Besides, F_{ST} and Bayesian Analysis of Population Similarity also support the separation of *Hypanus* **sp. 1** (Brazil) from the sister species *H. rudis* (Africa). Morphological and morphometric analyses also corroborated four morphotypes within the *H. americanus* species group and some relevant measurements to distinguish them are ventral fold height and length, interspiracle and interorbital lengths. Claspers of adult males also exhibit morphological dissimilarities, in which the distance from apopyle to clasper tip in relation to pelvic fin length has an intermediate proportion in *Hypanus*

sp. 1 (0.88), with *H. americanus* having the largest (0.98) and *H. longus* the smallest (0.81). The ecological niche modeling agreed with molecular and morphological analysis and delimits the distribution of *Hypanus* **sp. 1** to shallow areas close to the shore along the Brazilian coast, suggesting that the Amazon river and the Mid-Atlantic ridge might act as barriers. The integration of these data to describe a new species provides enough information for a conservation status, since all species of the *H. americanus* species group are under the 'data deficient' status.

INTRODUCTION

The genus *Hypanus* Rafinesque, 1818 of marine stingrays from Eastern and Western coasts of America and Western Africa was recently genetically differentiated from other Dasyatidae genera (Last *et al.* 2016; Chapter I) Within *Hypanus*, there are ten valid species, of which seven occur in Western Atlantic Ocean.

The species with the widest distribution, *H. americanus* (Hildebrand & Schroeder, 1928) from New Jersey (USA) to São Paulo (Brazil), was demonstrated to be non-monophyletic (Chapter I). Closely related species to it are *H. longus* (Garman 1880) from Baja California to Colombia and Galapagos, and *H. rudis* (Gunther 1870) found only in the Gulf of Guinea, at the Atlantic African coast, forming the *H. americanus* species group.

Bigelow and Schroeder (1953) did a very thorough description of these stingrays from North Atlantic: *Hypanus americanus, H. geijskesi* (Boeseman 1948), *H. guttatus* (Bloch and Schneider 1801), *H. sabinus* (Lesueur 1824), and *H. say* (Lesueur 1817). However, other *Hypanus* species were described after this revision: *H. marianae* (Gomes et al. 2000) and *H. colarensis* (Santos et al. 2004) from Northern and Northeastern coasts of Brazil, *H. longus* and *H. dipterurus* (Jordan and Gilbert 1880) from the Pacific coast of America, and *H. rudis* from Western Africa, which were not included in Bigelow and Schroeder (1953)'s revision. Besides, since the genus *Hypanus* was only recently resurrected (Last et al. 2016), these species were all considered as belonging to the genus *Dasyatis* (Rafinesque 1818), as well as other species currently allocated in other genera but occurring in Western South Atlantic: *Bathytoshia centroura* (Mitchill 1815), *Pteroplatytrygon violacea* (Bonaparte 1832), *Dasyatis hypostigma* Santos & Carvalho, 2004. Currently, *Hypanus*' species are grouped by morphological similarities and lack synapomorphic characteristics (Last et al. 2016). So, there is a need of morphological identification and differentiation of lineages comprised within the genus combined with their genetic information.

A mitochondrial genomes' analysis indicated a distinct genetic lineage of stingrays occurring in Tropical Southwestern Atlantic, at the Brazilian coast, traditionally identified as *H. americanus* (Menni and Stehmann 2000; Freitas et al. 2019), but more closely related to *H. rudis* in Western Africa than to *H. americanus* itself (Chapter I). The Pacific species *H. longus* is also genetically nested within this clade. A recent molecular identification of rays at the Brazilian Amazon coast has also identified two lineages in the clade that used to be known as *H. americanus* (Rodrigues Filho et al. 2020). Hence the urge to better define these species for fisheries management and conservation efforts (Dulvy et al. 2017; Almeida Marques et al. 2019; Lima et al. 2019).

Therefore, the combination of data from distinct sources such as genetics, morphology, and ecology is an outstanding methodology to delimit species as an evolutionary unit; thus, a specific character is not needed, but an association of many taxonomic features (Padial et al. 2010). To accomplish this, we aim to aggregate to the elasmobranch barcode gene, NADH dehydrogenase subunit 2 (ND2) (Naylor et al. 2012), the morphological and morphometric data, and ecological niche modeling (ENM) to better define the species. The ENM can also be used either as *a priori* analysis to raise hypotheses to be tested with genetic and morphological data, as well as to identify environmental variables that could allow or restrict a species distribution in certain areas (Alvarado-Serrano and Knowles 2014), such as the Amazon river discharge as a barrier for *H. americanus* sensu lato suggested in Chapter I. The requirement of accurate species' delimitation lies in the fact that all lineages within this clade are under 'data deficient' status at the Red List of IUCN (International Union for Conservation of Nature and Natural Resources) (Grubbs et al. 2016; Séret et al. 2016; Smith 2016) and for a better evaluation of their threatening status we need to solve some taxonomic uncertainties.

MATERIAL AND METHODS

Ecological Niche Modelling

We used 241 location records of the currently known species *Hypanus americanus* sensu lato and, of those selected 38 of the South American clade deposited in scientific collections from the online databases *species*Link (CRIA 2019), FishNet2 (FishNet2 2020), and GBIF (GBIF.org 2020), as well as locations of examined specimens for the morphological and molecular analyses.

Environmental variables with predictive potential for species' distribution were obtained from the database MARSPEC (Sbrocco and Barber 2013), that comprises a set of oceanic information developed for marine spatial ecology, and Bio-ORACLE (Assis et al.

2018), that provides data variety in a spatial resolution of 5 arcmin (9.2 km) in ESRI ascii format. We made a correlation analysis among 41 variables to observe information superposition that interferes in model development using the Variance Inflation Factor (VIF) and ended up using distance to shore, bathymetry, surface silicate, benthic salinity, benthic current velocity, East-West aspect, surface nitrate, North-South aspect, profile curvature, surface pH, surface calcite, and surface cloud cover.

The ecological niche modeling and spatial distribution (ENM) of both *Hypanus americanus* sensu lato and of *Hypanus* **sp. 1** were developed using the Maximum Entropy Modelling methodology (MaxEnt) (Phillips et al., 2006) implemented in R software (R Core Team 2018). We used the R package ENMeval (Muscarella et al. 2014) to select the block partition method for training and testing, choose the best feature class and regularization multiplier, and used evaluation metrics to select the best performance for modeling that dataset.

Molecular

We sampled 45 specimens of the clade *Hypanus americanus* species group, being eight of *H. americanus* from Massachusetts (USA) to Nicaragua, four of *H. longus* from Baja California Sur (Mexico), four of *H. rudis* from Senegal and Ghana, and 29 of *Hypanus* **sp. 1** from Pará to São Paulo (Brazil).

Genomic DNA was extracted from muscular tissue samples using the E.Z.N.A Tissue DNA Kit (Omega Bio-Tek). The mitochondrial gene NADH dehydrogenase subunit 2 (ND2) was amplified through the Polymerase Chain Reaction using a pair of primers proposed by Naylor, Ryburn, Fedrigo, & Lopez (2005), Ilem-Mustelus and Asn-Mustelus. The PCR reactions were done in 25 μl volume of 0.3 μM primers, 2.5 mM MgCl2, 200 μM each dNTP, 10X Ex Taq buffer (20 mM Tris- HCl pH 8.0, 100 mM KCl, 0.1mM EDTA, 1mM DTT, 0.5% Tween20, 05% Nonidet P-40, 50% Glycerol), 0.25 UTaKaRa Ex Taq (Takara, Mountain View, CA), and 50–100 ng template DNA. The reaction was denatured at 94°C for 3 minutes, then 35 cycles of denaturation at 94° C for 30s, annealing at 48° C for 30s and extension at 72° C for 90s. PCR products were bi-directionally Sanger sequenced at DNA Sequencing Facility (San Diego, USA) and Macrogen Inc. (Seoul, Korea). DNA sequences were edited using Geneious® v. 6.1.8 (Biomatters Ltd Auckland, New Zealand. Available at http://www.geneious.com).

All 45 sequences were aligned using the MUSCLE algorithm implemented in Geneious® v. 6.1.8. The alignment is a partial sequence of the gene ND2, since the final portion of tRNA-Gln and tRNA-Met before the protein-coding gene ND2 were also included, as well as tRNA-Trp and tRNA-Ala after the gene. The inclusion of tRNAs in the alignment was due to the poor resolution of a region at the end of ND2 sequences, forcing the removal of, approximately, 80 bp. Therefore, we decided to also use the already sequenced tRNAs, which are well-aligned and can bring useful relationship information. The full alignment has 1122 nucleotides. Using the software MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms (Kumar et al. 2018), the chosen molecular evolution model was HKY + G.

The alignment was analyzed in the software DNAsp (Rozas et al. 2017) for haplotypes and nucleotides' diversities. Then, the dataset was analyzed by a molecular variance analysis (AMOVA), using the software Arlequin (Excoffier and Lischer 2010), to test the lineages' grouping hypotheses provided in Chapter I, in which the *H. americanus* clade is formed by five lineages: two sympatric in North and Central America, one in the Pacific, one in South America and one in Eastern Atlantic. We used the software BAPS 6.0 (Bayesian Analysis of Population Structure) (Corander and Marttinen 2006) to determine the number of genetic clusters and test admixture levels by informing the geographic locations of each sample. Haplotypes and location information were combined in the software PopArt (Leigh and Bryant 2015) using the algorithm TSC (Clement et al. 2002) to build haplotype networks.

Morphology

After a broad *Hypanus* molecular phylogenetics analyses, 79 specimens of the clade *Hypanus americanus* species group were examined: 42 of *H. americanus*, 15 of *H. longus*, five of *H. rudis*, and 17 of *H.* **sp. 1**.) from the collections: Laboratório de Ictiologia de Ribeirão Preto, Universidade de São Paulo (LIRP), Museum of Comparative Zoology, Harvard University (MCZ), Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ), Museu de Zoologia da Universidade de São Paulo (MZUSP), Biodiversity Research and Teaching Collections, Texas A&M University (TCWC), Universidade do Estado do Rio de Janeiro (UERJ), University of Florida, Florida Museum of Natural History (UF), National Museum of Natural History, Smithsonian Institution (USNM) (collections' codes follow Sabaj, 2016). Holotype of *H. americanus* and syntypes of *H. longus* were analyzed; while the holotype of *H. rudis* is lost (Seret 1990). A list of the examined comparative material is in
COMPARATIVE MATERIAL
encompassing representatives from an extensive area of distribution and distinct ontogenetic stages of each species whenever specimens were available in collections. Measurements are

presented in Figure 10 except tail origin - spine origin length (TSL) and caudal height at P2 end (CH), which were measured in lateral view.

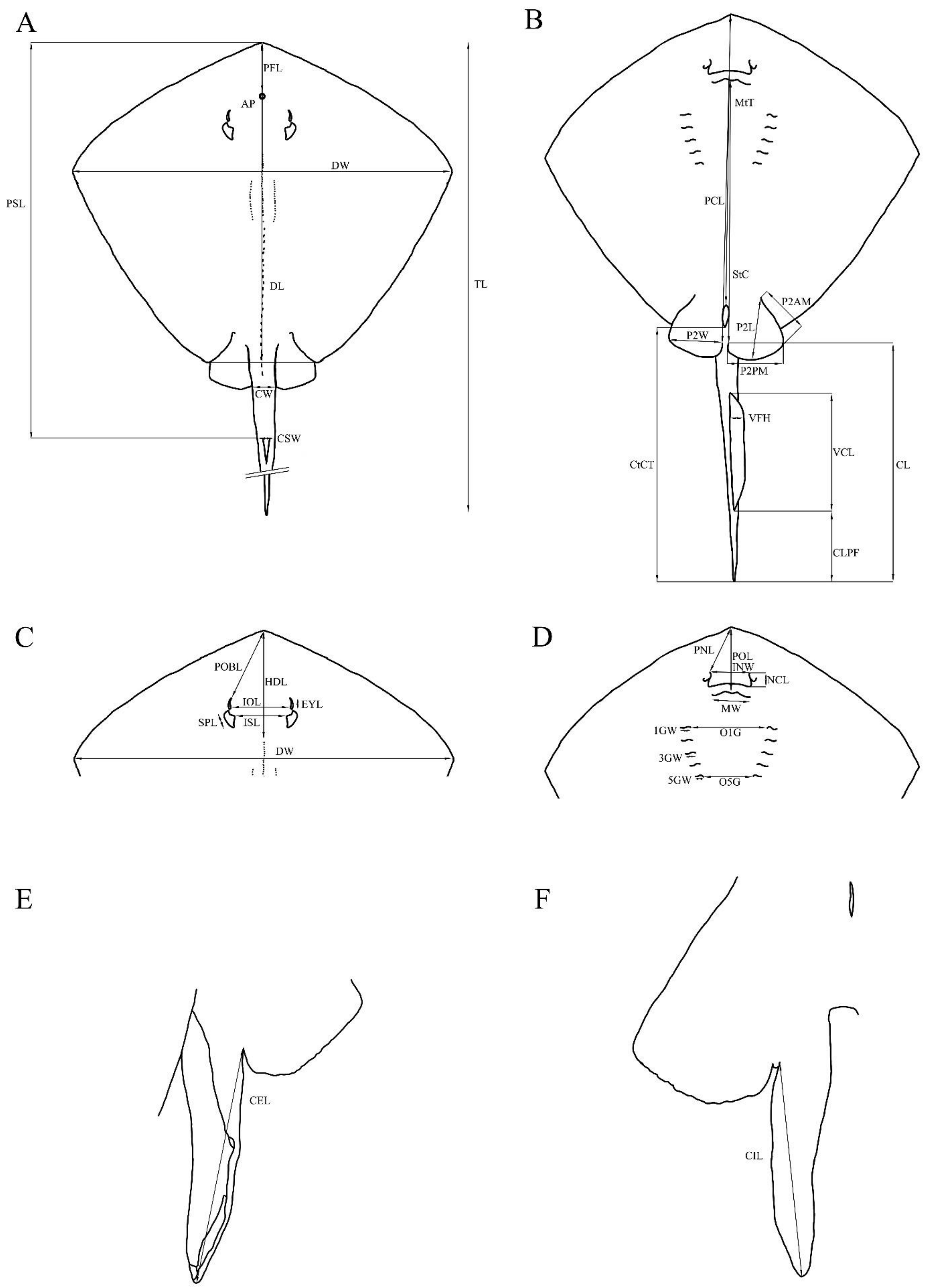

Figure 10. Measurements of *Hypanus* specimens. A, C, E dorsal views; B, D, F ventral views. Whole body (A and B), head (C and D), and male clasper (E and F). Total length (TL), Disc width (DW), Disc length (DL), Anterior projection (AP), Mouth to tail (MtT), Pre-caudal

length (PCL), Snout to cloaca length (StC), Cloaca to caudal tip length (CtCT), Pre-spine length (PSL), Caudal length (CL), Caudal width at spine (CSW), Tail origin - spine origin length (TSL), Caudal width at P2 end (CW), Caudal height at pelvic fin end (CH), Ventral caudal fold length (VFL), Ventral caudal fold height (VFH), Caudal length post ventral fold (CLPF), pelvic fin width (P2W), pelvic fin length (P2L), pelvic fin anterior margin (P2AM), pelvic fin posterior margin (P2PM), Clasper internal length (CIL), Clasper external length (CEL), Head length (HDL), Pre-orbital length (POBL), Pre-oral length (POL), Pre-nasal length (PNL), Internasal width (INW), Nasal curtain length (NCL), Nasal curtain height (NCW), Mouth width (MW), Eye length (EYL), Interorbital length (IOL), Interspiracle length (ISL), Spiracle length (SPL), Over 1st gill slits (O1G), Over 5th gill slits (O5G), 1st gill slit width (1GW), 3rd gill slit width (3GW), 5th gill slit width (5GW), Pre-fontanelle length (PFL).

Specimens were measured following Last, White, & Pogonoski (2008) and Manjaji (2004). External morphology descriptions followed Last & Stevens (2009) and Manjaji (2004); clasper descriptions in accordance with de Moreira *et al.* (2018). Morphometric data were standardized to reduce the allometric influence on data using the equation $Ms = M0\ (Ls/L0)^b$ (Konan et al. 2010), in which Ms is the specimen's standardized measure, M0 the original measure, Ls the arithmetic average of the disc width of all measured specimens, and L0 the disc width of the examined specimen. Allometric coefficient (b) is calculated by linear regression of M0 log *vs.* L0 log.

On a corrected-allometry dataset, a variance inflation factor (VIF) (Zuur et al. 2009) analysis was done to remove autocorrelated variables with a value higher than 5. Afterward, we detected outliers' presence and those specimens were eliminated from the dataset. Before starting statistical analyses, the data were tested for parametricity (homogeneity and homoscedasticity) using the function "mvn" of the R package *MVN* (Korkmaz et al. 2014). A principal component analysis (PCA) of the final dataset, with uncorrelated measurements and absence of outliers, was done to parameterizes the mean variation of samples (Schlager 2017); and to designate specimens to groups based on their characteristics. And a canonical variate analysis (CVA), that maximizes the distances between groups (Schlager 2017) and is a discriminant analysis for multiple groups (Mitteroecker and Bookstein 2011) was also performed. These analyses contributed to observe data distribution and ordination.

Finally, a Multivariate Analysis of Variance (MANOVA for parametric dataset, or PERMANOVA for non-parametric) using the R package *vegan* (Oksanen et al. 2019) was applied to test differences in the mean of compared species. For significant results, we did univariate tests to identify the most relevant measurements separating the groups. All analyses were developed using the R software (R Core Team 2018).

RESULTS

Ecological Niche Modeling of *Hypanus americanus* sensu lato

Using all location records of the currently known species *Hypanus americanus* to model a map of habitat suitability, there is a break near the Amazon river outflow (Figure 11). This ENM was used as *a priori* test to raise hypotheses to be tested by the integration of morphological and molecular data, as suggested by Alvarado-Serrano & Knowles (2014). The most relevant environmental variables for its occurrence are the closeness to coast (43.2% of permutation importance), surface phosphate values close to zero (19.5%), and benthic temperatures close to 25°C (8.6%). The Amazon river discharge in the Atlantic Ocean provides a salinity decrease to, approximately, 20 PSU (Gouveia et al. 2019), which is a value we realized may not be tolerated by these stingrays; therefore, the distribution discontinuity. Based on this, it is reasonable to assume both allopatric lineages north and south of this barrier, since the habitat is not adequate for them. As a consequence of the lack of gene flow, they could be undergoing independent evolutionary paths. We can use this model to test the hypothesis that *Hypanus americanus* is not a natural group.

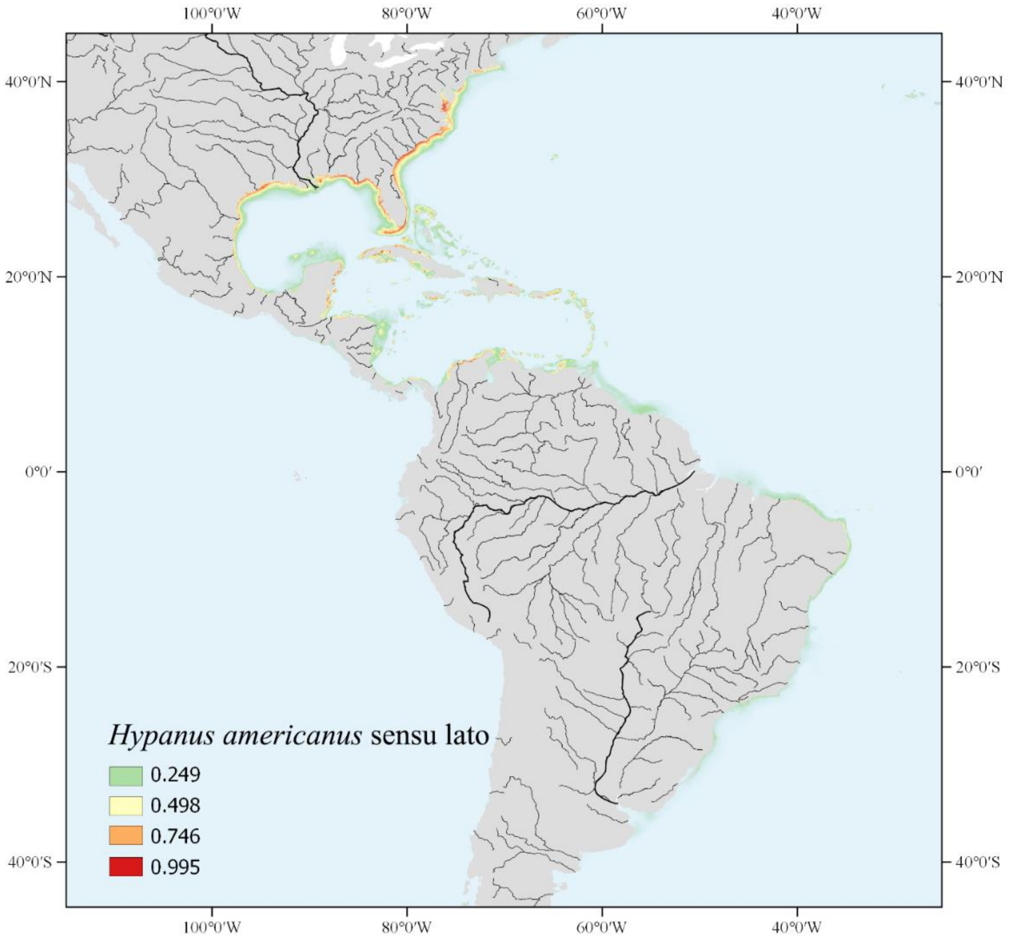

Figure 11. Ecological niche modeling of *Hypanus americanus* sensu lato with a lack of habitat suitability at the Amazon river discharge. Legend colors indicate habitat suitability for clade's occurrence.

Integrative taxonomy

Hypanus americanus species group

The haplotype network (Figure 12) of ND2 revealed the presence of five lineages within the clade *Hypanus americanus* species group: *H. americanus* + *H.* aff. *americanus* + *H. longus* + *H. rudis* + *Hypanus* **sp. 1**, a lineage called *Hypanus* aff. *rudis* in Chapter I. This grouping hypothesis was tested by an AMOVA and resulted in an explanation of 88.17% of variation ($p < 0.05$). Genetic distances based on ND2 between and within the lineages are in Table 7.

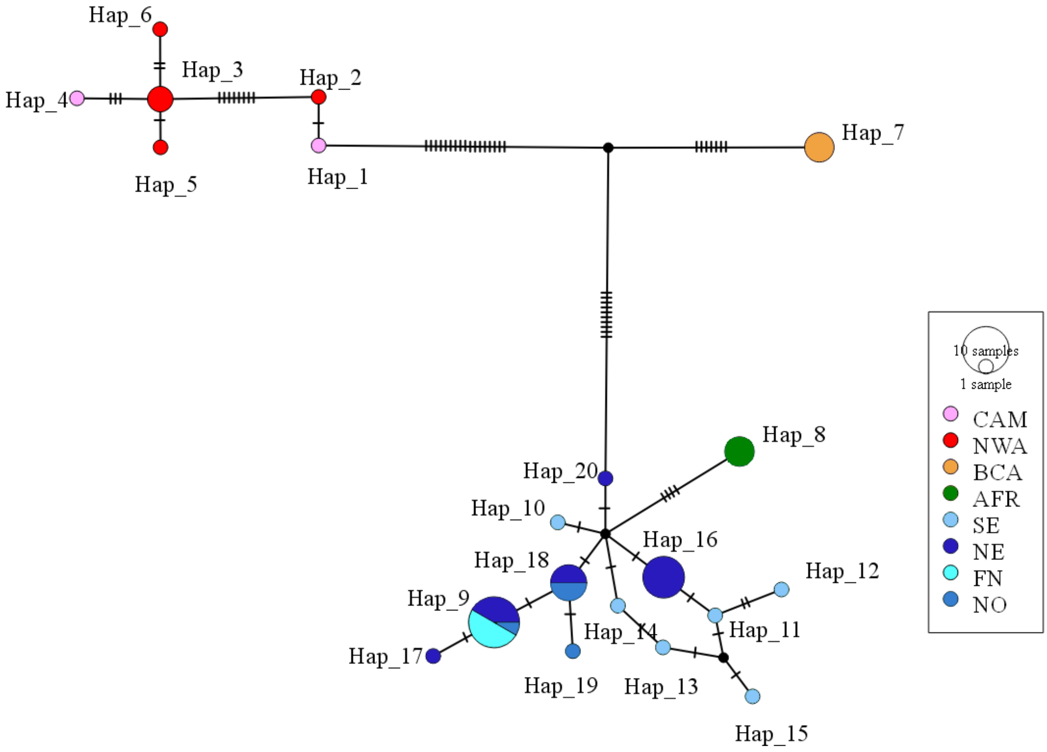

Figure 12. Haplotype network of specimens belonging to the lineages *H. americanus* from Northwestern Atlantic (NWA) and Central America (CAM) in red and pink (Haplotypes 3 to 6); *H.* aff. *americanus* also from Northwestern Atlantic (NWA) and Central America (CAM) in red and pink (Haplotypes 1 and 2); *H. longus* from Baja California in the Pacific coast (BCA) in orange (Haplotype 7); *H. rudis* from Africa (AFR) in green (Haplotype 8); and *Hypanus* **sp. 1** from Northern, Northeastern, Fernando de Noronha, and Southeastern Brazil (NO, NE, FN, SE) in shades of blue (Haplotypes 9 to 20). Black circles represent non-sampled haplotypes, and traces mutational steps.

Table 7. Genetic distances distance between *Hypanus americanus* species group lineages below diagonal; intraspecific lineages within each lineage in bold diagonal. In %.

	H. americanus	*H.* aff. *americanus*	*H. longus*	*H. rudis*	*Hypanus* **sp. 1**
H. americanus	**0.239**				
H. aff. *americanus*	1.135	**0.119**			
H. longus	3.106	2.927	**0.000**		
H. rudis	3.465	3.763	2.628	**0.000**	
Hypanus **sp. 1**	3.171	3.463	2.335	0.669	**0.253**

It is noticeable the smallest interspecific genetic distance between *H. rudis* and *Hypanus* **sp. 1** is still twice as large as the intraspecific distance within representatives of *Hypanus* **sp. 1**. Furthermore, as shown in Chapter I, lineages' delimitation analyses using mitochondrial genomes showed these stingrays are indeed two distinct species, which have been separated for 1.28 My.

To morphologically differentiate these species, we analyzed the morphometric measurements of 79 specimens of the clade *H. americanus* species group and got visual ordinations of CVA and PCA in Figure 13. The molecular clade of *H.* aff. *americanus* was not morphologically distinct from *H. americanus* and, therefore, considered as the same species. This dataset was parametric and MANOVA’s p-value was significative (5.558×10^{-10}), indicating there are differences among species’ averages, also indicating it is a species group where *H. americanus* from Central and North America is a different lineage from the South American one, and the South American is distinct from the African. The most important variables for these four species’ distinction are anterior projection (AP), ventral fold length (VFL) and height (VFH), pelvic fin anterior margin (P2AM), head length (HDL), internasal width (INW), interorbital length (IOL), interspiracle length (ISL), and spiracle length (SPL) (Table 8). Both clasper external (CEL) and internal lengths (CIL) by pelvic length are statistically significant ($p = 1.73 \times 10^{-7}$ and $p = 7.96 \times 10^{-11}$, respectively) in differentiating male specimens of this clade.

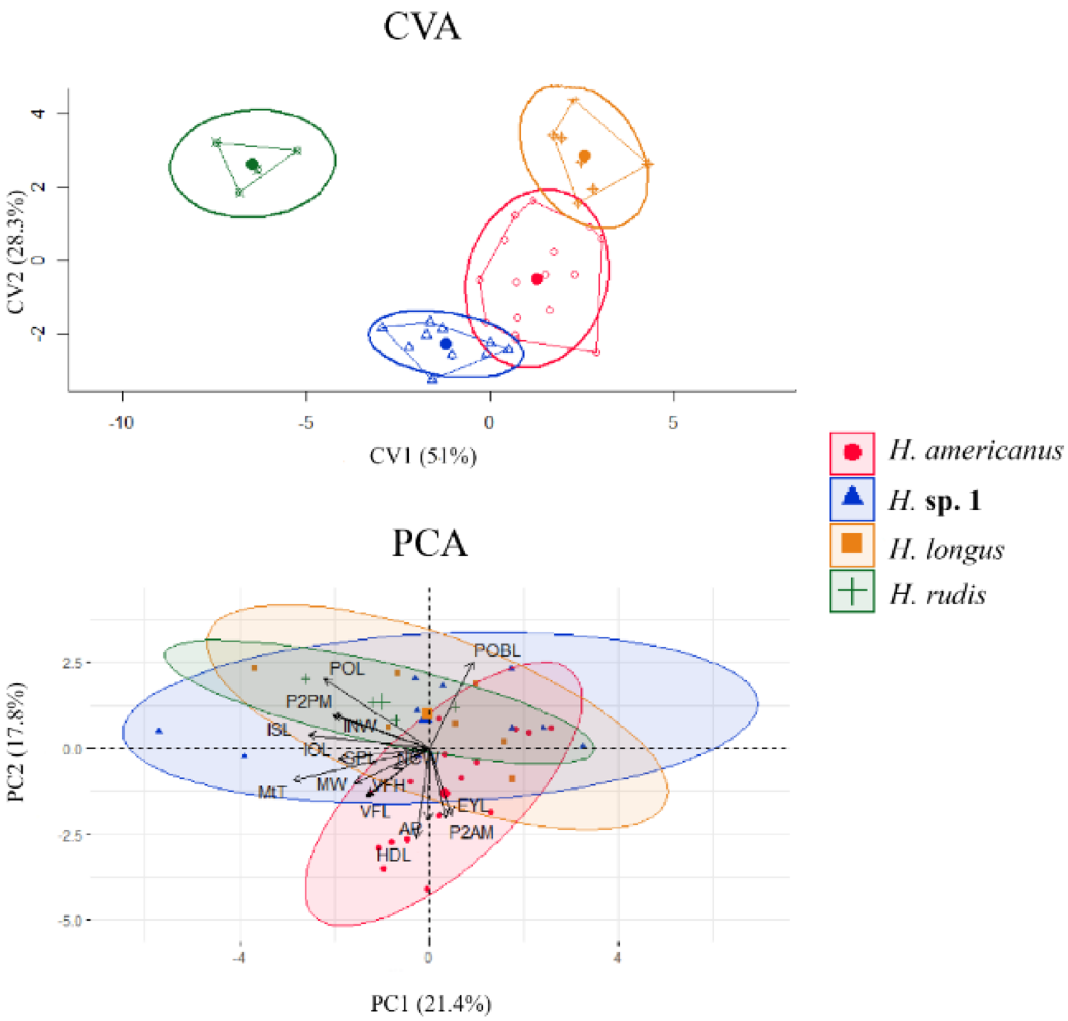

Figure 13. Canonical Variance Analysis (CVA) and Principal Component Analysis (PCA) of *Hypanus americanus* (red circles), *Hypanus* **sp. 1** (blue triangles), *H. longus* (orange squares), and *H. rudis* (green crosses), with visual representations of their morphometric measurements.

Table 8. Most statistically relevant morphometric measurements to distinguish the species *H. americanus*, *H. longus*, *H. rudis*, and *Hypanus* **sp. 1** (AP, VFL, VFH, P2AM, and HDL, in %DW; INW, IOL, ISL, and SPL in %HDL; CEL and CIL in %P2L).

	AP	**VFL**	**VFH**	**P2AM**	**HDL**	**INW**	**IOL**	**ISL**	**SPL**	**CEL**	**CIL**
H. americanus	45.43	54.43	1.82	15.44	33.09	31.17	53.32	49.79	19.76	46.29	37.72
H. longus	39.08	51.67	1.39	14.14	31.14	31.84	60.77	55.43	20.47	83.27	71.22
H. rudis	35.26	48.33	1.56	13.14	30.87	32.01	57.13	51.04	20.09	32.48	22.75
H. **sp. 1.**	35.46	46.47	1.92	14.63	31.73	32.35	52.84	50.26	18.73	63.34	49.57

***Hypanus rudis* x *Hypanus* sp. 1**

The haplotype network (Figure 12) of ND2 revealed the presence of two lineages in the clade *H. rudis* + *Hypanus* **sp. 1** clearly separated by the Atlantic Ocean. All *H. rudis* representatives share the same haplotype, while *Hypanus* **sp. 1** has 12 along the Brazilian coast from North to Southeast and Fernando de Noronha Archipelago. BAPS analysis (Figure 14) of ND2 recovered a similar genetic structure, even though the best clustering was performed for K=3, in which Brazilian Northeastern region has one population shared with Northern and Fernando de Noronha, and another shared with the Southeastern; *H. rudis*' specimens clustered in a third isolated lineage. Despite these analyses having shown a degree of genetic differentiation between Western and Eastern Atlantic lineages, the test of this hypothesis (AMOVA F_{CT}) was not significant. However, pairwise F_{ST} values between the three populations were significant (Table 9).

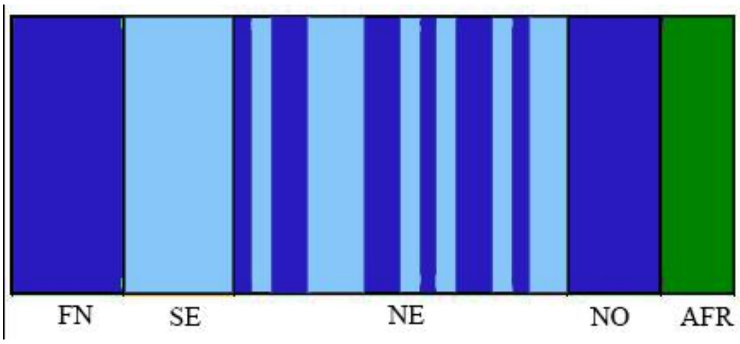

Figure 14. Genetic structure between *H. rudis* (AFR, in red) and *Hypanus* **sp. 1** (FN, SE, NE, NO in blue and yellow). Bayesian Analysis of Population Structure (BAPS) admixture based on ND2 sequences.

Table 9. Pairwise F_{ST} values between three lineages within the clade *H. rudis* (AFR) + *Hypanus* **sp. 1** (NO+NE+FE and SE). *****significative values.

	NO+NE+FN	**SE**	**AFR**
NO+NE+FN	0		
SE	0.41083*****	0	
AFR	0.76181*****	0.71859*****	0

Haplotype diversity of *Hypanus* **sp. 1** is 12, nucleotide diversity 1.33627 (s.d. = 1.20870), Table 10. The AMOVA for the hypothesis of the Southeastern Brazil population as distinct from all the other three was not significative, indicating we should not consider the existence of structured populations within the species *Hypanus* **sp. 1** using the barcode marker. Neutrality tests were also non-significant (except Fu's F for the Southeastern population).

Table 10. Summary statistics based on the ND2 mitochondrial molecular marker for each group of localities of *Hypanus* **sp. 1** N: sample size; π: nucleotide diversity; l: number of haplotypes; D: Tajima's test; Fs: Fu's test; -: no polymorphism. *p < 0.05.

	N	**π**	**l**	**D**	**Fs**
NO	5	0.07273	3	-0.9726	-0.8292
NE	18	0.15865	5	0.63454	0.15547
FN	6	0	1	0	-
SE	6	0.25455	6	0.37522	-3.4533*

For the comparison of these possible distinct species from Brazilian and African coasts, as already suggested by lineages' delimitation in Chapter I and barcode analyses herein, we analyzed only specimens with disc width (DW) up to 400 mm and selected five representatives of *Hypanus* **sp. 1** and four of *H. rudis*, even though performing a previous data allometric correction, since no larger specimen of *H. rudis* is deposited in collections. Morphometric data visualization with CVA and PCA are in Figure 15. Since the dataset was parametric, MANOVA test for differences in groups' average was significant ($p = 3.254 \times 10^{-4}$), suggesting they are statistically distinct.

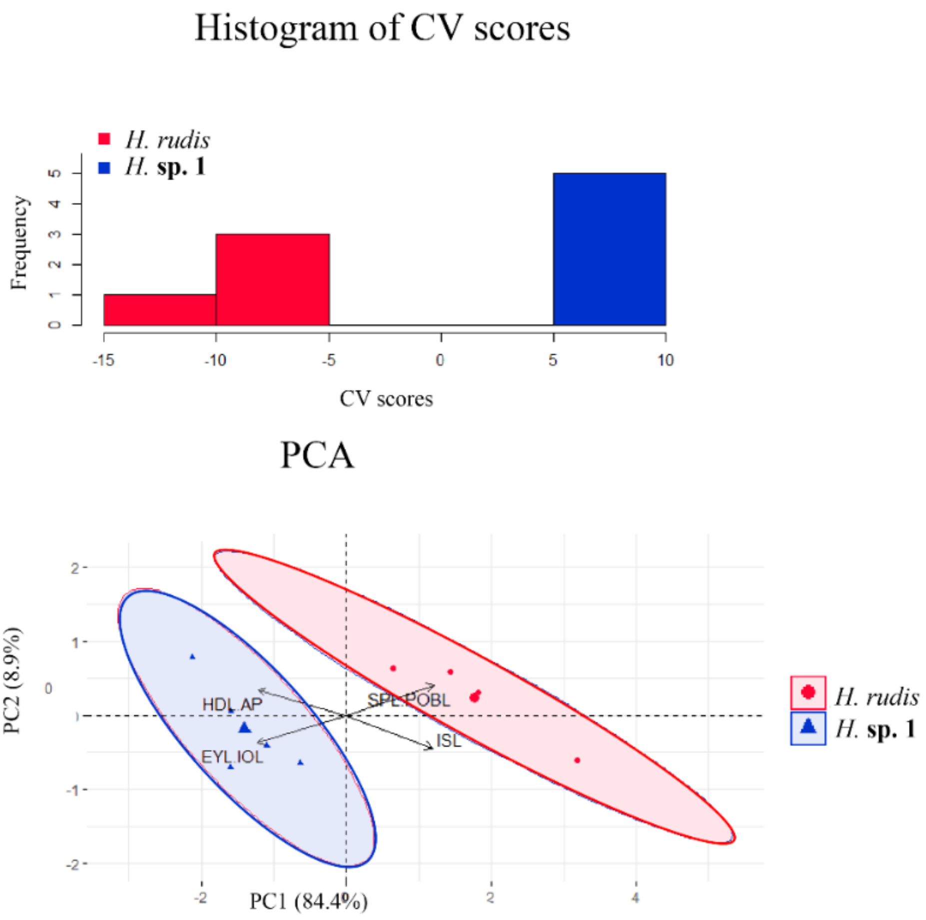

Figure 15. Histogram of Canonical Variates (CV) scores and Principal Component Analysis (PCA) of *Hypanus* **sp. 1** (red circles) and *Hypanus rudis* (blue triangles). Visual representations of their morphometric measurements in groups of species (CV and PCA) and locations (LDA).

We also tested the measurements for statistical significance between both groups and got interorbital length (IOL), spiracle length (SPL), and interspiracle length (ISL) in % of HDL, eye length (EYL) in % of SPL, head length (HDL) in % of anterior projection (AP), eye length (EYL) in % IOL, spiracle length (SPL) in % preorbital length (POBL) (Table 11). The combination of visual and statistical analyses supports the description of a new species for the Brazilian coast, distinct from *H. rudis,* its closest related species (Figure 16).

Table 11. Most statistically relevant ($p < 0.05$) morphometric measurements to distinguish the species *Hypanus* **sp. 1** and *H. rudis* (IOL, SPL, ISL in %HDL; EYL/SPL; HDL/AP; EYL/IOL; SPL/POBL).

	IOL	**SPL**	**ISL**	**EYL/SPL**	**HDL/AP**	**EYL/IOL**	**SPL/POBL**
Hypanus **sp. 1**	52.84	18.73	50.26	65.58	88.6	23.14	28.64
H. rudis	59.02	20.54	51.79	60.33	87.81	21.22	31.77

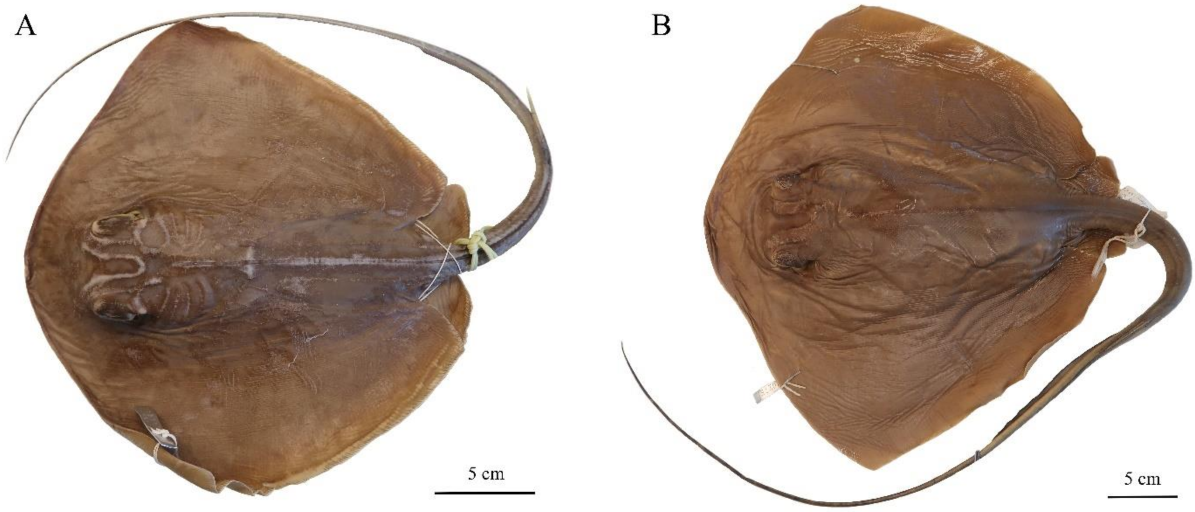

Figure 16. Dorsal view images of juveniles of A. *Hypanus* **sp. 1** (USNM 216825, female, 239 mm DW, from Maranhão coast, Brazil); and B. *H. rudis* (USNM 202758, male, 339 mm DW, from Guinea coast).

Ecological Niche Modeling of *Hypanus* sp. 1

The ENM suggests the most important environmental variables for the occurrence of *Hypanus* **sp. 1** are closeness to shore (80.8% of permutation importance), shallow areas (9.1%), low values of benthic phosphate (5.4%), and low range of sea surface salinity (2.5%) (Figure *17*). Besides analyzing the environmental variables suitable for its occurrence, we could also suggest the species distribution and update its geographic map based on verified occurrence records by morphological, molecular, and ecological tools.

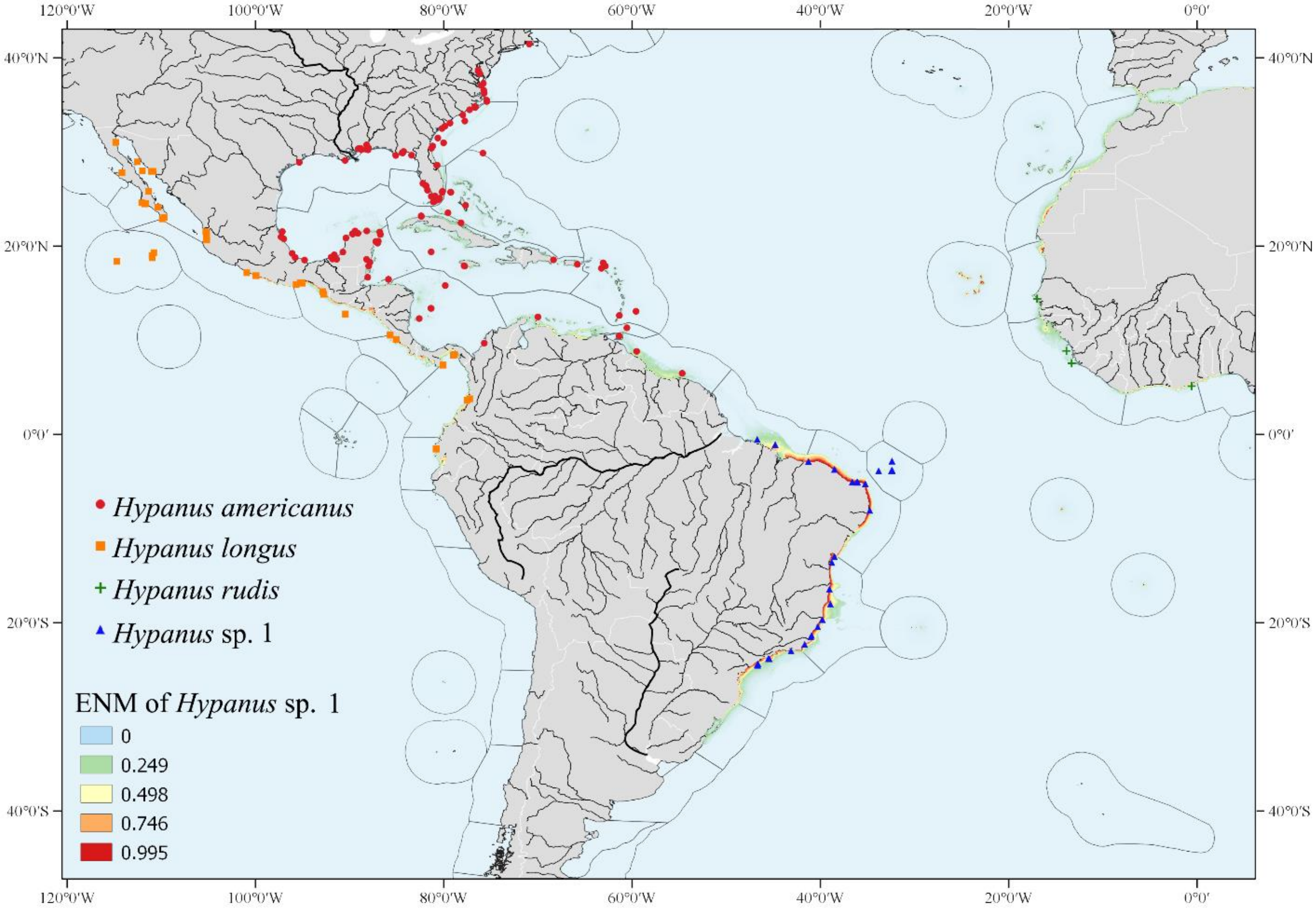

Figure 17. Ecological niche modeling of *Hypanus* **sp. 1**. Legend colors indicate habitat suitability for its occurrence. Marine ecoregions are highlighted in black lines following (Spalding et al. 2007). Location records of *H. americanus, H. longus*, *H. rudis*, and *H.* **sp. 1** in Supp. Mat. 5.

We notice the outflow of the Amazon and La Plata rivers are barriers for the occurrence of this species due to the amount of freshwater and sediments carried by them toward the Ocean. Besides, the preference for habitats closer to shore is demonstrated by its absence in Saint Peter and Saint Paul Archipelago (Vaske et al. 2005), Vitória-Trindade Seamount Chain (Pinheiro et al. 2015), Ascension Island (Wirtz et al. 2017), and Cape Verde Islands (Wirtz et al. 2013).

A literature review showed no more than nine studies of ecological niche modeling have been done in Elasmobranchs, and most of them are concentrated in Gulf of Mexico with very few in Southwestern Atlantic Ocean (Melo-Merino et al. 2020). Hence the relevance of modeling the niche and distribution of the new species *Hypanus* **sp. 1**.

Description of a new *Hypanus* species

Based on the combination of molecular, morphological, and ecological data, besides the previous suggestion of lineages' delimitation based on mitochondrial genomes (Chapter I), we have enough support to describe a new species of the *Hypanus* from the Southwestern Atlantic, at the Brazilian coast. These stingrays used to be known as *H. americanus* but it will be described under a new specific epithet since the holotype of *H. americanus* is from Chesapeake Bay (MD, USA). We suggest a holotype for the new species a specimen from Fernando de Noronha (PE, Brazil).

Order Myliobatiformes Compagno (1973)

Family Dasyatidae Jordan & Gilbert (1879)

Genus *Hypanus* Rafinesque (1818)

***Hypanus* sp. 1** (Figure 18, Table 12)

Holotype

MNRJ XXXX, female, 618 mm DW, Praia do Porto, Fernando de Noronha, Pernambuco, Brazil, 3°50'02.5"S 32°24'05.3"W, Col. Date: 27 Jul 2019, Cols.: Petean, F.F., Lima, S.M.L., Costa, T.A., Di Dario, F., Mendes, L.F., Araújo, T.F.P.

Paratypes

USNM 216825, female, 239 mm DW Off coast of northeastern Brazil. Maranhão, Brazil,1°04'48.0"S 44°48'00.0"W, Col. Date: 17 May 1975, Col.: Collette, B. B.; MNRJ 18910, female, 591 mm DW, Praia de Iracema, Fortaleza, Ceará, Brazil; UERJ 2045, male, 680 mm DW, Praia de Iracema, Fortaleza, Ceará, Brazil; MZUSP 9925, male, Atol das Rocas, Rio Grande do Norte, Brazil, Col. Date: Feb 1972, Col: N.A. Menezes; UERJ 373, female, 536 mm DW, Bahia, 13°36'S 38°47'W, Col. Date: 24 Jul 1999; UERJ 1880, female, 501 mm DW, Ilha Grande, Rio de Janeiro, Brazil; UERJ 2047, male, 364 mm DW, Muriqui, Mangaratiba, Rio de Janeiro, Brazil, Col. Date: 1 Feb 2004, Col.: L.R.G. Rodrigues; UERJ 2048, male, 370 mm DW, Muriqui, Mangaratiba, Rio de Janeiro, Brazil, Col. Date: 18 Mar 2002, Col.: L.R.G. Rodrigues; UERJ 2051, female, 364 mm DW, Muriqui, Mangaratiba, Rio de Janeiro, Brazil, Col. Date: 8 Mar 2004, Col.: L.R.G. Rodrigues; MNRJ 17709, female, 345 mm DW, Col. Date: 21 Aug 1998; LIRP 1554, female, 668 mm DW, Ponta do Jarobá, Canal de São Sebastião, Município de São Sebastião, São Paulo, Brazil, 23,8288°S 45,4233°W, Col. Date: 4 May 1996, Col.: Lev. Canal S. Seb. 1993-96; LIRP 1555, female, 565 mm DW, Praia das Pitangueiras, Canal de São Sebastião, São Paulo, Brazil, 23,8288°S 45,4233°W, Col. Date: 14 Mar 1996, Col.: Lev. Canal S. Seb. 1993-96; LIRP 1556, female, 536 mm DW, Ponta do Jarobá, Canal de São Sebastião, Município de São Sebastião, São Paulo, Brazil, 23,8288°S 45,4233°W, Col. Date: 25 Jul 1995, Col.: Lev. Canal S. Seb. 1993-96; LIRP 1557, female, 515 mm DW, Ponta do Jarobá, Canal de São Sebastião, Município de São Sebastião, São Paulo, Brazil; Additional examined specimens in Comparative Material section.

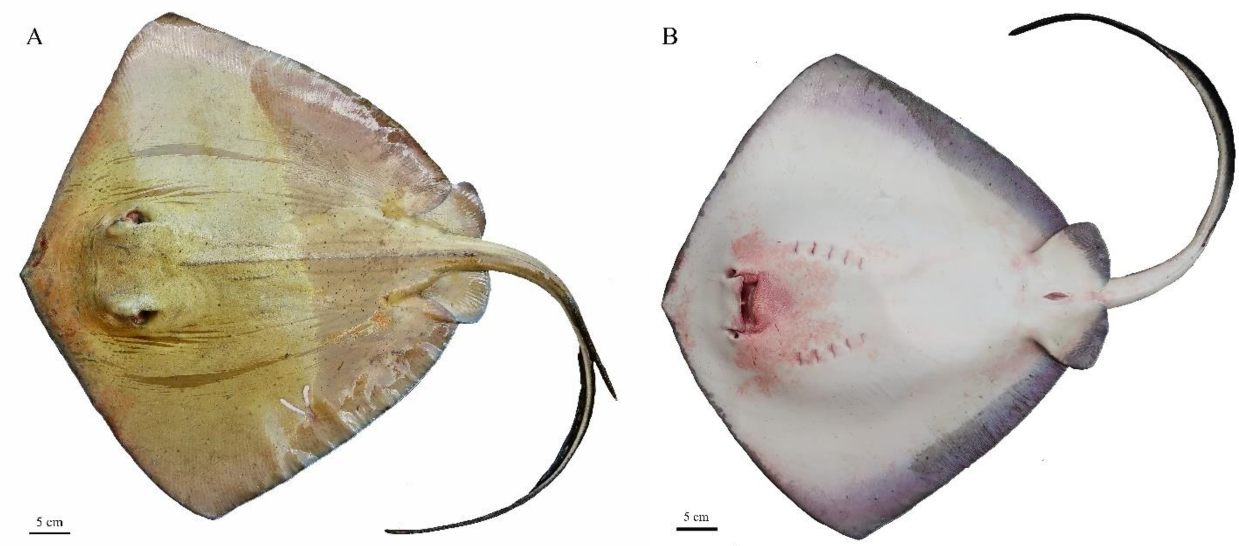

Figure 18. A. Dorsal and B. Ventral views of the holotype MNRJ XXXX, female, 618 mm DW, of *Hypanus* **sp. 1** from Fernando de Noronha, PE, Brazil.

Table 12. Morphometric measurements of specimens of *Hypanus* **sp. 1**. Body measurements in relation to disc width (DW), and head measurements in relation to head length (HDL), n: number of measured specimens for each measurement. MNRJ XXXX: holotype.

	MNRJ XXXX		**n**	**Range**				**Mean**	
	mm	**%**		**mm**		**%**		**%**	**sd**
				min	**max**	**min**	**max**		
TL	1150		13	661	1430			991.0714	227.20
HDL	210		15	87.25	230			158.4581	56.58
DW	618		14	345	680			493	120.27
					%DW				
DL	550.00	89.00	15	231	642	81.62	96.11	86.01	3.66
AP	256.00	41.42	15	125	256	29.10	41.42	35.46	2.59
MtT	394.00	63.75	13	173	481	55.95	73.10	64.18	6.34
PCL	528.00	85.44	15	221	635	60.29	96.46	83.38	9.84
StC	470.00	76.05	14	199	556	69.03	84.60	74.76	5.38
CtCT	653.00	105.66	13	441	877	87.82	151.48	126.91	17.67
PSL	674.00	109.06	13	306	817	105.68	122.31	110.29	4.39
CL	640.00	103.56	13	431	844	84.43	148.22	125.14	17.66
CSW	18.83	3.05	14	7.66	22.25	2.38	3.47	2.87	0.32

TSL	160.00	25.89	13	78.37	225	24.76	33.68	28.25	2.54
CW	35.94	5.82	15	14.28	40.4	4.39	6.15	5.31	0.53
CH	24.13	3.90	15	10.17	28.76	3.20	4.32	3.76	0.41
VFL	278.00	44.98	14	119	380	39.46	56.89	46.47	5.44
VFH	12.13	1.96	14	3.73	14.2	1.37	2.30	1.92	0.27
CLPF	192.00	31.07	13	93	338	15.74	85.21	50.06	15.85
P2W	95.30	15.42	15	38.33	128.84	9.85	20.30	14.99	3.45
P2L	105.47	17.07	15	38.37	106.65	11.65	17.54	15.43	1.49
P2AM	97.71	15.81	15	38.43	100.81	12.22	17.30	14.63	1.27
P2PM	91.47	14.80	15	30.66	116.05	9.24	17.37	13.51	2.70
CIL	-		3	9.95	119.42	2.69	17.56	7.79	8.46
CEL	-		3	14.99	143.7	4.05	21.13	9.96	9.68
HDL	210.00	33.98	15	87.25	230	30.22	49.84	31.73	1.17
					%HDL				
POBL	142.47	67.84	15	54.24	143	46.26	68.89	64.89	3.13
POL	130.20	62.00	15	49.83	157	42.27	75.76	62.07	6.50
PNL	98.15	46.74	15	36.27	117	31.87	57.30	48.42	4.92
INWM	59.13	28.16	15	29.05	72.06	19.20	36.70	32.35	2.68
NCL	64.71	30.81	14	28.32	73.12	21.01	38.42	33.09	2.84
NCW	35.02	16.68	15	14.54	36.71	11.37	20.51	17.08	1.72
MW	49.95	23.79	15	24.35	59.81	16.22	31.23	26.74	2.20
EYL	23.18	11.04	15	13.19	24	7.53	15.12	12.44	1.26
IOL	107.98	51.42	15	47.79	116.21	35.06	61.64	52.84	3.81
ISL	101.90	48.52	15	45.8	115.86	33.08	55.55	50.26	2.51
SPL	34.04	16.21	15	18.07	43	11.05	24.16	18.73	1.84
O1G	148.05	70.50	15	63.45	180.33	48.07	84.61	74.75	5.14
O5G	98.29	46.80	15	43.12	115.71	31.91	55.65	49.12	3.09
1GW	16.93	8.06	15	7.31	22.39	5.50	10.53	8.80	1.02
3GW	18.21	8.67	15	7.8	22.91	5.91	10.65	9.36	0.89
5GW	12.94	6.16	15	5.38	14.46	4.20	7.32	6.09	0.63
PFL	101.00	48.10	13	45.79	111	32.79	55.76	51.07	3.91

Synonymy

Pastinaca hastata (DeKay 1842) (*in partim*): p.373 (original description)

Dasibatis hastata (Garman 1883) (*in partim*): p.70 (brief description)

Dasyatis hastata (Jordan and Evermann 1896) (*in partim*), p.83 (brief description)

Dasybatus hastatus (Garman 1913) (*in partim*): p.391 (brief description)

Dasyatis americana (Hildebrand & Schroeder 1928) (*in partim*): p.64 (original description)

Hypanus americanus (Last et al. 2016) (*in partim*): p.356 (brief description)

Diagnosis

The species *Hypanus* **sp. 1** can be distinguished from non-*Hypanus* Dasyatidae species that occur in Western Atlantic by the presence of dorsal caudal ridge (*vs.* absence in *Bathytoshia* *centroura*, *vs.* dorsal caudal fold in *Dasyatis hypostigma*); absence of a notch w-shaped in central ventral disc (*vs.* presence in *D. hypostigma*); anterior portion of disc with angle varying from 125 ˚ to 135˚ and clear ventral disc (*vs.* 150˚ and dark ventral disc in *Pteroplatytrygon violacea*). From most *Hypanus* species, except those from *H. americanus* species group, *Hypanus* **sp. 1** can be discriminated by the diamond-shaped body with short snout (*vs.* long snout in *H. guttatus, H. colarensis, H. sabinus vs.* very long snout in *H. geijskesi*), presence of ventral caudal fold and dorsal caudal ridge (*vs.* ventral and dorsal caudal folds in *H. marianae, H. dipterurus, H. say, H. sabinus*), white spot between the eyes anterior to precerebral fontanelle (*vs.* absence of this clear spot in *H. marianae, H. geijskesi, H. guttatus, H. colarensis, H. dipterurus, H. say, H. sabinus*). It can be differentiated from species of the *H. americanus* clade (*H. americanus, H. longus* and *H. rudis*) by sparse black spots on dorsal disc and by eleven morphometric measurements (Table 8), the most relevant: spiracle length (18.73% of HDL *vs.* 19.76% in *H. americanus vs.* 20.47% in *H. longus vs.* 20.09% in *H. rudis*), anterior projection (35.46% of DW *vs.* 45.43% in *H. americanus vs.* 39.08% in *H. longus vs.* 35.26% in *H. rudis*), caudal ventral fold height (1.92% of DW *vs.* 1.82% in *H. americanus vs.* 1.39% in *H. longus vs.* 1.56% in *H. rudis*). In male adults, the pelvic fin length (P2L) is smaller than the distance from apopyle to clasper tip (ap-tip), and the measurement P2L/ap-tip is 0.88% in *Hypanus* **sp. 1** (*vs.* 0.98% in *H. americanus*, and 0.81% in *H. longus*).

Description

Angular snout, straight pectoral fins anterior margins, moderately angular lateral; free rear tips angular. Disc wider than longer with disc length 86% of width. Sinuous ventral gill openings, the first 8.8%, third 9.36%, and fifth 6.09% of HDL. Presence of a dorsal caudal ridge and a ventral caudal fold of almost the same length, which begins below spine origin and measures 46.47% (s.d. 5.44%) of DW (Figure 19). When complete, tail can measure up to 146% of DW and can either taper towards a thin or thick caudal at the end depending on its length.

Protruded eyes above head; laterally positioned spiracles with rectangular shape in diagonal direction with upper portion more posterior than lower. Preorbital length 29% larger than interspiracle length; eye length 23% of interorbital length. Lower jaw slightly convex with corners somewhat posterior than medial portion; subtle medial indentation. Upper teeth fairly exposed with closed mouth. Mouth floor with three central papillae and two smaller ones at the corners.

Nasal curtain weakly concave with subtle invagination at the center and rounded corners; do not expand laterally beyond oral groove and the external limit of nostril. Anterior nasal distance almost the same as the free posterior margin of nasal curtain. Fringes at posterior margin of nasal curtain with subtle gap at midline; oval nostril. Adult teeth cusps acute in males and non-cuspidate in females. Teeth larger and in more rows medially, which decrease in size and number laterally.

Pelvic fins almost entirely dorsally exposed by the pectoral; squared shape with rounded corners, which are more laterally expanded in males. Dorsal terminal cartilage of clasper with serrated lateral margin and absence of pseudosiphon; clasper external (CEL) and internal lengths (CIL) 9.96% and 7.79 % of DW, respectively (Figure 20).

A few rostral and stellar interorbital and interspiracle thorns; a scapular thorn (only one) and two scapular rows (from none to 14 thorns on each side); dorsal disc covered by sparse thorns close to midline, and a row from mid-scapular to caudal sting (from none to 24 thorns); caudal fin with minute sparse thorns. Smooth pelvic fins.

Dorsal disc color greenish with small black spots distributed on surface. Lateral and posterior margin of pectoral fins and posterior margin of pelvic with a white stripe dorsally Ventral disc surface whitish, with darker pectoral and pelvic fins' margins. Preserved specimens are greyish or brownish, and the black spots usually disappear. Ventral surface keeps clearer than dorsal. White rounded mark anterior to fontanelle region half head length. Largest disc width measured was 680 mm in a mature male, and 668 mm in a mature female; largest immature male 370 mm. Larger specimens were observed alive in Fernando de Noronha Archipelago, but not measured.

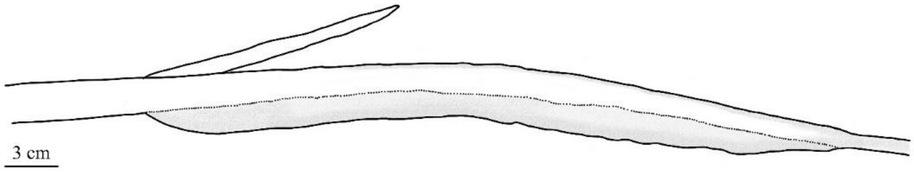

Figure 19. Schematic lateral view drawing of caudal fin of *Hypanus* **sp. 1** showing ventral fold and dorsal ridge.

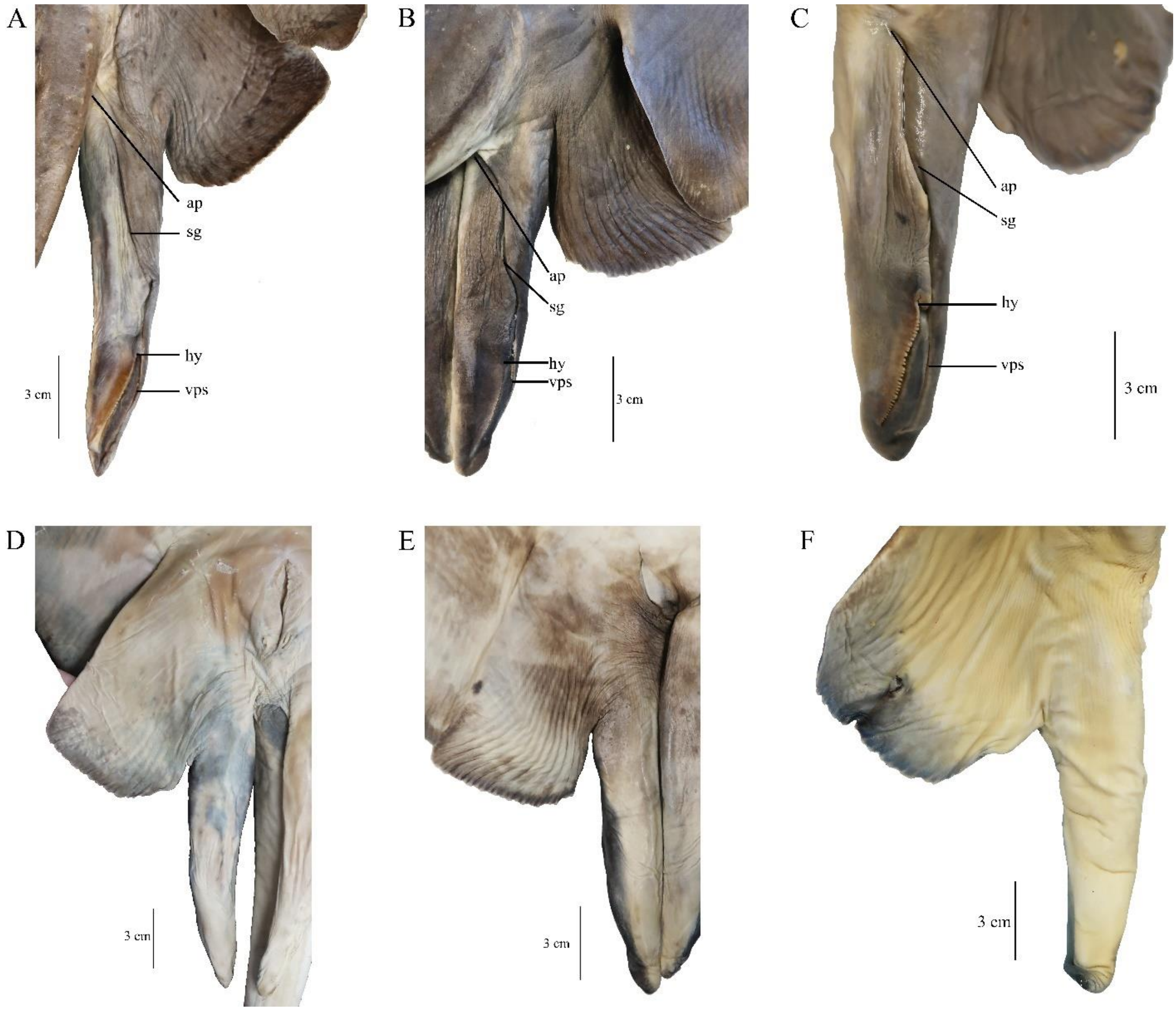

Figure 20. Right clasper images of *Hypanus* **sp. 1** (UERJ 2045 from Ceará, Brazil, 680 mm of DW) in A. dorsal and D. ventral views; *H. americanus* (USNM 395424 from Gulf of Mexico, 602 mm of DW) in B. dorsal and E. ventral views; *H. longus* (TCWC 12102.01 from Gulf of California). ap, apopyle; hy, hypopyle; sg, spermatic groove; vps, ventral pseudosiphon.

Geographic distribution

This species is distributed from the Amazon river mouth, with confirmed occurrence at the Parcel de Manuel Luiz in Maranhão (Rocha and Rosa 2001), to São Paulo state coast in Brazil (Menni and Stehmann 2000), besides the oceanic Archipelago of Fernando de Noronha and Rocas Atoll (Rosa and Moura 1997) at Northeastern Brazil. It ranges from Tropical Atlantic to Temperate South America Realms, at the Provinces of North Brazil Shelf, Tropical Southwestern Atlantic, and Warm Temperate Southwestern Atlantic according to the marine delimitations by Spalding *et al.* (2007) (Figure 17). Despite being known from localities far from the coast up to 250 km (Fernando de Noronha), it is a mostly coastal species occurring usually at the continental shelf in marine environments. This species is limited at its northern and southern distribution by the freshwater and sediments brough by the Amazon and La Plata rivers, respectively, to the Atlantic Ocean. Besides, the cold-water temperature (0.8% of importance) of Cape Frio current (Valentin 2001) and the increase in phosphate concentration, since *Hypanus* **sp. 1** prefers values < 0.5 mmol/m^3, are also variables that negatively affect its southernmost distribution.

Common names

In Brazil (Portuguese language) these stingrays are usually known by the names “Raia de Pedra” (rockray) and “Raia Manteiga” (butterray).

Natural history

Most examined specimens of *Hypanus* **sp. 1** in fish collections and observed living stingrays in Fernando de Noronha, PE, Brazil were female. This unequal sex ratio of 4:1 has already been observed in Fernando de Noronha by Aguiar (2005) and a great disproportion of 52:1 in Rocas Atoll (Agra 2009). A ratio female:male of 3:1 in Isla de Margarita, Venezuela (Tagliafico *et al.* 2013) and in Glovers Reef Atoll, Belize (Tilley and Strindberg 2013) were also reported for *Hypanus americanus*. Since all these observations were on shallow habitats, it is possible female stingrays use these areas for reproduction and/or parturition (Pikitch et al. 2005). It is probably not a reflection of unequal birth sex ratio as neonates born in captivity have the same proportion of males and females (Henningsen and Leaf 2010), suggesting it could be a behavioral grouping of females the reason for such unequal ratios.

Besides, these stingrays are usually seen with an accompanying fauna such as the teleosts *Caranx bartholomei* Cuvier, 1833 (Figure 21) and *Abudefduf saxatillis* (Linnaeus 1758), sharks *Ginglymostoma cirratum* (Bonnaterre 1788) and *Negaprion brevirostris* (Poey 1868), and even the marine turtle *Chelonia mydas* (Linnaeus 1758) (Agra 2009).

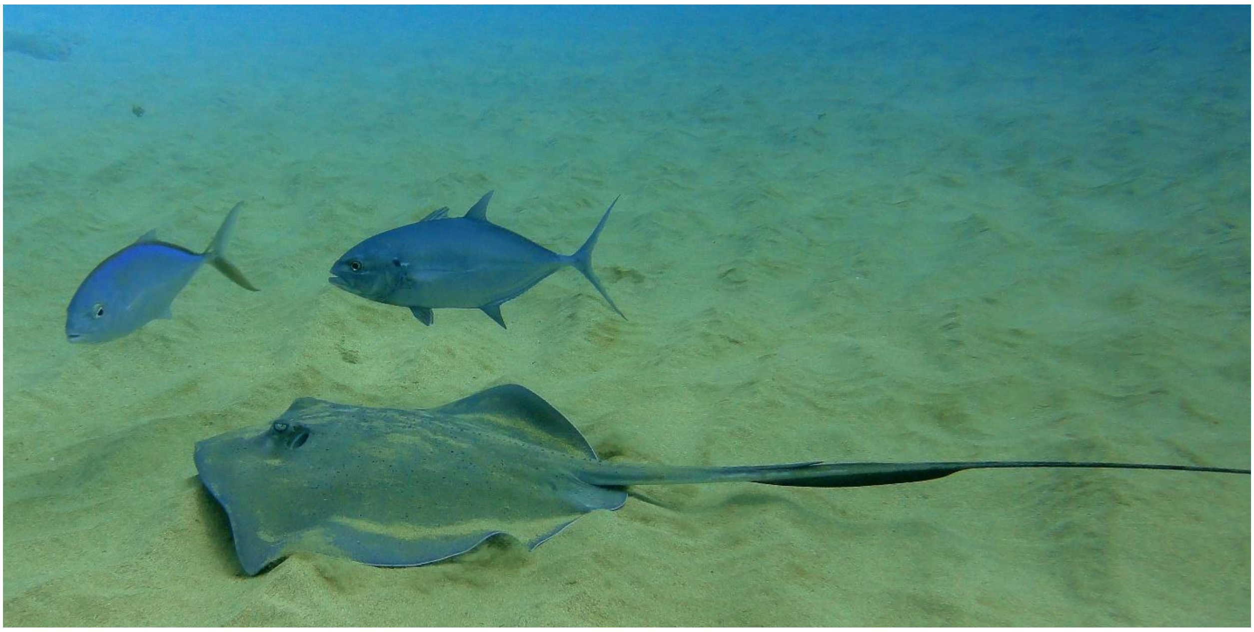

Figure 21. A female specimen of *Hypanus* **sp. 1** and two representatives of *Caranx bartholomei* in Fernando de Noronha, PE, Brazil. Photo by Tiego A. Costa.

Remarks

For DeKay (1842) the genus *Pastinaca* Cuvier did not have any caudal fins, but specimens possessed edentate spines. He described the species *Pastinaca hastata* (DeKay 1842) from Rhode Island (USA), which was synonymized to the currently valid species *Bathytoshia centroura* (Mitchill 1815) by Bigelow & Schroeder (1953), considered invalid by Compagno (2005), and valid by Seret (1990) occurring in Eastern Atlantic, Africa.

Garman (1883) wrote a list of *Dasibatis* species occurring in America and added *Dasibatis hastata* to it, mentioning it was known from Florida (USA) to Rio de Janeiro (Brazil). Later, Hildebrand & Schroeder (1928) described *Hypanus americanus* and suggested *Dasibatis hastata* as a synonym to the newly described species, besides expanding its distribution from Chesapeake Bay (USA) to Brazil. In 1984, Compagno & Roberts suggested that *H. americanus* also occurs in Western Africa, which was referred to as *Dasyatis hastata* by Seret (1990).

The validity of *Dasyatis hastata* in Western Africa is yet to be studied; however, so far, we can assume there are two similar morphotypes of *Hypanus* at the American Atlantic coast: *H. americanus* from New Jersey (USA) to the Amazon river outflow, and *Hypanus* **sp. 1** from the Amazon mouth to São Paulo (Brazil).

DISCUSSION AND CONCLUSIONS

Descriptions of new species provoke a taxonomic instability which might compromise ecological and conservation studies (Padial and De La Riva 2006); however, the more data we acquire, the more integrative research can be made to infer relationships among lineages, as well as describing new ones (Padial et al. 2010). Even though speciation is a process and each character change at a distinct rate (Padial et al. 2010), within the *Hypanus americanus* species group we observed differences in three levels, molecular, morphological and ecological, leading to the description of a new species from Southwestern Atlantic Ocean. This species was previously identified as *H. americanus*, herein restricted to the North Atlantic and Caribbean Sea, however it is more closely related to the African coastal species, *H. rudis*.

In molecular analyses, the Chondrichthyan DNA barcode ND2 (Naylor et al. 2012) and mitochondrial genomes (Chapter I) suggest five lineages in a group of stingrays in which there used to be recognized only three: *H. americanus* in Western Atlantic, *H. longus* in Eastern Pacific, and *H. rudis* in Eastern Atlantic. The hypothesis of five lineages is supported by an AMOVA and explains 88.17% of the variance. The genetic distances of *Hypanus* **sp. 1** and the others vary from 0.67% (to *H. rudis*) to 3.46% (*H.* aff. *americanus*). Even though this distance is usually considered low, since general barcode analyses using COI suggest a threshold of 2.0% for fishes, a recent integrative analysis of the catfish genus *Hypostomus* Lacepède, 1803 showed the interspecific value can vary within the genus and could be decreased to 0.5% (Jardim de Queiroz et al. 2020). Therefore, based on the support of mitochondrial genomes' lineages' delimitation from Chapter I, we can accept the interspecific distance based on ND2 in *Hypanus* to be 0.67%.

We investigated morphologically four of the five lineages identified genetically and got congruent results supporting their distinctiveness. The most relevant characters for identifying *H. americanus*, *H. longus*, *H. rudis*, and *Hypanus* **sp. 1** are the presence of black spots on the dorsal side of live stingrays, and the morphometric measurements: anterior projection, ventral fold length and height, internasal width, interorbital, interspiracle, and spiracle lengths. Besides, the clasper external (CEL) and internal lengths (CIL) of males are also important features to differentiate the newly described species, especially when compared to the pelvic fin length (P2L): 63.34% (CEL/P2L) and 49.57% (CIL/P2L) (Figure 20).

There is a significant difference in clasper morphology between *H. americanus* and *Hypanus* **sp. 1**, as already observed by Santos (2007), and *H. longus*. In terms of size, clasper external length (CEL) is larger in *Hypanus* **sp. 1** than in *H. americanus* (143.7 mm *vs.* 134.28

mm) in specimens of approximately the same disc width (680 mm *vs.* 678 mm). The ventral pseudosiphon (vps) is entirely visible in *Hypanus* **sp. 1** while it is larger in *H. longus*, and only its proximal portion is dorsally observable in *H. americanus* (Figure 20). Besides, in the new species, the distance from hypopyle (hy) to the clasper distal portion is 46.61 mm (*vs.* 42.7 mm in *H. americanus vs.* 67.97 mm in *H. longus* – unknown disc width), showing the clasper glans is proportionately smaller in *Hypanus* **sp. 1** than in *H. americanus* and *H. longus* (34.17% *vs.* 38.41% *vs.* 36.53% of distance from apopyle to clasper tip).

The two most closely related lineages within the *H. americanus* species group are *H. rudis*, from Eastern Atlantic, and *Hypanus* **sp. 1**, from Western Atlantic which were already proven to be distinct by mitochondrial genome analyses (Chapter I). Therefore, we performed specific analyses of this clade to investigate genetic, morphological, and ecological differences between them, besides the allopatric distribution in which they are separated by the Atlantic Ocean. Pairwise F_{ST} between all populations of *Hypanus* **sp. 1** and *H. rudis* suggested a structuration divided by the Atlantic Ocean besides a possible separation of the new species in two sympatric populations: one in Southeastern and Northeastern Brazil and another in Northeastern, Northern and Fernando de Noronha. This result is also supported by a Bayesian Analysis of Population Structure (BAPS) (Corander and Marttinen 2006), which takes into account the geographical distribution of haplotypes as well. These phylogeographic studies regarding the new species should be further investigated using more genetic data, including the relation of specimens of Fernando de Noronha to those from the Northeastern coast of Brazil. These two genetic lineages, on Tropical Southwestern and in Tropical Eastern Atlantic, are congruently morphologically distinct, in which interorbital, interspiracle, and spiracle lengths, and the proportion of eye length by the spiracle length (65.58% in *Hypanus* **sp. 1** and 60.33% *H. rudis*) are relevant measurements. Despite the absence of adult specimens of *H. rudis* in fish collections, only juveniles (DW < 400 mm) of both species were analyzed for a more precise comparison.

The ecological niche modeling of *Hypanus* **sp. 1** suggested that the most suitable habitats for this species are those closer to the coast, low depths and phosphate values, and low ranges of sea surface salinity. Its absence in most oceanic islands and seamounts (Vaske et al. 2005; Wirtz et al. 2013, 2017; Pinheiro et al. 2015), with the exception of Fernando de Noronha, between the Brazilian and African coasts shows it would be unlike for these stingrays to cross the Atlantic and meet specimens of *H. rudis* on Eastern Atlantic. As suggested in Chapter I, these lineages diverged 1.28 Ma and have already been through diversifications of many species concepts by De Queiroz (2007). Given these lines of evidence that suggest distinct geographic distribution, independent molecular evolutionary processes, morphological differences in clasper with probable consequences in reproduction, we have enough support to formally describe a new species.

The former *H. americanus* sensu lato is currently under the status of 'data deficient' in IUCN (Grubbs et al. 2016) and taxonomic studies were a priority research for better delimitation of the evolutionary unit and posterior evaluation of its threatening category. We now provided information that this species has a more restricted distribution than previously known, occurring from New Jersey (USA) to the Amazon river mouth, and could even be two sympatric lineages to be morphologically and ecologically delimited with more studies.

Hypanus **sp. 1** is herein described using the combination of three distinct sources that, together, support its taxonomic status as new species of the genus *Hypanus* which is distributed from the Amazon river mouth to São Paulo (Brazil), as well as Fernando de Noronha, Rocas Atoll (Rosa and Moura 1997), and Parcel de Manuel Luiz (Rocha and Rosa 2001), preferring habitats closer to the coast and shallow environments. These stingrays are fished in Northeast Brazil for eating purposes, but there is a well-preserved group in Fernando de Noronha due to environmental protection in conservation units. An evaluation of the conservation status could be now undertaken for *Hypanus* **sp. 1** with its taxonomic and geographic delimitation.

COMPARATIVE MATERIAL

Hypanus americanus

USNM 88378 (holotype), male, 432 mm DW, Crisfield, Maryland, USA, Col. Date: 15 Sep 1921, Col.: Schroeder, W. C.; USNM 438857, male, 236 mm DW, Maryland, Talbot County, Black Walnut Point, Mouth of Choptank River, Maryland, USA, 38°42'50.4"N 76°21'18.0"W, Col. Date: 29 Aug 2015, Col.: Aguilar, R.; MCZ S-410, male, 444 mm DW, Penikese Island, Massachusetts, USA, 41°27'04.6"N 70°55'15.7"W, Col. Date: 1873; USNM 433348, male, 467 mm DW, East of Cape Hatteras National Seashore, North Carolina, USA, 35°30'24.1"N 75°26'35.9"W, Col. Date: 08 Sep 2006, Col.: National Oceanic and Atmospheric Administration; USNM 433349, female, 426 mm DW, East of Cape Hatteras National Seashore, North Carolina, USA, 35°30'24.1"N 75°26'35.9"W, Col. Date: 08 Sep 2006, Col.: National Oceanic and Atmospheric Administration; USNM 396895, male, 252 mm DW, East of Pamlico Sound, North Carolina, USA, 35°21'54.0"N 75°25'54.1"W, Col. Date: 09 Sep 2006, Col.: National Oceanic and Atmospheric Administration; UF 29988, male, 302 mm DW, SSE of Cape Fear, North Carolina, USA, 33°17'00.0"N 77°46'00.0"W, Col. Date: 11 Dec 1961, Col.: R/V Silver Bay; UF 29966, female, 297 mm DW, East of Sapelo Island, Georgia, USA, 31°29'00.0"N 80°38'00.0"W, Col. Date: 11 Dec 1963, Col.: US Fish and Wildlife Service; USNM 204772, male, 357 mm DW, North Causeway Bridge, New Smyrna Beach, Florida, USA, Col. Date: 06 Jul 1963, Col.: Gcm; USNM 316632, female, 153 mm DW, Florida, 30°37'23.9"N 81°10'48.0"W, Col. Date: 27 Jan 1980, Col.: South Carolina Department of Natural Resources;

TCWC 2793.01, male, Atlantic Ocean, 28°35'24.0"N 80°42'00.0"W, Col. Date: 25 Sep 1977; TCWC 2794.01, male, Atlantic Ocean, 28°35'24.0"N 80°42'00.0"W, Col. Date: 16 Jun 1977; TCWC 13311.01, male, 402 mm DW, Western Atlantic Ocean, 29°52'48.0"N 75°50'24.0"W, Col. Date: 1985, Col.: Gruber, S.; USNM 74427, male, 110 mm DW, West of Loggerhead, Florida, USA, Col.: Gudger, E. W.; UF 29978, male, 328 mm DW, Bear Cut, Rickenbacker Causeway, Biscayne Bay, Florida, USA, 25°43'47.5"N 80°09'29.2"W, Col. Date: 14 Apr 1954; UF 164248, female, 200 mm DW, East Cape, Florida Bay, Everglades National Park, Monroe County, Florida, USA, 25°18'45.0"N 80°56'15.0"W, Col. Date: 26 Jun 1973, Col.: Schmidt, Tom; UF 116124, female, 158 mm DW, Bay side of Long Key, Florida Keys, USA, 26°23'15.0"N 81°52'00.0"W, Col. Date: 02 May 1959; USNM 125797, male, 378 mm DW, Key West, Florida, USA; UF 51383, male, 368 mm DW, Plantation Key, rock piles off ocean beach, ca 4.0 mi SW of Tavernier, Florida Keys, USA, 24°57'00.0"N 80°33'00.0"W, Col. Date: 03 Aug 1972, Col.: Yerger, R; *et al.*; USNM 47376, male, 350 mm DW, Tampa, Florida, USA, Col.: Henshall, J. A.; UF 61160, male, 162 mm DW, Alligator Harbor, Gulf of Mexico, Florida, USA, 29°54'52.1"N 84°25'16.9"W, Col. Date: 28 Jun 1964, Cols.: Datesman, G; Blaney, R.; USNM 395424, male, 602 mm DW, Dauphin Island, Alabama, USA, 30°13'22.1"N 88°02'30.1"W, Col. Date: 14 Aug 2008; TCWC 2184.01, male, 320 mm DW, Gulf of Mexico, Moblie Ship channel, Alabama, USA, 30°27'00.0"N 88°00'36.0"W, Col. Date: 10 May 1960; USNM 127338 Bay Des Ilettes, Louisiana, USA, Col.: Ginsburg, I.; TCWC 4376.01, male, 306 mm DW, Gulf of Mexico, Freeport, Texas, USA, 28°54'36.0"N 95°16'12.0"W, Col. Date: 05 Nov 1979, Col.: Chittenden, M. E.; TCWC 4407.01, male, 350 mm DW, Gulf of Mexico, Freeport, Texas, USA, 28°54'36.0"N 95°16'12.0"W, Col. Date: 22 Oct 1980, Col.: Chittenden, M. E.; TCWC 4382.01, female, 259 mm DW, Gulf of Mexico, Freeport, Texas, USA, 28°54'36.0"N 95°16'12.0"W, Col. Date: 14 Jul 1980, Col.: Chittenden, M. E.; TCWC 4391.01, female, 400 mm DW, Gulf of Mexico, Freeport, Texas, USA, 28°54'36.0"N 95°16'12.0"W, Col. Date: 05 Apr 1981, Col. Chittenden, M. E.; TCWC 3115.03, male, 678 mm DW, Gulf of Mexico, Freeport, Texas, USA, 28°54'36.0"N 95°16'12.0"W, Col.: Rockett, M. *et al*.; MCZ 39605, female, 411 mm DW, Yucatán, Mexico, 21°36'17.2"N 89°25'23.3"W, Col. Date: 1950; USNM 204771, male, 394 mm DW, Guanaja Is., W. of Brick Point, Honduras, 16°27'50.0"N 85°52'19.9"W, Col. Date: 29 Apr 1967; USNM 37700, male, Jamaica, Greater Antilles, Public Museum, Institute of Jamaica; UF 29989, female, 281 mm DW, South of St. Elizabeth, Jamaica, 17°52'00.0"N 77°46'30.0"W, Col. Date: 18 May 1965, Col.: R/V Oregon; UF 29990, male, 282 mm DW, South of Black River, Jamaica, 17°55'00.0"N 77°51'00.0"W, Col. Date: 18 May 1965, Col.: R/V Oregon; MCZ 63252, female, 179 mm DW, between E. point of South Bimini and Bonefish Hole, 1/2 mile offshore, 25°43'03.0"N 79°15'07.4"W, Col. Date: 22 Jan 1986; USNM 52828, female, Hucares, Puerto Rico, Greater Antilles; MCZ 35142, female, 255 mm DW, Cuba; MCZ 40700, male, 160 mm DW, Havana, Cuba, 23°12'29.0"N 82°24'09.8"W; USNM 331981, female, 263 mm DW, Cayo Paredon Grande, Northwest Coast, Cuba, 22°28'12.0"N 78°10'12.0"W, Col. Date: 15 Apr 1994, Cols.: Parenti, L. R.; Collette, B. B.; Arteaga, J. P.; Bustamante, G.; Jewett, S. L.; Claro, R.; MCZ S-811, female, 295 mm DW, Trinidad y Tobago, 10°26'06.6"N 61°22'54.6"W, Col. Date: Jan 1884; UERJ 2077, male, 489 mm DW, Caribbean Sea 08 Jan 2000; USNM 204656 Northeast South America, off Guyana. 8°49'00.1"N 59°31'00.1"W;

Hypanus longus

MCZ 126 (syntype), female, 363 mm DW, Acapulco, Guerrero, Mexico, 16°51'30.0"N 99°56'30.0"W, Col. Date: Dec 1859; USNM 28188, male, 360 mm DW, Mazatlán, Sinaloa, Mexico, Col.: Gilbert, C. H.; TCWC 7562.02

(2 specimens), female (75 mm DW), male (74 mm DW), Puertecitos, Baja California, Gulf of California, Mexico, 28°00'00.0"N 112°00'00.0"W, Col. Date: 26 Jul 1993, Cols.: McEachran, J.D., J.N. Caira, *et al*.; TCWC 7578.01, female, 322 mm DW, Bahia de Los Angeles, Baja California, Gulf of California, Mexico, 28°57'00.0"N 112°33'36.0"W, Col. Date: 02 Ago 1993, Cols: McEachran, J.D., J.N. Caira, *et al*.; TCWC 12102.01, male, Gulf of California, Mexico, 28°00'00.0"N 112°00'00.0"W, Col. Date: 1993, Cols.: McEachran, J.D., J.N. Caira, *et al*.; TCWC 12108.01, male, San Jose del Cabo, Baja California, Gulf of California, Mexico, 31°01'12.0"N 114°49'48.0"W, Col. Date: 20 Sep 1993, Cols.: McEachran, J.D., J.N. Caira, *et al*.; TCWC 12109.01, male, San Jose del Cabo, Baja California, Gulf of California, Mexico, 31°01'12.0"N 114°49'48.0"W, Col. Date: 20 Sep 1993, Cols.: McEachran, J.D., J.N. Caira, *et al*.; TCWC 13653.01, male, Bahia de Los Angeles, Baja California, Gulf of California, Mexico, 28°57'00.0"N 112°33'36.0"W, Col. Date: 1993, Col.: McEachran, J.D.; **MCZ 554 (syntype)**, female, 290 mm DW, Panama Gulf, 7°21'26.0"N 80°04'32.8"W, Col. Date: 24 Jun/7 Jul 1972; USNM 131020, female, Panama City Market, Panama, Col. Date: 10 Feb 1912, Cols.: Meek, S. E.; Hildebrand, S. F.; MCZ 1069, female, 418 mm DW, San Miguel, Panama, 8°27'30.0"N 78°51'30.0"W, Col. Date: 1904/1905; USNM 206990, female, 384 mm DW, Colombia, 3°37'48.0"N 77°31'48.0"W, Col. Date: 30 Jan 1969, Col: FAO; TCWC 13240.01, female, 713 mm DW, off coast of Colombia near Buenaventura, Col. Date: 21 Sep 1969, Col: R/V Cacique.

Hypanus rudis

USNM 202739, female, 330 mm DW, Off Guinea, 8°52'12.0"N 13°52'12.0"W, Col. Date: 24 Nov 1963, Col: Collette, B. B.; USNM 202757, female, 336 mm DW, Off Guinea, 8°52'12.0"N 13°52'12.0"W, Col. Date: 24 Nov 1963, Col: Collette, B. B.; USNM 202758, male, 339 mm DW, Off Guinea, 8°52'12.0"N 13°52'12.0"W, Col. Date: 24 Nov 1963, Col: Collette, B. B.; USNM 202759, female, 346 mm DW, Off Guinea, 8°52'12.0"N 13°52'12.0"W, Col. Date: 24 Nov 1963, Col: Collette, B. B.; USNM 202730 Sierra Leone, 7°33'29.9"N 13°19'00.1"W, Col. Date: 17 Nov 1963, Col: Collette, B. B. *et al*.; USNM 202760, female, 390 mm DW, Sierra Leone, 7°33'29.9"N 13°19'00.1"W, Col. Date: 17 Nov 1963, Col: Collette, B. B. *et al*.

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REFERENCES

Agra G (2009) Organização social de elasmobrânquios na Reserva Biológica Do Atol das Rocas, Brasil. Universidade Federal de Pernambuco

Aguiar AA (2005) Estrutura e densidade populacional e uso de hábitat por *Dasyatis americana* Hildebrandt & Schroeder, 1928 (Chondrichthyes; Dasyatidae) no Arquipélago de Fernando de Noronha, Brasil. Universidade Federal da Paraíba

Almeida Marques R, Guimarães Julio T, Sole-Cava AM, Vianna M (2019) A new strategy proposal to monitor ray fins landings in south-east Brazil. Aquat Conserv Mar Freshw Ecosyst 1–18. https://doi.org/10.1002/aqc.3203

Alvarado-Serrano DF, Knowles LL (2014) Ecological niche models in phylogeographic studies: Applications, advances and precautions. Mol Ecol Resour 14:233–248. https://doi.org/10.1111/1755-0998.12184

Assis J, Tyberghein L, Bosch S, et al (2018) Bio-ORACLE v2.0: Extending marine data layers for bioclimatic modelling. Glob Ecol Biogeogr 27:277–284. https://doi.org/10.1111/geb.12693

Bigelow HB, Schroeder WC (1953) Fishes of the western North Atlantic Part II. Sawfishes, guitarfishes, skates and rays

Blickenstaff JC (2005) Women and science careers: Leaky pipeline or gender filter? Gend Educ 17:369–386. https://doi.org/10.1080/09540250500145072

Bloch ME, Schneider JG (1801) Systema Ichthyologiae Iconibus cx Ilustratum. Post obitum auctoris opus inchoatum absolvit, correxit, interpolavit Jo. Gottlob Schneider, Saxo. Sumtibus Auctoris Impressum et Bibliopolio Sanderiano Commissum, Berolini

Boeseman M (1948) Some preliminary notes on Surinam sting rays, including the description of a new species. Zool Meded 30:31–47

Bonaparte CL (1832) Iconografia delle fauna italica per le quattro classi degli animali vertebrati. Tomo III. Pesci., Fasc. 1, p. Roma

Chapter I: Petean FF, Yang L, Corrigan S, et al (2020) The role of the American continent on the diversification of the stingrays' genus *Hypanus* Rafinesque (1818) (Myliobatiformes: Dasyatidae). In: História evolutiva do gênero de raias marinhas Hypanus Rafinesque, 1818 (Myliobatiformes, Dasyatidae). Ph.D. Thesis. Universidade Federal do Rio Grande do Norte

Clement M, Snell Q, Walke P, et al (2002) TCS: estimating gene genealogies. In: Proc 16th Int Parallel Distrib Process Symp 2. p 184

Compagno L (1973) Interrelationships of living elasmobranchs. Zool J Linn Soc 53:15–61

Compagno LJ V., Roberts TR (1984) Marine and freshwater stingrays (Dasyatidae) of West Africa, with description of a new species. Proceeding Calif Acad Sci 43:283–300

Compagno LJ V (2005) Checklist of Living Chondrichthyes. In: Hamlet W (ed) Reproductive Biology and Phylogeny of Chondrichthyes, 3rd edn. Science Publishers, Plymouth, pp 503–548

Corander J, Marttinen P (2006) Bayesian identification of admixture events using multilocus molecular markers. Mol Ecol 15:2833–2843. https://doi.org/10.1111/j.1365-294X.2006.02994.x

CRIA (2019) specieslink. http://www.splink.org.br

de Moreira RA, Loboda TS, de Carvalho MR (2018) Comparative anatomy of the clasper of the subfamily Potamotrygoninae (Chondrichthyes: Myliobatiformes). J Morphol 279:598–608. https://doi.org/10.1002/jmor.20795

De Queiroz K (2007) Species concepts and species delimitation. Syst Biol 56:879–886. https://doi.org/10.1080/10635150701701083

DeKay JE (1842) Zoology of New-York, or the New-York fauna; comprising detailed descriptions of all the animals hitherto observed within the state of New-York, with brief notices of those occasionally found near its borders, and accompanied by appropriate illustrations. . W. & A. White & J. Visscher, Albany

Dulvy NK, Simpfendorfer CA, Davidson LNK, et al (2017) Challenges and Priorities in Shark and Ray Conservation. Curr Biol 27:R565–R572. https://doi.org/10.1016/j.cub.2017.04.038

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564–567

FishNet2 (2020) FishNet 2 Portal. http://www.fishnet2.net. Accessed 31 Jan 2020

Freitas RHA, Aguiar AA, Freitas AKCHA, et al (2019) Unravelling the foraging behavior of the southern stingray, *Hypanus americanus* (Myliobatiformes: Dasyatidae) in a southwestern atlantic MPA. Neotrop Ichthyol 17:1–14. https://doi.org/10.1590/1982-0224-20180131

Garman S (1880) New species of selachians in the museum collection. Bull Museum Comp Zool 6:167–172

Garman S (1883) The American species of the genus *Dasibatis*. In: Jordan DS, Gilbert CH (eds) Synopsis of the fishes of North America. Government Printing Office, Washington, D.C., pp 65–72

Garman S (1913) The Plagiostomia. (Sharks, Skates, and Rays). Memoirs of the Museum of Comparative Zoology at Harvard College XXXVI, Cambridge, USA

GBIF.org (2020) GBIF. https://www.gbif.org. Accessed 31 Jan 2020

Gomes UL, Rosa RS, Gadig OBF (2000) *Dasyatis marianae* sp. n.: A new species of stingray (Chondrichthyes: Dasyatidae) from the Southwestern Atlantic. Copeia 2000:510–515

Gouveia NA, Gherardi DFM, Wagner FH, et al (2019) The Salinity Structure of the Amazon River Plume Drives Spatiotemporal Variation of Oceanic Primary Productivity. J Geophys Res Biogeosciences 124:147–165. https://doi.org/10.1029/2018JG004665

Grubbs RD, Snelson FF, Piercy A, et al (2016) *Hypanus americanus*. The IUCN Red List of Threatened Species. In: e.T60149A104123038

Gunther A (1870) Catalogue of the fishes in the British Museum. Catalogue of the Physostomi, containing the families Gymnotidae, Symbranchidae, Muraenidae, Pegasidae, and of the Lophobranchii, Plectognathi, Dipnoi, ...[thru] ... Leptocardii, in the British Museum. v. 8.

Henningsen AD, Leaf RT (2010) Observations on the Captive Biology of the Southern Stingray. Trans Am Fish Soc 139:783–791. https://doi.org/10.1577/t09-124.1

Hildebrand SF, Schroeder WC (1928) Fishes of Chesapeake Bay. US Government Printing Office

Jardim de Queiroz L, Cardoso Y, Jacot-des-Combes C, et al (2020) Evolutionary units delimitation and continental multilocus phylogeny of the hyperdiverse catfish genus Hypostomus. Mol Phylogenet Evol 145:106711. https://doi.org/10.1016/j.ympev.2019.106711

Jordan DS, Evermann BW (1896) The fishes of North and Middle America: Descriptive catalogue of the species of fish-like vertebrates found in the waters of North America, North of the Isthmus of Panama. Bull United States Natl Museum 47:1240

Jordan DS, Gilbert CH (1879) Notes on the fishes of Beaufort Harbor, North Carolina. Proc United States Natl Museum 1:365–388. https://doi.org/10.1126/science.9.231.776

Jordan DS, Gilbert CR (1880) Notes on a collection of fishes from San Diego, California. Proc United States Natl Museum 3:23–34. https://doi.org/10.1017/CBO9781107415324.004

Konan KM, Adépo-Gourène AB, Ouattara A, et al (2010) Morphometric variation among male populations of freshwater shrimp *Macrobrachium vollenhovenii* Herklots, 1851 from Côte d'Ivoire Rivers. Fish Res 103:1–8. https://doi.org/10.1016/j.fishres.2010.01.005

Korkmaz S, Goksuluk D, Zararsiz G (2014) MVN: An R package for assessing multivariate normality. R J 6:151–162. https://doi.org/10.32614/rj-2014-031

Kumar S, Stecher G, Li M, et al (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol 35:1547–1549

Last PR, Naylor GJP, Manjaji-Matsumoto BM (2016) A revised classification of the family Dasyatidae (Chondrichthyes: Myliobatiformes) based on new morphological and molecular insights. Zootaxa 4139:345–368. https://doi.org/10.11646/zootaxa.4139.3.2

Last PR, Stevens JD (2009) Sharks and Rays of Australia, Second Edi. CSIRO Publishing

Last PR, White WT, Pogonoski JJ (2008) Resurrection of the genus *Neotrygon* Castelnau (Myliobatoidei: Dasyatidae) with the description of *Neotrygon picta* sp. nov., a new species from northern Australia. In: Last PR, White WT, Pogonoski JJ (eds) Descriptions of New Australian Chondrihcthyans. CSIRO Marine and Atmospheric Research, Hobart, p 358

Leigh JW, Bryant D (2015) PopART: Full-feature software for haplotype network construction. Methods Ecol Evol 6:1110–1116

Lesueur CA (1824) Description of several species of the Linnaean genus *Raia*, of North America. J Acad Nat Sci 4:100–121, Pls. 4-6

Lesueur CA (1817) Description of three new species of the genus *Raja*. J Acad Nat Sci 1:41–45

Lima JS, Zappes CA, Di Beneditto APM, Zalmon IR (2019) Ethnoecology and socioeconomic around an artificial reef: The case of artisanal fisheries from southeastern Brazil. Biota Neotrop 19:. https://doi.org/10.1590/1676-0611-BN-2018-0620

Manjaji BM (2004) Taxonomy and Phylogenetic Systematics of the Indo-Pacific Whiptailed Stingray Genus *Himantura* Müller & Henle 1837 (Chondrichthyes: Myliobatiformes: Dasyatidae). University of Tasmania

Melo-Merino SM, Reyes-Bonilla H, Lira-Noriega A (2020) Ecological niche models and species distribution models in marine environments: A literature review and spatial analysis of evidence. Ecol Modell 415:35. https://doi.org/10.1016/j.ecolmodel.2019.108837

Menni RC, Stehmann MFW (2000) Distribution, environment and biology of batoid fishes off Argentina, Uruguay and Brazil. A review. Rev del Mus Argentino Ciencias Nat Nueva Ser 2:69–109

Mitchill SL (1815) The fishes of New-York, described and arranged. Trans Lit Philos Soc New-York 1:355–492, Pls. 1-6.

Mitteroecker P, Bookstein F (2011) Linear Discrimination, Ordination, and the Visualization of Selection Gradients in Modern Morphometrics. Evol Biol 38:100–114. https://doi.org/10.1007/s11692-011-9109-8

Muscarella R, Galante PJ, Soley-Guardia M, et al (2014) ENMeval: An R package for conducting spatially independent evaluations and estimating optimal model complexity for <scp>Maxent</scp> ecological niche models. Methods Ecol Evol 5:1198–1205. https://doi.org/10.1111/2041-210X.12261

Naylor GJP, Caira JN, Jensen K, et al (2012) A DNA Sequence-based approach to the identification of shark and ray species and its implications for global elasmobranch diversity and parasitology. Bull Am Museum Nat Hist 367:262

Naylor GJP, Ryburn J a, Fedrigo O, Lopez a (2005) Phylogenetic relationships among the Major Lineages of Modern Elasmobranchs. Reprod Biol Phylogeny Chondrickthyes 576

Oksanen J, Blanchet FG, Friendly M, et al (2019) vegan: Community Ecology Package

Padial JM, De La Riva I (2006) Taxonomic inflation and the stability of species lists: The perils of ostrich's behavior. Syst Biol 55:859–867. https://doi.org/10.1080/10635150600081588

Padial JM, Miralles A, Riva ID la, Vences M (2010) The integrative future of taxonomy. Front Zool 7:1–14

Pikitch EK, Chapman DD, Babcock E a, Shivji MS (2005) Habitat use and demographic population structure\rof elasmobranchs at a Caribbean atoll (Glover's\rReef, Belize). Mar Ecol Prog Ser 302:187–197. https://doi.org/10.3354/meps302187

Pinheiro HT, Mazzei E, Moura RL, et al (2015) Fish biodiversity of the Vitória-Trindade seamount chain, southwestern Atlantic: An updated database. PLoS One 10:1–17. https://doi.org/10.1371/journal.pone.0118180

R Core Team (2018) R: A language and environment for statistical computing.

Rafinesque CS (1818) [Review of] Journal of the Academy of Natural Sciences of Philadelphia. Vol. I. Part I. Am Mon Mag Crit Rev 3:269–274. https://doi.org/10.1177/1077546305060158

Rocha LA, Rosa IL (2001) Baseline assessment of reef fish assemblages of Parcel Manuel Luiz Marine State Park, Maranhão, north-east Brazil. J Fish Biol 58:985–998. https://doi.org/10.1006/jfbi.2000.1504

Rodrigues Filho LF da S, Feitosa LM, Silva Nunes JL, et al (2020) Molecular identification of ray species traded along the Brazilian Amazon coast. Fish Res 223:105407. https://doi.org/10.1016/j.fishres.2019.105407

Rosa RS, Moura RL (1997) Visual assessment of reef fish community structure in the Atol das Rocas Biological Reserve, off northeastern Brazil. In: Proceedings of the 8th International

Coral Reef Symposium. International Society for Reef Studies, pp 983–986

Rozas J, Ferrer-Mata, A. Sánchez-DelBarrio JC, Guirao-Rico S, et al (2017) DnaSP 6: DNA Sequence Polymorphism Analysis of Large Datasets. Mol Biol Evol 34:3299–3302

Sabaj MH (2016) Standard symbolic codes for institutional resource collections in herpetology and ichthyology: an Online Reference. Version 6.5

Santos HRS (2007) Revisão taxonômica das espécies viventes de Dasyatidae (Chondrichthyes, Myliobatiformes) do Atlântico Ocidental. Universidade Federal do Rio de Janeiro, Museu Nacional

Santos HRS, Carvalho MR (2004) Description of a new species of whiptailed stingray from the Southwestern Atlantic Ocean (Chondrichthyes, Myliobatiformes, Dasyatidae). Bol do Mus Nac 516:1–24

Santos HRS, Gomes UL, Charvet-Almeida P (2004) A new species of whiptail stingray of the genus *Dasyatis* Rafinesque, 1810 from the Southwestern Atlantic Ocean (Chondrichthyes: Myliobatiformes: Dasyatidae). Zootaxa 492:1–12. https://doi.org/10.5281/zenodo.157371

Sbrocco EJ, Barber PH (2013) MARSPEC: ocean climate layers for marine spatial ecology. Ecology 94:979. https://doi.org/10.1890/12-1358.1

Schlager S (2017) Morpho and Rvcg - Shape Analysis in R: R-Packages for Geometric Morphometrics, Shape Analysis and Surface Manipulations, 1st edn. Elsevier Ltd

Seret B (1990) Dasyatidae. In: Lévêque C, Paugy D, Teugels GG (eds) Faune des poissons d’eaux douces et saumâtres de l’Afrique de l’Ouest, v. 1. Paris, pp 62–75

Séret B, Couzens G, Valenti S V. (2016) *Hypanus rudis*. The IUCN Red List of Threatened Species. In: e.T161620A104133548

Smith WD (2016) *Hypanus longus*. The IUCN Red List of Threatened Species. In: e.T60157A104126060

Spalding MD, Fox HE, Allen GR, et al (2007) Marine Ecoregions of the World: A Bioregionalization of Coastal and Shelf Areas. Bioscience 57:573. https://doi.org/10.1641/B570707

Tagliafico A, Rago N, Salomé Rangel M (2013) Aspectos biológicos de las rayas *Dasyatis guttata* y *Dasyatis americana* (Myliobatiformes: Dasyatidae) capturadas por la pesquería artesanal de la isla de Margarita, Venezuela. Rev Biol Mar Oceanogr 48:365–373. https://doi.org/10.4067/S0718-19572013000200015

Tilley A, Strindberg S (2013) Population density estimation of southern stingrays *Dasyatis americana* on a Caribbean atoll using distance sampling. Aquat Conserv Mar Freshw

Ecosyst 23:202–209. https://doi.org/10.1002/aqc.2317

Valentin JL (2001) The Cabo Frio Upwelling System, Brazil. In: Seeliger U, Kjerfve B (eds) Coastal Marine Ecosystems of Latin America. Ecological Studies (Analysis and Synthesis), vol 144. Springer Berlin Heidelberg

Van Oosten EB, Buse K, Bilimoria D (2017) The leadership lab for women: Advancing and retaining women in STEM through professional development. Front Psychol 8:1–5. https://doi.org/10.3389/fpsyg.2017.02138

Vaske T, Lessa RP, de Nóbrega M, et al (2005) A checklist of fishes from Saint Peter and Saint Paul Archipelago, Brazil. J Appl Ichthyol 21:75–79. https://doi.org/10.1111/j.1439-0426.2004.00600.x

Wirtz P, Bingeman J, Bingeman J, et al (2017) The fishes of Ascension Island, central Atlantic Ocean - new records and an annotated checklist. J Mar Biol Assoc United Kingdom 97:783–798. https://doi.org/10.1017/S0025315414001301

Wirtz P, Brito A, Falcón JM, et al (2013) The coastal fishes of the Cape Verde Islands - New records and an annotated check-list: (Pisces). Spixiana 36:113–142

Zuur AF, Ieno EN, Elphick CS (2009) A protocol for data exploration to avoid common statistical problems. Methods Ecol Evol 1:3–14. https://doi.org/10.1111/j.2041-210x.2009.00001.x

SUPPLEMENTARY MATERIAL

Supp. Mat. 5. Geographical coordinates in decimal degrees of specimens and tissue samples of *Hypanus americanus*, *H. longus*, *H. rudis*, and *H.* **sp. 1** used in the integrative taxonomy from the online databases FishNet 2 (FishNet2 2020), GBIF (GBIF.org 2020), and *sp*Link (CRIA 2019), from analyzed specimens deposited in museums and tissue samples used herein. Lon: longitude, lat: latitude.

	H. americanus		*H. longus*		*H. rudis*		*Hypanus* **sp. 1**	
	lon	lat	lon	lat	lon	lat	lon	lat
FishNet 2	-90.4811	29.09222	-111.05	27.93333				
	-89.0841	30.34303	-111	19				
	-62.85	17.8833	-110.8	19.31667				
			-105.283	21.51667				
			-105.233	21.16667				
			-90.45	12.78333				
GBIF	-99.1	19.37	-114.733	18.36667			-19.6789	-39.7842
	-97.2875	20.97361	-114.183	27.8			-3.84771	-32.4433
	-97.15	21.53333	-112.083	24.59167			-3.82162	-32.3875
	-96.9965	20.76961	-111.733	24.48333			-3.80556	-32.3769
	-96.1	19.22639	-111.667	24.5				
	-95.7833	18.78333	-110.967	18.75				
	-94.7819	18.51594	-110.9	27.93333				
	-91.9583	18.825	-110.34	24.13				
	-91.823	18.633	-109.917	22.88333				
	-91.6286	19.06861	-105.197	20.65				
	-91.38	18.6	-105.192	21.44167				
	-90.723	19.358	-105.19	20.65				
	-90.3942	20.85889	-100.93	17.16				
	-89.7092	21.3149	-95.68	15.9				
	-89.6667	21.28333	-95.2404	16.09957				
	-89.1189	21.35783	-95.0183	16.06889				
	-88.8502	30.37326	-92.85	15.16028				
	-88.2078	18.59278	-92.7505	14.8833				
	-88.2	30.75	-85.7	10.55				
	-88.1963	30.24825	-85.05	10.08333				
	-88.1754	21.60893	-80.817	-1.55				
	-87.9738	17.88693	-79	8.416667				
	-87.843	18.276	-81.9256	26.45				
	-87.2215	20.4827	-81.7265	25.93651				
	-87.0255	20.3123	-81.3441	19.3845				
	-86.9587	20.5156	-81.3388	13.37693				
	-86.798	21.492	-81.3283	25.28				
	-86.7353	21.2372	-81.3189	30.39313				
	-85.8722	16.4639	-81.1124	24.69254				
	-85.1013	29.62765	-80.9639	24.75417				
	-84.3672	29.89364	-80.7139	24.88944				
	-84.2689	30.04755	-80.4436	25.08865				

	-83.4217	29.64878	-80.1667	25.735				
	-82.3849	23.15183	-80.1413	25.8096				
	-82.2236	26.65549	-80.0167	30.96667				
	-81.9256	26.45	-79.8476	15.80754				
	-81.7265	25.93651	-79.5551	23.53677				
	-81.3441	19.3845	-79.3397	33.0649				
	-81.3388	13.37693	-79.2794	25.73365				
	-81.3283	25.28	-77.9818	33.93511				
	-81.3189	30.39313	-76.691	34.82843				
	-81.1124	24.69254	-76.6638	34.7181				
	-80.9639	24.75417	-76.1791	38.3143				
	-80.7139	24.88944	-76.0103	37.06363				
	-80.4436	25.08865	-75.76	36.55				
	-80.1667	25.735	-75.7032	9.694265				
	-80.1413	25.8096	-75.7	36.23333				
	-80.0167	30.96667	-75.4317	35.365				
	-79.8476	15.80754	-70.921	41.45128				
	-79.5551	23.53677	-70.0003	12.4708				
	-79.3397	33.0649	-68.3534	18.53759				
	-79.2794	25.73365	-63.2622	17.63839				
	-77.9818	33.93511	-63.0753	18.2222				
	-76.691	34.82843	-61.355	12.63167				
	-76.6638	34.7181	-60.55	11.32				
	-76.1791	38.3143	-59.5847	13.06794				
	-76.0103	37.06363	-54.6833	6.483333				
	-75.76	36.55						
	-75.7032	9.694265						
	-75.7	36.23333						
	-75.4317	35.365						
	-70.921	41.45128						
	-70.0003	12.4708						
	-68.3534	18.53759						
	-63.2622	17.63839						
	-63.0753	18.2222						
	-61.355	12.63167						
	-60.55	11.32						
	-59.5847	13.06794						
	-54.6833	6.483333						
Molecular analyses	-88.748	30.231	-111.33	25.8311	14.40224	-16.9818	-0.52132	-46.7106
	-88.081	16.6907	-109.69	23.0458	14.4	-16.9667	-2.86582	-41.2709
	-82.609	12.3028	-88.748	30.231	5.128356	-0.57258	-16.4313	-39.0405
	-80.194	32.4929	-88.081	16.6907			-3.70702	-38.5032
	-79.891	32.7292	-82.609	12.3028			-5.04661	-36.6193
	-77.262	34.4815	-80.194	32.4929			-5.08728	-36.1671
	-75.784	37.2804	-79.891	32.7292			-5.05546	-36.0557
			-77.262	34.4815			-5.23278	-35.2302

			-75.784	37.2804			-24.5272	-46.6603
							-24.4469	-46.7153
							-24.3714	-46.6283
							-23.8236	-45.5364
							-22.99	-43.116587
							-3.84831	-32.441819
							-3.84726	-32.442215
							-3.84446	-32.4281
							-3.84015	-32.4155
							-3.83403	-32.401477
							-3.83369	-32.400302
							-2.83403	-32.401478
Morphological analyses	-95.3486	28.89361	-114.832	31.0225	8.87	-13.87	-23.83	-45.4233
	-89.4231	21.60478	-112.56	28.94806	7.5583	-13.3167	-1.08	-44.8
	-88.0417	30.2228	-112	28			-21.484	-41.015
	-88.0142	30.45361	-99.9417	16.85833			-21.34	-40.939
	-85.8722	16.4639	-80.0758	7.357215			-20.428	-40.285
	-84.4214	29.91448	-78.8583	8.458333			-13.6	-38.783
	-82.4027	23.20805	-77.53	3.63			-3.7174	-38.514
	-81.8667	26.3875	-77.2506	3.781749			-2.834	-32.401478
	-81.18	30.6233	-95.3486	28.89361			-1.08	-44.8
	-80.9375	25.3125	-89.4231	21.60478			8.8167	-59.517
	-80.75	28.58333	-88.0417	30.2228				
	-80.7	28.59167	-88.0142	30.45361				
	-80.6333	31.48333	-85.8722	16.4639				
	-80.55	24.95	-84.4214	29.91448				
	-80.1581	25.72987	-82.4027	23.20805				
	-79.2521	25.71749	-81.8667	26.3875				
	-78.17	22.47	-81.18	30.6233				
	-77.85	17.91667	-80.9375	25.3125				
	-77.775	17.86667	-80.75	28.58333				
	-77.7667	33.28333	-80.7	28.59167				
	-76.355	38.714	-80.6333	31.48333				
	-75.8392	29.87861	-80.55	24.95				
	-75.7557	36.5506	-80.1581	25.72987				
	-75.4433	35.5067	-79.2521	25.71749				
	-70.921	41.45128	-78.17	22.47				
	-61.3818	10.43518	-77.85	17.91667				
	-59.5167	8.8167	-77.775	17.86667				
			-77.7667	33.28333				

			-76.355	38.714				
			-75.8392	29.87861				
			-75.7557	36.5506				
			-75.4433	35.5067				
			-70.921	41.45128				
			-61.3818	10.43518				
			-59.5167	8.8167				
***sp*Link**	-81.3737	13.39394	-81.3737	13.39394			-22.2972	-41.6733
	-77.6861	24.32821	-77.6861	24.32821			-18.0058	-38.9389
	-65.8145	18.05726	-65.8145	18.05726			-12.9711	-38.5108
							-8.0699	-34.7533
							-3.8598	-33.818

CONCLUSÕES GERAIS

O gênero de raias marinhas *Hypanus* é monofilético com 14 linhagens, sendo algumas já reconhecidas morfologicamente e com nomes de espécies válidos, como *H. colarensis, H. dipterurus, H. geijskesi, H. longus, H. marianae* e *H. sabinus*. Dessas, *H. geiksesi* estava posicionada no gênero *Fontitrygon* e foi realocada para *Hypanus* após análises filogenéticas usando genomas mitocondriais; *H. colarensis*, apesar da ausência de material genético para análises, também foi transferida para *Hypanus* devido às similaridades morfológicas e de distribuição geográfica. Algumas espécies até o momento válidas são formadas por mais de uma linhagem: *H. americanus* + *H.* aff. a*mericanus, H. guttatus* + *H.* aff. *guttatus*, *H. rudis* + *H.* aff. *rudis*, e *H. say* + *H.* aff. *say*. Essas divisões são bem suportadas por análises de delimitações de linhagens.

A espécie *H. americanus* senso lato foi reconhecida como parafilética, pois os exemplares que ocorrem no Atlântico Oeste, entre o deságue do Rio Amazonas e o sul do Estado de São Paulo (Brasil), e que antes eram identificados como *H. americanus*, pertencem a uma linhagem distinta daquela do Atlântico Norte Ocidental (*H. americanus* senso stricto) e mais proximamente relacionadas à espécie *H. rudis*, do Atlântico Oriental. Porém, análises do genoma mitocondrial mostraram que essas linhagens se separaram há 1,28 Ma, logo após grandes mudanças no Oceano Atlântico devidas ao fechamento do Istmo do Panamá. Correntes, temperatura, salinidade ficaram alteradas e permitiram que alguns exemplares de uma população de raias viventes na costa brasileira pudessem alcançar a costa do continente Africano. Contudo, como mostrou a análise de modelagem de nicho ecológico, essas raias preferem hábitats costeiros e de baixa profundidade. Dessa forma, o fluxo constante de indivíduos através do Atlântico não é esperado, corroborando a ideia de que tenha sido um evento estocástico e que sejam espécies distintas.

Estudos morfológicos e morfométricos comparando essa linhagem de raias da costa brasileira às outras do mesmo clado, *H. rudis*, *H. americanus* sensu stricto e *H. longus*, mostraram que há diferenças entre as quatro, como por exemplo, na morfologia do clásper de machos adultos e algumas medidas na região da cabeça. Assim, a integração dos dados genéticos, morfológicos e ecológicos sustentam a descrição de uma nova espécie que ocorre na costa brasileira.

Análises biogeográficas do gênero *Hypanus* sugerem que sua origem tenha sido no Atlântico Ocidental Tropical há, aproximadamente, 30,97 Ma, durante um período de resfriamento global em que uma linhagem ancestral de raias possa ter buscado refúgio em águas mais quentes. Como o gênero está distribuído ao redor do continente Americano, testamos as grandes barreiras impostas por este às raias e observamos que as barreiras terrestres (fechamento do Istmo do Panamá e Península da Florida durante as glaciações do Pleistoceno), salinidade e sedimentos (deságue do Rio Amazonas), distância da costa (Barreiras do Meso-Atlântico e Pacífico Oriental), e temperatura (águas frias em grandes latitudes) foram muito importantes para sua diversificação.

Com os dados dos exomas nucleares já sequenciados, tomografias computadorizadas e modelagens de nicho de todas as linhagens de *Hypanus* já identificadas com os genomas mitocondriais será possível fazer uma revisão taxonômica integrativa completa do gênero. Esses estudos já foram iniciados, porém não completados pela limitação de tempo. Para compreender a diversificação dos organismos, uma das abordagens é o estudo filogeográfico unindo variações fenotípicas e ambientais. O primeiro passo é identificar as barreiras de distribuição geográfica e correlacioná-las aos estudos genéticos para avaliar se são coincidentes com variações fenotípicas. Assim, podem-se levantar hipóteses acerca da influência ambiental sobre os fenótipos de populações em áreas distintas, mas com características ambientais e genéticas similares (Zamudio et al., 2016). A delimitação e descrição das espécies de *Hypanus*, assim como suas áreas de distribuição, são requisitos básicos para a avaliação de seus estados de conservação. O fornecimento dessas informações para especialistas terá efeito direito na conservação dessas raias.