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Genetic evidence supports a range extension for the Brazilian cownose ray *Rhinoptera brasiliensis* in the western North Atlantic

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Ethics statement

Collection of tissue samples in all locations complied with animal welfare laws, guidelines, and policies. Sample collection in the Indian River Lagoon, Florida was conducted under protocols approved by the Florida Atlantic University Institutional Animal Care and Use Committee (Animal Use Protocol #A16-16) and in accordance with federal and Florida state laws and regulations under the following permits: FWC Special Activity Licenses SAL-16-1785-SRP,

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SAL-17-1785-SRP, SAL-18-1785A-SRP, and SAL-19-1785-SRP; Florida Department of Environmental Protection Florida Park Service Scientific Research Permits 07261610 and 07241710A; and U.S. Fish and Wildlife Service Special Use Permits 41572-2016-04 and 41572-2017-07. Sample collections in St. Andrew, St. Joseph, and Apalachicola bays as well as Santa Rosa Sound were permitted under Florida Fish and Wildlife Conservation Commission Special Activity Licenses SAL-11-1292-SRP, SAL-12-1292-SRP, SAL-13-1292-SRP, SAL-14-1292-SRP, SAL-15-1292-SRP, SAL-16-1292-SRP, SAL-13-1464-SR; Florida Park Service Permit number 14100111; and National Parks Service Gulf Islands Scientific Research and Collecting Permit GUIS-2013-SCI-0024. Sample collection in Chesapeake Bay, Virginia was conducted in accordance with the San Francisco State University Institutional Animal Care and Use Committee (IACUC) protocol A15-09R2. No permits were required for collection of tissue samples by researchers with Florida Fish and Wildlife Conservation Commission or the North Carolina Division of Marine Fisheries.

Abstract

We report 24 new records of the Brazilian cownose ray *Rhinoptera brasiliensis* outside its accepted geographic range. Sequencing of a 442-base pair portion of the mitochondrial NADH dehydrogenase subunit 2 gene for 282 *Rhinoptera* samples revealed eight records off the east coast of the USA and 16 from the eastern Gulf of Mexico. Both sexes of all life stages were documented in all seasons over multiple years in the Indian River and Lake Worth lagoons,

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Florida, indicating that their range extends further in the western North Atlantic than previously described.

KEYWORDS

American cownose ray, Elasmobranch, Indian River Lagoon, mitochondrial DNA, ND2, *Rhinoptera bonasus*

The cownose rays (Rhinopteridae) are comprised of eight morphologically similar species in the genus *Rhinoptera* (Last *et al*., 2016). Rhinopterids are circumglobally distributed, with two recognized species in the western Atlantic: the Endangered Brazilian cownose ray *Rhinoptera brasiliensis* Müller 1836 (Vooren & Lamónaca, 2004) and the Near Threatened American cownose ray *Rhinoptera bonasus* (Mitchill 1815) (Barker, 2006). Both species are benthopelagic and indigenous to tropical and temperate shallow waters along continental shelves (Last *et al*., 2016). Historically, tooth series counts were used to distinguish between these two species, despite ambiguity arising from overlap in series counts (Bigelow & Schroeder, 1953). Recent research documented changes in tooth morphology with age, making this characteristic unreliable (Jones *et al*., 2017). More reliable methods of distinguishing *R. brasiliensis* from *R. bonasus*, such as cranial anatomy and number of spiral valve lamellae, necessitate either dissection or advanced analytical techniques, such as computed tomography or magnetic resonance imaging (Jones *et al*., 2017).

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In lieu of clear, external diagnostic characteristics to distinguish *Rhinoptera* spp., species identifications in the western North Atlantic have largely relied on assumed geographic ranges. This approach, however, grossly underestimated the range of *R. brasiliensis* in the western Atlantic. The range of *R. bonasus* extends from New England in the United States (USA) to northern Argentina (Last *et al*., 2016). Historically, *R. brasiliensis* was considered endemic to Brazil, restricted to the waters of southern Brazil (Barker, 2006; Bigelow & Schroeder, 1953). Recent literature extended the range of *R. brasiliensis* to include Central America (McEachran & de Carvalho, 2002), the southern Gulf of Mexico (Palacios-Barreto *et al*., 2017), and the northern Gulf of Mexico (Jones *et al*., 2017), using a combination of morphological and molecular methods in species identifications. This range extension of *R. brasiliensis* substantially increased overlap in the ranges of *R. brasiliensis* and *R. bonasus*, and given the absence of clear field characteristics, the range of *R. brasiliensis* is still poorly understood. Given this uncertainty, existing datasets of *Rhinoptera* spp. in the western Atlantic may reflect a species complex rather than single species, which could lead to the use of spurious data from biological and ecological studies in their management. Here, we used genetic methods to identify *Rhinoptera* spp. to evaluate the extent of occurrence of *R. brasiliensis* beyond its currently described range in the western North Atlantic; specifically, in the eastern Gulf of Mexico (hereafter eGOM) and along the east coast of the USA (hereafter Atlantic).

A total of 282 archived or opportunistically collected *Rhinoptera* spp. tissue samples collected between 2002 and 2018 were analysed from 12 locations in the eGOM and Atlantic,

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spanning from Santa Rosa Sound, Florida, to Chesapeake Bay, Virginia (Figure 1). *Rhinoptera* spp. were caught throughout the year, primarily by gillnet, and fin clips were collected and stored in 95% ethanol. Total genomic DNA was extracted from ~15 mg of tissue using a Qiagen DNeasy^TM DNA extraction kit (Qiagen, USA), according to the manufacturer's protocol with the exception that tissue samples were digested overnight. A 442-base pair region of the mitochondrial (mtDNA) NADH dehydrogenase subunit 2 (ND2) gene was targeted using a forward primer (RhinND2F1: 5'-GAACCCYTTAATCCTCTYCATC-3') designed by McDowell and Fisher (unpubl. data) and a reverse primer (RayND2R: 5'-GGATTGATAGTACGCCTATGG-3') designed for this study. Polymerase Chain Reaction (PCR) mixtures contained 25–50 ng template DNA, 10 mM TAQ buffer (Invitrogen), 1.5 mM MgCl$_2$, 0.3 μM of each primer, 0.1 mM dNTPs (Promega mix), 1 U of *Taq* polymerase (Invitrogen), and PCR-grade water for a final reaction volume of 25 μL. PCR cycling conditions consisted of an initial denaturation at 95˚C for 5 min, then 35 cycles of: 30 sec denaturation at 95˚C, 30 sec annealing at 58˚C, 30 sec extension at 72˚C, followed by a 5 min final extension at 72˚C. Amplicons were cleaned using 3 μL of ExoSAP-IT (ThermoFisher) and sequenced in the forward and reverse directions on an Applied Biosystems™ 3730XL DNA Analyzer using a BigDye™ Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems™) following the manufacturer's protocol except that all sequences were run using half reactions.

Consensus sequences were generated by aligning the forward and reverse sequences for each individual in CodonCode v 9.0.1 (CodonCode Corporation, Dedham, USA). Resultant

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haplotypes were compared to mtDNA ND2 haplotypes generated, using the methods described

here, for 10 specimens each of *R. brasiliensis* and *R. bonasus* collected from Mississippi and

Alabama waters (USA), which were verified using a suite of 21 external morphological

measurements and genetic analysis of the CO1 gene (see Jones *et al*., 2017). The Jukes-Cantor

substitution model (Jukes & Cantor, 1969) was determined to be the nucleotide substitution

model of best fit for the data in jModelTest 2 v 2.1.10 using Bayesian Information Criterion

(BIC) values (Darriba *et al*., 2012). Phylogenetic relationships between these haplotypes were

inferred using a maximum likelihood approach with 10,000 bootstrap replicates and the Jukes-

Cantor substitution model in MEGA v X (Kumar *et al*., 2018), with the bat ray *Myliobatis*

californica Gill 1865 (GenBank accession no. KM364985), serving as an outgroup.

Relationships among haplotypes were estimated using the maximum parsimony method of

Polzin and Daneshmand (2003) and visualized as a median-joining haplotype network using

Network v 10.1.10 (Bandelt *et al*., 1999).

The 282 tissue samples revealed 14 haplotypes that formed two distinct genetic clades.

Three haplotypes representing 24 individuals clustered together with the single haplotype

(RBRA1) sequenced for the 10 verified *R. brasiliensis* (GenBank accession nos. MT410205–

MT410207) and 11 haplotypes representing 258 individuals clustered with the single haplotype

sequenced for the 10 verified *R. bonasus* (RBON1) (GenBank accession nos. MT410194–

MT410204) (Supporting Information Figure S1). The sampled *R. brasiliensis* and *R. bonasus*

haplotypes were differentiated by 5.43–7.24% sequence divergence, based on the number of base

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pair differences (Supporting Information Figure S2). In contrast, the maximum sequence differentiation between sampled haplotypes within each species was much less; 0.68% and 1.13% for *R. brasiliensis* and *R. bonasus*, respectively (Supporting Information Figure S2). The minimum sequence differentiation between *M. californica* and *R. brasiliensis* and *R. bonasus* was 13.80% and 12.90%, respectively.

The 24 genetically identified *R. brasiliensis* were collected from eight locations in the western North Atlantic; eight were from two estuaries in the Atlantic and 16 were from six estuaries in the eGOM (Figure 1). In the Atlantic, five *R. brasiliensis* were caught at the mouth of the St. Sebastian River in the Indian River Lagoon, Florida (hereafter IRL) and three were caught in Lake Worth Lagoon, Florida. In the eGOM, six were caught in Apalachicola Bay, five in Santa Rosa Sound, two in Charlotte Harbor, and one in each of St. Andrew Bay, St. Joseph Bay, and Cedar Key. Individuals included males and females, juveniles and adults, and were caught across all seasons in multiple years between 2003 and 2018 (Table 1). Evidence of reproductive habitat use in the Atlantic was identified by the presence of two particular *R. brasiliensis* near the mouth of the St. Sebastian River, a gravid female caught in summer (August), and a juvenile male caught in winter (January). None were identified from estuaries in the Atlantic north of the St. Sebastian River in the IRL, including the Altamaha River, Georgia (n = 2), Pamlico Sound and its tributaries, North Carolina (n = 66), and Chesapeake Bay, Virginia (n = 23).

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The discovery of 24 *R. brasiliensis* based on mtDNA ND2 sequence data across sites in Florida waters further confirms its occurrence in the eGOM (see Carney *et al*., 2017; Jones *et al*., 2017; McDowell & Fisher, unpubl. data) and documents that the range extends further in the western North Atlantic than previously described, at least as far north as the St. Sebastian River in the IRL. Since mtDNA is maternally inherited, this finding assumes that these individuals are not hybrid crosses between female *R. brasiliensis* and male *R. bonasus*, which would require analysis of additional nuclear data. Hybridization has not yet been documented in cownose rays, but has been shown in freshwater rays (Potamotrygonidae, Cruz *et al*., 2015) and fiddler rays (Rhinobatidae, Donnellan *et al*., 2015). While it is possible to detect patterns of introgression from mtDNA markers alone, robust evidence necessitates powerful datasets that typically use much larger DNA fragments (*e.g*. Rosenzweig *et al*., 2016).

These new records are part of a growing body of literature documenting the presence of *R. brasiliensis* in the western North Atlantic. Prior species accounts that used genetic identifications of *R. brasiliensis* in the Atlantic include four records with no life history data from Georgia in fall (October) 2007 (Quattro, unpubl. data), one of unknown size from North Carolina with an unknown capture date in 2008 (Naylor *et al*., 2012), and one from a deceased specimen of unknown size found on a beach in New Jersey in summer (August) 2017 (Stoeckle *et al*., 2020). There is one additional historic record from North Carolina, however, since this identification was based on tooth series counts alone (Bigelow & Schroeder, 1953), it is not considered reliable. Despite these records, the Atlantic was not considered part of the accepted

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range of *R. brasiliensis* in subsequent accounts (*e.g.* Last *et al.*, 2016), possibly because they were discounted as 'vagrants'. The term vagrant was recently defined for elasmobranchs by Grant *et al.* (2019) as 'individuals found outside of the species' distribution, in a habitat not biologically utilized by the species'. Our work has shown the presence of both male and female *R. brasiliensis* of all life stages, documented across all seasons over multiple years as far north as the St. Sebastian River in the IRL in the Atlantic. This indicates that *R. brasiliensis* is unlikely to be a vagrant, at least in the Atlantic off Florida, and that this region is part of its range, although the northernmost extent of its range remains uncertain. Whether *R. brasiliensis* have long occurred in these waters but were obscured by the presence of morphologically similar *R. bonasus*, or this reflects a more recent range expansion, remains unknown.

Juvenile *R. brasiliensis* occurred near the mouth of the St. Sebastian River during winter, in St. Andrew and Apalachicola bays during summer and fall, and in Charlotte Harbor during fall and winter. The presence of juveniles in these estuaries along both coasts of Florida, as well as the presence of a gravid *R. brasiliensis* in the IRL during the time associated with their parturition (Rangel *et al.*, 2017), suggests these bays may serve as nurseries. Juvenile *R. bonasus* are abundant in estuaries in the northern Gulf of Mexico and have been shown to tolerate low temperatures (*e.g.* <12℃, reported down to 8.3–9.1℃), potentially residing in estuaries over winter, and possibly until they reach maturity (Ajemian, 2011; Ajemian & Powers, 2016; Bigelow & Schroeder, 1953). Additional research is needed to better understand how and to what extent *R. brasiliensis* uses and relies on these estuarine habitats in the Atlantic and eGOM.

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The opportunistic nature of the sampling regime in this study likely limited our understanding of the occurrence and frequency of *R. brasiliensis* throughout the study area. For example, for some locations, four samples were collected at a single site in a single day and represented the same life stage, while elsewhere 52 samples were selected from archived samples to include all life stages, seasons, and sexes over nine years. Despite these differences, the presence of *R. brasiliensis* at locations outside of its accepted range, detected over multiple years, implies this species has a wider range than previously thought.

As documented here, the range of *R. brasiliensis* extends at least to the St. Sebastian River in the IRL in the Atlantic. More comprehensive, year-round surveys paired with genetic species identifications are still needed to clarify the northern extent of the range of *R. brasiliensis* in the Atlantic and seasonal patterns of occurrence in these waters. This range extension further increases range overlap between *R. brasiliensis* and *R. bonasus,* emphasizing the need to verify species identities using genetic methods prior to undertaking biological or ecological studies of either species. Further, existing biological and relative abundance datasets require careful consideration as they may reflect a *Rhinoptera* species complex rather than single species. Future studies should use a suite of approaches, including tagging and molecular methods, to better understand and disentangle the biology and ecology of these morphologically similar, sympatric species.

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Contributions

HKW, CMJ, and NMP conceived and designed the study. HKW completed the lab work, data

analysis, and drafted the manuscript with support from NMP and CMJ. CMJ, MJA, MPM, BLW,

GRP, DMB, LDH, DZ, JDS, JMH, and TDE provided the tissue samples used in this study and

assisted in writing the manuscript.

Significance statement

In the western Atlantic, the range of the Brazilian cownose ray *Rhinoptera brasiliensis* has been

obscured by the sympatric, morphologically similar, *R. bonasus.* Here, genetic evidence supports

a range extension for *R. brasiliensis* to the east coast of Florida, USA. This finding emphasizes

the need to genetically verify species identities prior to undertaking biological and ecological

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studies of *Rhinoptera* spp. and that existing datasets may reflect a species complex rather than

single species.

References

Ajemian, M. J. (2011). *Foraging ecology of large benthic mesopredators: effects of myliobatid rays on shellfish resources*. (Doctoral thesis, University of South Alabama, USA).

Ajemian, M. J., & Powers, S. P. (2016). Seasonality and ontogenetic habitat partitioning of cownose rays in the northern Gulf of Mexico. *Estuaries and Coasts*, **39**, 1234—1248

Bandelt, H. J., Forster, P., & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48

Barker, A. S. (2006). *Rhinoptera bonasus*. In *IUCN Red List of Threatened Species* 2006: e.T60128A12310195. Available at https://www.iucnredlist.org/species/60128/12310195 (last accessed 22 May 2020)

Bigelow, H. B., & Schroeder, W. C. (1953). *Fishes of the western North Atlantic, Part 2. Sawfishes, guitarfishes, skates, rays, and chimaeroids*. *Memoir of the Sears Foundation for Marine Research Number 1*. New Haven: Yale University

Carney, S. L., McVeigh, D. M., Moss, J. B., Ferrier, M. D., & Morrissey, J. F. (2017). Insights on mitochondrial genetic variation in Chesapeake Bay summer-resident cownose rays. *Transactions of the American Fisheries Society*, **146**, 478–484

Crus, V. P., Vera M., Mendonca, F. F., Pardo, B. G., Martinez, P., Oliveira, C., & Foresti, F. (2015). First identification of interspecies hybridization in the freshwater stingrays *Potamotrygon motoro* and *P. falkneri* (Myliobatiformes, Potamotrygonidae). *Conservation Genetics*, **16**, 241–245

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

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Donnellan, S. C., Foster, R., Junge, C., Huveneers, C., Rogers, P., Kilian, A., & Bertozzi, T. (2015). Fiddling with the proof: the magpie fiddler ray is a colour pattern variant of the common Southern Fiddler Ray (Rhinobatidae: *Trygonorrhina*). *Zootaxa*, **3981**, 367–384

Grant, M. I., Kyne, P. M., Simpfendorfer, C. A., White, W. T., & Chin, A. (2019). Categorising use patterns of non-marine environments by elasmobranchs and a review of their extinction risk. *Reviews in Fish Biology and Fisheries*, **29**, 689–710

Jones, C. M., Hoffmayer, E. R., Hendon, J. M., Quattro, J. M., Lewandowski, J., Roberts, M. A., Poulakis, G. R., Ajemian, M. J., Driggers III, W. B., de Carvalho, M. R., Rêgo, M. G., Hazin, F. H. V., & Márquez-Farías, J. F. (2017). Morphological conservation of rays in the genus *Rhinoptera* (Elasmobranchii, Rhinopteridae) conceals the occurrence of a large batoid, *Rhinoptera brasiliensis* Müller, in the northern Gulf of Mexico. *Zootaxa*, **4286**, 499–514

Jukes, T. H., & Cantor, C. R. (1969). Evolution of protein molecules. In: H. N. Munro (Ed.), *Mammalian Protein Metabolismprotein metabolism, Volume III* (pp. 21–132). New York: Academic Press

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, **35**, 1547–1549

Last, P. R., White, W. T., de Carvalho, M. R., Séret, B., Stehmann, M. F. W., & Naylor, G. J. P. (eds.) (2016). *Rays of the World*. Australia: CSIRO publishing

McEachran, J. D., & de Carvalho, M. R. (2002). Batoid fishes. In: K. E. Carpenter (Ed.), *The living marine resources of the western central Atlantic. Volume 1: Introduction, molluscs, crustaceans, hagfishes, sharks, batoid fishes, and chimaeras. FAO Species identification guide for fishery purposes* (pp. 508–589). Rome: Food and Agriculture Organization of the United Nations

Naylor, G. J. P., Caira, J. N., Jensen, K., Rosana, K. A. M., White, W. T., & Last, P. R. (2012). 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**, 1–263

Palacios-Barreto, P., Cruz, V. P., Foresti, F., Rangel, B. S., Uribe-Alcocer, M., & Díaz-Jaimes, P. (2017). Molecular evidence supporting the expansion of the geographical distribution

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of the Brazilian cownose ray *Rhinoptera brasiliensis* (Myliobatiformes: Rhinopteridae) in the western Atlantic. *Zootaxa*, **4341**, 593–600

Polzin, T., & Daneshmand, S. V. (2003). On Steiner trees and minimum spanning trees in hypergraphs. *Operations Research Letters*, **31**, 12–20

Poulakis, G. R. (2013). Reproductive biology of the cownose ray in the Charlotte Harbor estuarine system, Florida. *Marine and Coastal Fisheries*, **5**, 159–173

Rangel, B. S., Cruz, V. P., Rodrigues, A., Araujo, M. L. G., Oliveira, C., Foresti, F., & Moreira, R. G. (2017). Sympatric and syntopic occurrence of cownose rays: neonatal strategies for survival? *Journal of Applied Ichthyology*, **33**, 542–545

Rosenzweig, B. K., Pease, J. B., Besansky, N. J., & Hahn, M. W. (2016). Powerful methods for detecting introgressed regions from population genomic data. *Molecular Ecology*, **25**, 2387–2397

Stoeckle, M. Y., Das Mishu, M., & Charlop-Powers, Z. (2020). Improved environmental DNA reference library detects overlooked marine fishes in New Jersey, United States. *Frontiers in Marine Science*, **7**, 226

Vooren, C. M., & Lamónaca A. F. (2004). *Rhinoptera brasiliensis*. In *IUCN Red List of Threatened Species* 2004: e.T44595A10912274. Available at https://www.iucnredlist.org/species/44595/10912274 (last accessed 22 May 2020)

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Figure 1 *Rhinoptera* spp. sampling sites in the western North Atlantic. (a) triangles represent general locations where the Brazilian cownose ray *R. brasiliensis* Müller 1836 and the American cownose ray *R. bonasus* (Mitchill 1815) co-occurred and circles represent general locations where only *R. bonasus* occurred (TAM = Tampa Bay, n = 22; ALT = Altamaha River, n = 2; PAM = Pamlico Sound and its tributaries, n = 66; CHE = Chesapeake Bay, n = 23). For locations of co-occurrence, inset maps b–d show sites where each species was caught, with overall sample size for each location indicated (*R. brasiliensis* = triangles; *R. bonasus* = circles; SSB = St. Sebastian River)

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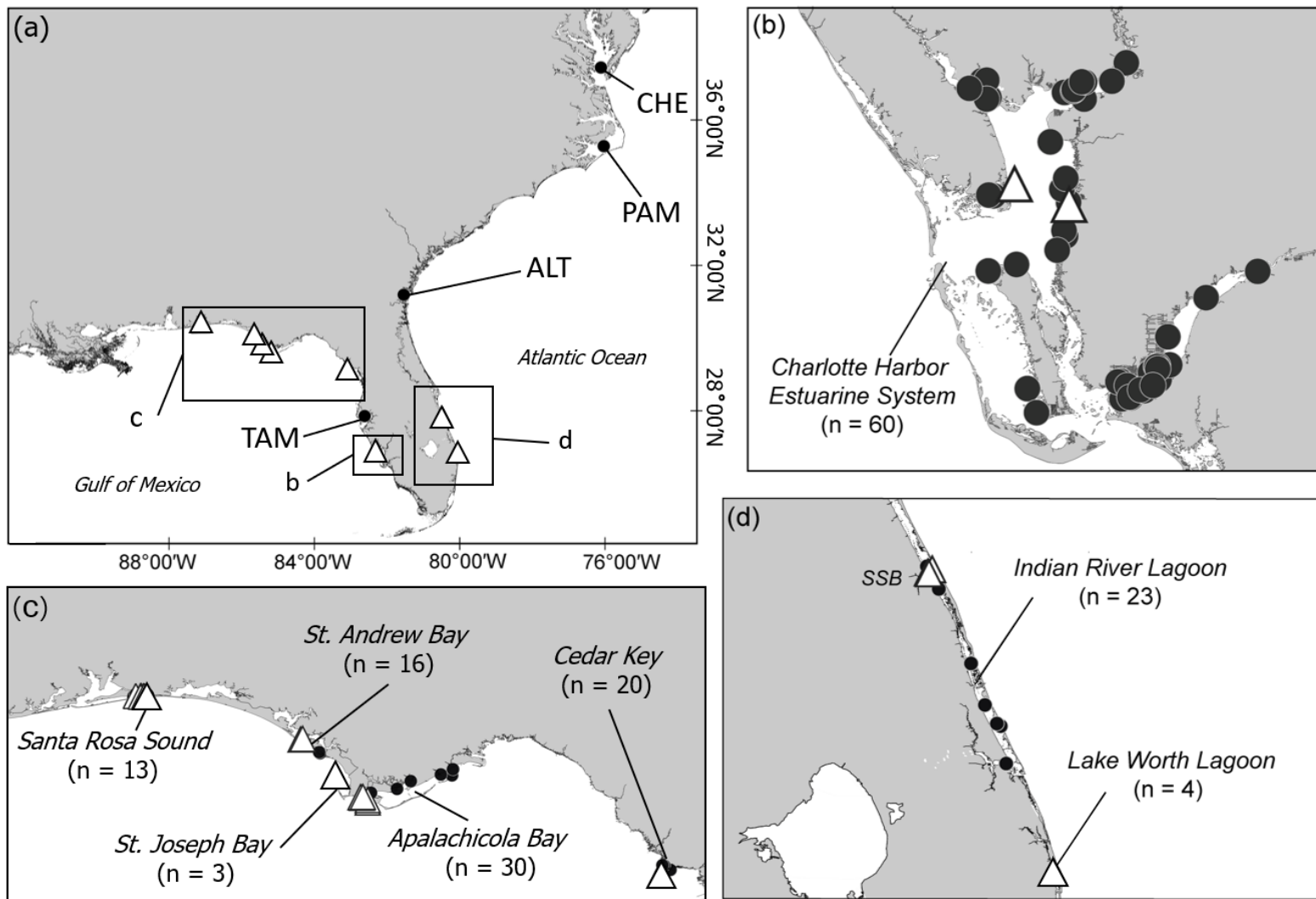
Figure 1

Significance statement

In the western Atlantic, the range of the Brazilian cownose ray *Rhinoptera brasiliensis* has been obscured by the sympatric, morphologically similar, *R. bonasus.* Here, genetic evidence supports a range extension for *R. brasiliensis* to the east coast of Florida, USA. This finding emphasizes the need to genetically verify species identities prior to undertaking biological and ecological studies of *Rhinoptera* spp. and that existing datasets may reflect a species complex rather than single species.

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Table 1. Biological details for the Brazilian cownose ray *Rhinoptera brasiliensis* Müller 1836 genetically identified, via a 442-base pair fragment of the mitochondrial NADH dehydrogenase subunit 2 gene, from sites off Florida in the eastern Gulf of Mexico (eGOM) and along the east coast of the USA (Atlantic). Specific dates and biological details were not available (NA) for samples collected from Santa Rosa Sound.

Region	Location	Date collected[a]	Season[b]	Sex	Life stage[c]	Disc width (cm)	Haplotype
eGOM	Santa Rosa Sound	2013–2017	NA	NA	NA	NA	RBRA1
		2013–2017	NA	NA	NA	NA	RBRA1
		2013–2017	NA	NA	NA	NA	RBRA1
		2013–2017	NA	NA	NA	NA	RBRA1
		2013–2017	NA	NA	NA	NA	RBRA1
	St. Andrew Bay	25/10/2013	Fall	F	Juvenile	55.0	RBRA1
	St. Joseph Bay	14/08/2012	Summer	M	Adult	90.0	RBRA1
	Apalachicola Bay	20/06/2013	Summer	F	Adult	88.5	RBRA1
		20/06/2013	Summer	F	Adult	71.5	RBRA1
		20/06/2013	Summer	F	Juvenile	49.0	RBRA1
		14/06/2012	Summer	F	Juvenile	48.0	RBRA1
		14/06/2012	Summer	M	Juvenile	40.0	RBRA1
		24/10/2013	Fall	M	Juvenile	49.0	RBRA1
	Cedar Key	06/12/2017	Winter	F	Adult	77.5	RBRA3
	Charlotte Harbor	17/10/2003	Fall	M	Juvenile	62.5	RBRA1
		15/12/2008	Winter	M	Juvenile	66.7	RBRA1
Atlantic	Lake Worth Lagoon	14/02/2018	Winter	M	Adult	74.0	RBRA1
		14/02/2018	Winter	F	Adult	78.4	RBRA1
		14/02/2018	Winter	F	Adult	80.2	RBRA1
	Indian River Lagoon	12/01/2018	Winter	M	Juvenile	61.3	RBRA1
		12/04/2017	Spring	M	Adult	87.0	RBRA5
		12/04/2017	Spring	M	Adult	87.5	RBRA1
		12/04/2017	Spring	M	Adult	85.1	RBRA1
		07/08/2017	Summer	F[†]	Adult	93.2	RBRA1

[a]DD/MM/YYYY
[b]Spring = March–May; summer = June–August; fall = September–November; winter = December–February
[c]Life stage of *R. brasiliensis* was inferred from disc width at 100% maturity for both sexes (> 71.2 cm disc width) of *R. bonasus* (Mitchill 1815) from Charlotte Harbor, Florida by Poulakis (2013)
[†]Gravid