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Sphyrna alleni sp. nov., a new hammerhead shark (Carcharhiniformes, Sphyrnidae) from the Caribbean and the Southwest Atlantic.

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Abstract

Hammerhead sharks (Family Sphyrnidae) comprise a monophyletic Miocene radiation of carcharhiniform sharks characterized by their laterally expanded and dorsoventrally compressed head ('cephalofoil'). The bonnethead shark (*Sphyrna tiburo*) is currently described as a single amphi-American hammerhead species composed of the subspecies *Sphyrna tiburo tiburo* in the Western Atlantic Ocean (WA) and *S. tiburo vespertina* in the Eastern Pacific Ocean (EP). Variation in mitochondrial DNA and cephalofoil shape suggest a species complex, with *S. tiburo* occurring in the U.S., Mexico, and Bahamas; *S.* aff. *tiburo* occurring from Belize to Brazil; and *S. vespertina* occurring in the EP. Morphometric, meristic, and genetic variation was used to resolve the bonnethead shark complex in the Western Atlantic. Twenty-three specimens (12 *S.* aff. *tiburo* from Belize and 11 *S. tiburo* from U.S.) were subject to sixty-one morphometric measurements and three meristic characters (counts of the number of precaudal vertebrae, lower and upper rows of functional teeth). An allometric formula was used to standardize any effect caused by differences in size of the individuals and data were analyzed with univariate and multivariate statistics. *Sphyrna* aff. *tiburo* and *S. tiburo* have non-overlapping vertebral counts (80-83 and 71-74 respectively) but no morphometric differences were detected. Although not captured in morphometric analysis, the cephalofoil of *S.* aff. *tiburo* has a more pointed anterior margin than *S. tiburo* that together with lobule shaped posterior margins gives the cephalofoil a distinctive shovel-shaped appearance. Concatenated mitochondrial sequences and 12 nuclear microsatellite markers clearly separated *S.* aff. *tiburo* and *S. tiburo.* We conclude that this complex comprises two species in the Western Atlantic, *S. tiburo* and *S. alleni* sp. nov., and we provide a description of the latter, which is distinguished by precaudal vertebral counts (80-83), a shovel-shaped cephalofoil with rounded posterior margins, and robust differences in mitochondrial and nuclear genetic markers. We suggest nuclear genetic and meristic examination of EP bonnetheads is needed to update the taxonomical status and redescribe *S. vespertina*.

Key words: Shovelbill shark, morphology, mtDNA, nDNA, phylogenetics, species complex, taxonomy

Introduction

Hammerhead sharks (Family Sphyrnidae) are a monophyletic lineage of carcharhiniform sharks first appearing in the Miocene (Gilbert 1967; Lim *et al.* 2010; Naylor 1992). They are characterized by their laterally expanded, dorsoventrally compressed head or 'cephalofoil' and currently comprise nine named species (Gilbert 1967; Lim

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et al. 2010; Quattro *et al.* 2013). Hammerhead sharks are one of the most threatened shark families mainly due to overexploitation, with all species but one (*Sphyrna gilberti*) being Globally listed as 'Vulnerable', 'Endangered', or 'Critically Endangered' by the International Union for the Conservation of Nature (IUCN. 2021). There are four species of small-bodied hammerheads (< 1.5 m total length at first maturity) that are endemic to the Americas (*Sphyrna tiburo*, *Sphyrna tudes*, *Sphyrna corona*, and *Sphyrna media*), with one species occurring only in the Eastern Pacific ("EP"; *S. corona*), one species occurring only in the Western Atlantic ("WA"; *S. tudes*) (Compagno 1984b; Gilbert 1967; Springer 1940), and two species occur in both oceanic basins, including the scoophead shark (*S. media*) and the bonnethead shark (*S. tiburo*).

The bonnethead shark (*S. tiburo*; Linnaeus, 1758) is unique among the sharks of the genus *Sphyrna* because it has only a slightly expanded cephalofoil. This species is distributed in the EP from California (U.S.) to Ecuador, and in the WA from North Carolina (U.S.) to southern Brazil, including the Gulf of Mexico and the Caribbean (Compagno 1984b; Jawad 2013). It was originally named and described as *Squalus tiburo* by Linnaeus in 1758 based on a dried specimen collected in an unknown location in 'America'. There are no type specimens. Almost two centuries later Springer (1940) described the bonnetheads in the EP as a different species, separating them from *S. tiburo* based on cephalofoil shape, but no further description of the species was made. While *S. vespertina* has a cephalofoil that comes to a point on the anterior margin, *S. tiburo* has a slightly narrower and more evenly rounded cephalofoil (Springer 1940). The holotype for *S. vespertina* is CAS-SU 11584 with paratypes CM 5675 and CAS-SU 11881 (Springer 1940). Gilbert (1967) revised the hammerhead family and synonymized *S. vespertina* and *S. tiburo* following Bigelow and Schroeder (1948) on the grounds that cephalofoil shape variation within WA *S. tiburo* collected from a wide geographic area included pointed forms that resembled *S. vespertina*. Gilbert (1967) recognized the subspecies *S. tiburo tiburo* for the WA (Linnaeus, 1758), and *S. tiburo vespertina* for the EP (Springer 1940). However, a recent study comparing bonnethead shark specimens from the WA and EP support the species status of *S. vespertina* based on diagnostic differences in cephalofoil shape, electroreceptor densities, and mitochondrial genetic markers separating it from all WA bonnethead sharks (Aroca *et al.* 2022).

Genetic studies conducted on WA bonnethead sharks using up to three mitochondrial markers: cytochrome oxidase I (COI), control region (mtCR), and NADH dehydrogenase subunit 2- (NADH2), and one nuclear marker: internal transcribed spacer (ITS-2) indicate that a species complex comprised of at least two divergent lineages occurs in the region, hereafter referred as *S. tiburo* and *S.* aff. *tiburo* (Fields *et al.* 2016; Gonzalez *et al.* 2019; Naylor *et al.* 2012). *S. tiburo* occurs in the U.S., Mexico, and The Bahamas, while *S.* aff. *tiburo* has been found in Belize, Panama, Colombia, Trinidad & Tobago, and Brazil (Aroca *et al.* 2022; Fields *et al.* 2016; Gonzalez *et al.* 2019, 2021; Naylor *et al.* 2012). Morphometric analyses revealed distinct cephalofoil shapes for *S. tiburo*, *S.* aff. *tiburo*, and *S. vespertina* (Aroca *et al.* 2022). The cephalofoil of *S.* aff. *tiburo* has a more pointed anterior margin than *S. tiburo* and resembles but is still quantitively distinct from that of *S. vespertina,* usually because the latter has relatively straight rather than rounded posterior cephalofoil margins (Aroca *et al.* 2022). The probable inclusion of *S. tiburo* and *S.* aff. *tiburo* in bonnethead shark specimens examined by Bigelow and Schroeder (1948) explains why cephalofoil shape within WA *S. tiburo* appeared to be sufficiently variable to synonymize it with *S. vespertina.* No other morphological or meristic comparisons have been made between these WA cryptic species.

The present study combines classic taxonomy with genetics to describe *S.* aff. *tiburo* as a new species: "*Sphyrna alleni* sp. nov.". We performed 61 morphometric measurements and 3 meristic counts to evaluate potential differences in the morphology of these cryptic species. We also expand genetic analyses using two mitochondrial markers, cytochrome oxidase I (COI) and the control region (mtCR), and 17 nuclear microsatellite markers specific for *S. tiburo* (Price *et al.* 2014). We discuss the status of the broader bonnethead complex, suggesting that similar meristic and nuclear genetic analyses are needed in the EP to formally resurrect and redescribe *S. vespertina*. As the small hammerhead sharks are a common component of the local fisheries in many Latin-American countries and have been reported as collapsed, extirpated, or data deficient (Cardeñosa *et al.* 2020; Gonzalez *et al.* 2021; Harper *et al.* 2014; Reis-Filho *et al.* 2014) it is essential to resolve their taxonomy and phylogeography to support conservation.

Materials and methods

Specimen Collection and Preservation. Twelve mature bonnethead sharks (six males and six females) diagnosable in the field as *S.* aff. *tiburo* based on capture location and having cephalofoils with pointed anterior margins were

provided by fishermen from Robinson's Point and Riversdale Village, Belize (Table 1, Figure 1). Another 11 mature bonnethead sharks (six males and five females) identified as *S. tiburo* based on capture location and having a semi-circular cephalofoil were collected in Panama City and Sarasota, Florida, U.S (Table 1, Figure 1). For all the specimens, a set of samples (fin, muscle, liver) was collected and preserved (95% ethanol, RNAlater, and frozen). The whole sharks were kept in ice baths to avoid decay. Sixty-one morphometric measurements were performed on each shark and meristic data were collected. Specimens were then injected with formalin 10% and fixed for three months in a container with formalin 10%. The final step of the preservation was made at the Florida Museum of Natural History and consisted of preserving the specimens in a 95% ethanol pool for another three months. Details of all specimens can be found in the 'Materials Examined" section. Fin and muscle samples were subsequent used for genetic analyses (see mitochondrial and nuclear markers sections).

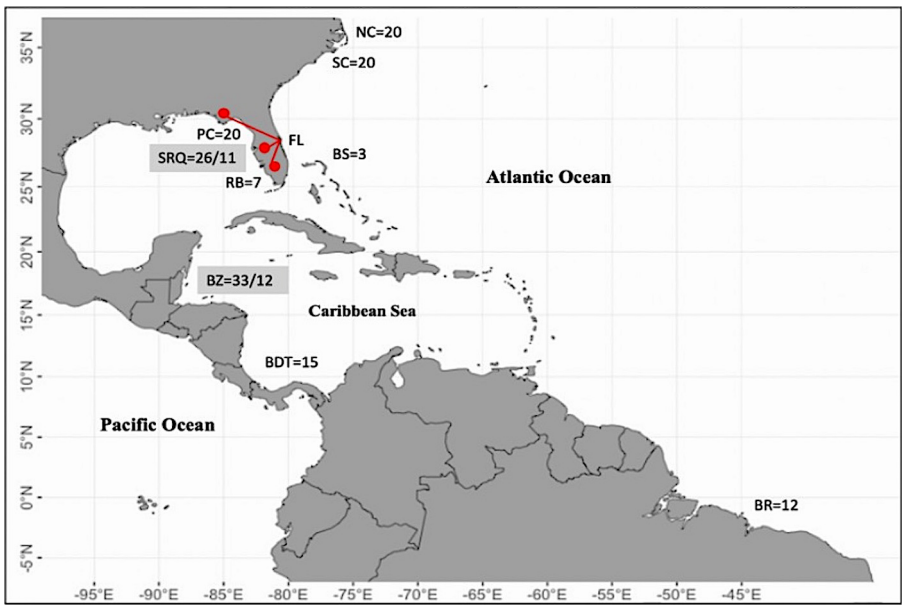

FIGURE 1. Sampling localities. Western Atlantic (WA): U.S. North Carolina (NC), South Carolina (SC), Florida (FL): Panama City (PC), Sarasota (SRQ), Rookery Bay (RB), Bahamas (BS); Caribbean: Belize (BZ), Bocas del Toro, Panama (BDT); Southwestern Atlantic: Brazil (BR). Grey boxes represent the two localities where specimens were also collected for morphometric, meristic, and genetic analyses (*S.* aff. *tiburo* N=12; *S. tiburo* N= 11). The information outside of boxes depict localities and number of samples used in the analysis of microsatellite DNA markers (*S.* aff. *tiburo* N=60; *S. tiburo* N=96).

Morphometric data. Measurements of precaudal (PCL), fork (FL), total (TL) and stretch total (STL) lengths were taken on a straight line along the axis of the body for 12 *S.* aff. *tiburo* and 11 *S. tiburo*. Subsequently, 61 morphometric features (see description) were measured following the methods from (Compagno 1984a).

Meristic data. Three meristic traits of all the specimens were counted: the total number of teeth on the upper and lower functional rows of the jaws, and the number of precaudal vertebrae (Springer & Garrick 1964). A dissection pin was inserted into the anterior margin of the precaudal pit and perpendicular to the body axis. Precaudal vertebrae counts (PVC) were then made in one of two ways. All the soft tissue was removed from 12 sharks (six *S.* aff. *tiburo* and six *S. tiburo*) from the tail to the brainstem, cleaning all the spine and counting the PCV by hand. The second approach consisted of taking X-rays of six well-preserved specimens (three *S.* aff. *tiburo* and three *S. tiburo*). Radiographs were taken of each specimen's vertebral column at the Veterinary Hospital of the University of Florida. Precaudal vertebrae counts were then performed by hand from the X-ray images obtained (Table 1).

Morphometric and Meristic Data Analysis. To remove any size effect on the 61 morphological traits, the data were size standardized by implementing an allometric formula, and later analyzed by using univariate and multivariate statistics following the methods by Elliot *et al.*, (1995) and Quattro *et al.*, (2013). Stretched total length (STL) was used as the measure of body length. All individual character measurements were standardized according to the formula (Elliott *et al.* 1995):

$M_{(Adj)} = M_o (L_s / L_o)^b$; where $M_{(Adj)}$ = *size-adjusted* measurement, *Mo* = original morphometric measurement, Ls= mean STL of each bonnethead from all samples for each variable, Lo= STL of specimen. and *b* was estimated by

the allometric growth equation $M = aL^{b}$. Parameter b was estimated as the slope of the regression of log M_o, on log L_o, for each character from the observed data.

TABLE 1. Summary of *S.* aff. *tiburo* and *S. tiburo* specimens used in this study. PVC= precaudal vertebrae count.

Species	Sex	TL (cm)	PVC	Specimen	UF-Museum Catalog #	Type	Collection site	Sample ID	Collector
S. aff. *tiburo*	F	103	83		UF245705;	Holotype		001BZN	
S. aff. *tiburo*	M	60.5	82	Preserved Formalin 10%	UF245723;			002BZN	Cindy Gonzalez
S. aff. *tiburo*	F	83	80		UF245724;			003BZN	
S. aff. *tiburo*	F	71.5	81		UF245725;	Paratype		004BZN	
S. aff. *tiburo*	M	63	83		UF245726;		Robinson's Point, Riversdale, Belize	005BZN	
S. aff. *tiburo*	M	64.5	82		UF245727;			006BZN	
S. aff. *tiburo*	F	85	81					017BZO	
S. aff. *tiburo*	F	77.5	80					008BZO	
S. aff. *tiburo*	F	74.8	82	Dissected	Collected 2016	Not Preserved		001BZO	Demian Chapman
S. aff. *tiburo*	M	81.6	81					019BZO	
S. aff. *tiburo*	M	90.5	80					005BZO	
S. aff. *tiburo*	M	88.7	80					009BZO	
S. tiburo	F	86	73	Preserved Formalin 10%	UF 247324;	Comparative material	Terra Ceia Bay, Sarasota, Panama City FL, U. S	6FLN	Jayne Gardiner, Tonya Wiley, John Carlson
S. tiburo	F	74.5	72		UF 247324;			12FLN	
S. tiburo	F	82	72		UF 247324;			10FLN	
S. tiburo	M	80.5	74		UF 247325;			7FLN	
S. tiburo	M	73.5	73		UF 247325;			8FLN	
S. tiburo	M	59.5	72		UF 247326;			11FLN	
S. tiburo	F	71.6	72					023FLO	
S. tiburo	F	82	72					008FLO	
S. tiburo	M	82.3	73	Dissected	Collected 2015			017FLO	
S. tiburo	M	76	74					006FLO	
S. tiburo	M	83.3	73					004FLO	

Principal Component Analysis (PCA) was used to independently examine the morphometric (continuous variables), and the meristic traits (discontinuous variables). The morphological variation to define groups among *S. tiburo* and *S.* aff. *tiburo* was assessed by examining principal components with eigenvalues greater than one and assessing the proportion of variation explained by those components, character weightings on components, and plots of component scores. A Discriminant Function Analysis (DFA) was used to evaluate the meristic and adjusted morphometric characters to identify the variables that explain differentiation between groups. Finally, the original data (Mo) were standardized as percent of STL and used to test for interspecific differences between means of individual morphometrics by using an ANOVA. When data were not normally distributed a Kruskal-Wallis was used to test whether samples originated from the same distribution. All statistical tests were considered significant at $\alpha = 0.05$.

Genetic Data and Analysis

Mitochondrial markers

Fin clip tissue samples from the *S. tiburo* and *S.* aff. *tiburo* specimens described earlier from Sarasota, Florida U.S (n=11) and Riversdale, Belize (n= 11) respectively, were stored in 95% ethanol and total DNA extraction was

performed with the Qiagen DNeasy Blood and Tissue Kit following the manufacturer´s protocol (Qiagen, Valencia, CA). A 563 base pair (bp) fragment of the mitochondrial cytochrome oxidase I (COI) was amplified for all the samples using the primers FishCoxI F (5´TCWACCAACCACAAAGAYATYGGCAC3´), and FishCoxI R (5´TARACTTCWGGGTGRCCRAAGAATCA3´), modified from Ward *et al.* (2005) and following their PCR conditions and thermal cycling profiles. A 579 bp fragment of the mitochondrial control region (mtCR) was amplified for all samples using the primers Pro-L (5`AGGGRAAGGAGGGTCAAACT3´), and 282H (5´AAGGCTAGGACCAAACCT3´) and the reaction and thermal cycling conditions described in Keeney *et al.* (2003). PCR products were purified using Exo-SAP (Thermo Scientific) and sequenced with both amplifying primers on an ABI 3730 DNA analyzer.

All sequences (COI and mtCR) were aligned, edited, and checked manually using Geneious v.2020.2.2 (http://www.geneious.com) software, which was also used to identify haplotypes (Maddison & Maddison 2000). Haplotype diversity (*h*), and nucleotide diversity (π) of the COI and mtCR fragments were calculated in Arlequin 3.5.1.2 (Excoffier & Lischer 2010) for the sampled localities. Ten sequences (five of each cryptic species) were selected and a concatenated alignment of both the COI and the CR was built. The program jModelTest v.2.3.1 was used to obtain the best model for DNA substitution (Posada 2008). After selecting the best model (TrN+I), PAUP (Excoffier & Heckel 2006) was used to build a concatenated neighbor joining tree, using *Sphyrna lewini* as the outgroup.

Nuclear Markers

PCR amplification and microsatellite genotyping. Fin clip tissue samples from *S. tiburo* (n=96) and *S.* aff. *tiburo* (n= 60) collected from a wide geographic area (Figure 1) were stored in 95% ethanol for genetic analyses, and genomic DNA extraction was performed using the Qiagen DNeasy Blood and Tissue Kit following the manufacturer´s protocol (Qiagen, Valencia, CA). They were genotyped for 17 polymorphic microsatellite markers (Price *et al.* 2014). Microsatellite primer and multiplexing details are provided in Table S1. PCR for microsatellite amplification were conducted using the Type-it Kit Qiagen Multiplex PCR Master Mix®, 25–70 ng of DNA and 0.4 μM of each primer in 5 μl of final reaction volume. Loci were organized into four multiplex sets per PCR reaction and were performed with sets of *S. tiburo* or *S.* aff. *tiburo*. The PCR protocol consisted on an initial step of 5 min at 95°C, followed by 29 cycles of 30 s at 95°C for denaturation, 90 s at 60°C for annealing, and 30 s at 72°C for extension, with an additional step for final extension of 30 min at 60°C PCR (Díaz-Jaimes *et al.* 2021). The PCR products for microsatellite loci were visualized using capillary electrophoresis and sized with Alexa725™ to score with the Geneious v.2020.2.2 software (http://www.geneious.com). The software Micro-Checker 2.2 (Van Oosterhout *et al.* 2004) was used to identify possible genotyping errors.

All loci successfully amplified were tested for presence of null alleles using the software Micro-Checker 2.2 (Van Oosterhout *et al.* 2004) and confirmed using GenePop (Rousset 2008). Nuclear genetic diversity was estimated as the number of alleles observed (N_a), and the average number of alleles per locus. Deviations from the Hardy–Weinberg equilibrium (HW) and linkage disequilibrium between all pairs of loci overall and within each cryptic species and sampling site were assessed by the exact test implemented in ARLEQUIN v. 3.5.2.2 (Excoffier & Lischer 2010), that was also used to calculate the observed heterozygosity (*HO*), expected heterozygosity (*HE*), and estimated inbreeding coefficient (F_{IS}) for each sampling site (burn-in period: 10,000 iterations, MCMC 100,000). Genetic differentiation between *S. tiburo* and *S.* aff. *tiburo* was tested using pairwise F_{ST} values calculated in ARLEQUIN v. 3.5.2.2, and *Jost's D* values (Jost *et al.* 2018) calculated using the software GENODIVE (Meirmans & Van Tienderen 2004). Average genetic diversity (AGD) defined as the gene diversity over all loci in each population, was calculated in ARLEQUIN v. 3.5.2.2. Loci or samples with more than 15% of missing data were removed from the analyses. All p-values were Bonferroni corrected.

We tested the hypothesis of two separate gene pools for *S. tiburo* and *S.* aff. *tiburo* by implementing the admixture model with correlated allele frequencies to estimate the most likely number of 'populations' (*K* clusters) in the data by using the software STRUCTURE (Pritchard *et al.* 2000). The Evanno method implemented in STRUCTURE HARVESTER v.1.0 (Earl & vonHoldt 2012) was used to estimate the best number of *K* clusters. Three independent runs were conducted (length burn-in period: 50,000; MCMC 500,000). The results were summarized in CLUMPP v.1.0 (Jakobsson & Rosenberg 2007) and were formatted and visualized in DISTRUCT (Rosenberg 2004).

Results

Morphometric analysis. The possible effects of the size on the 61 morphometric variables analyzed were eliminated. None of the correlation coefficients were significant, indicating that body size has no effect on any of the variables. PCA analysis extracted 14 components with eigenvalues greater than 1.0, accounting for 94.33% of the variability. The proportion of variation for the individual components and among components indicated that these continuous morphological characters cannot resolve *S. tiburo* and *S.* aff. *tiburo* (Figure 2).

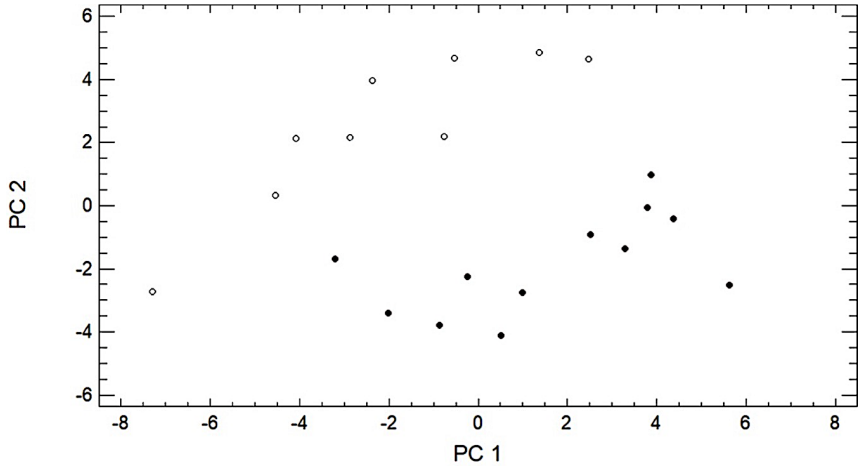

FIGURE 2. Plot of the first two principal components for 61 size-adjusted morphometric variables analyzed. Black circles indicate *S.* aff. *tiburo* specimens from Riversdale, Belize and white circles indicate *S. tiburo* specimens from Florida, U.S.

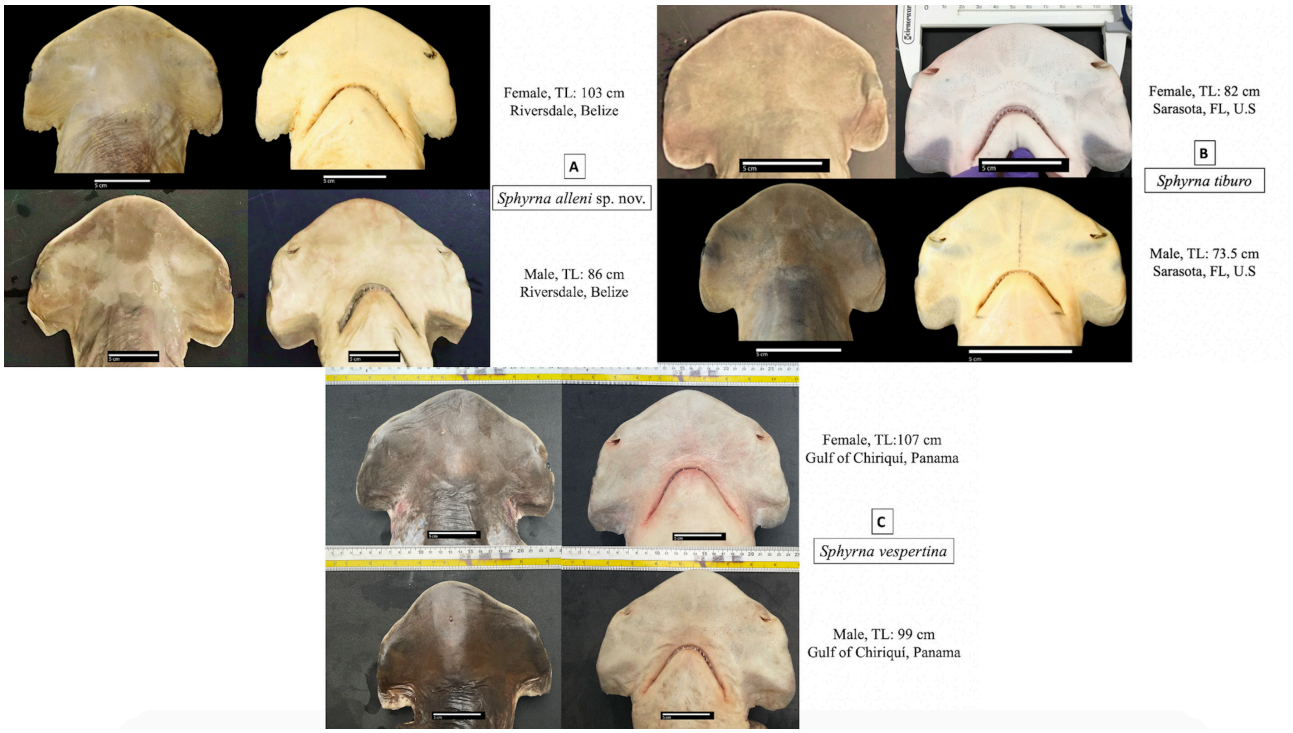

FIGURE 3. Cephalofoil comparison (dorsal and ventral views of each specimen are provided). Bar scale: 5cm. TL: Total length of specimen. A) *S. alleni* sp. nov.; B) *S. tiburo*; C) and *S. vespertina*. Photos: Cindy Gonzalez.

However, the anterior margin of all examined *S.* aff. *tiburo* cephalofoils have a noticeable triangular apex, with an additional bulge present in that of adult males (Figure 3). They also had noticeable lobule shaped head posterior margins, making the entire cephalofoil appear shovel shaped. In comparison, all *S. tiburo* specimens had a more

evenly rounded, semi-circular cephalofoil, with mature males having a bulge on the anterior margin (Figure 3). When comparing *S.* aff. *tiburo* and *S. vespertina* they also have triangular/ pointed cephalofoils, but the posterior margins of the head are straight, and the lobules are not present (Figure 3). These observations are all in line with the geometric morphometric analyses reported by Aroca *et al.* (2022).

Meristic Analysis. PCA analysis was run with three variables (PVC number of precaudal vertebrae, UT- upper functional teeth row, and LT- lower functional teeth row). The purpose of the analysis is to obtain a small number of linear combinations of the three variables, which account for most of the variability in the data. In this case, one component was extracted since only one component had an eigenvalue greater than or equal to 1.0. It accounted for 41.1213% of the variability in the original data.

The DFA of the meristic characters was also run with the three variables PVC, UT, and LT and was used to classify the meristic characters into two groups corresponding to *S. tiburo* and *S.* aff. *tiburo*. The rows of the UT and the LT were 25 in average for both species therefore this character was not useful to discriminate between species. The size adjusted data DFA identified the number of precaudal vertebrae as the only discriminator of *S. tiburo* and *S.* aff. *tiburo* (Figure 4), with 100% of specimens classified correctly (Table 2).

TABLE 2. Discriminant Function Analysis (DFA) of the percent of cases/specimens correctly classified: 100.00%.

Species	Size (n)	Species assigned	
		S.* aff. *tiburo	***S. tiburo***
S.* aff. *tiburo	12	12	0
		100.00%	0.00%
S. tiburo	11	0	11
		0.00%	**100.00%**

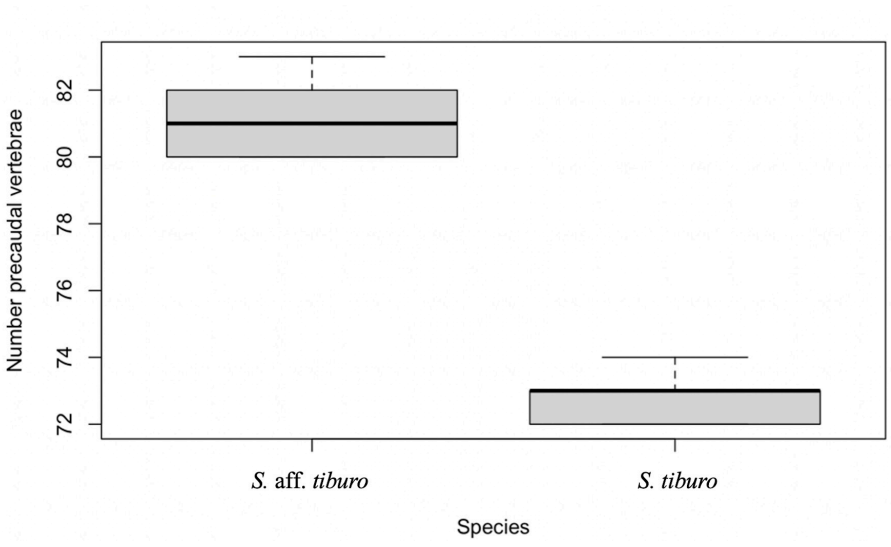

FIGURE 4. Boxplot of the number of precaudal vertebrae for *S.* aff. *tiburo* (Belize, n = 12) and *S. tiburo* (Florida, U.S, n = 11) specimens.

Mitochondrial DNA. The neighbor joining tree based on the concatenated 1,142 bp sequence (COI + mtCR) revealed *S. tiburo* and *S.* aff. *tiburo* to be reciprocally monophyletic with 89% bootstrap support (Figure 5).

Microsatellites. Twelve microsatellite loci were successfully amplified for *S. tiburo* (*n*= 96) and *S.* aff. *tiburo* (*n*= 60). After excluding individuals with >15% missing genotypes, 117 samples were included in our analyses: *S. tiburo* (*n*= 63), and *S.* aff. *tiburo* (*n*= 54). No evidence of null alleles was detected. The number of alleles ranged from 2 (locus Spti4) to 16 (locus Spti41) for *S. tiburo,* and between two (locus Spti44) and 16 (locus Spti48) for *S.* aff. *tiburo*. Genetic diversity values including the observed H_O and expected heterozygosities $H_{E,}$ were obtained for all loci and by locality across all loci (Table 3), along with deviations from HW equilibrium (Table 4). Allele frequencies only peripherally overlapped, and most alleles were unique to putative species (Figure 6).

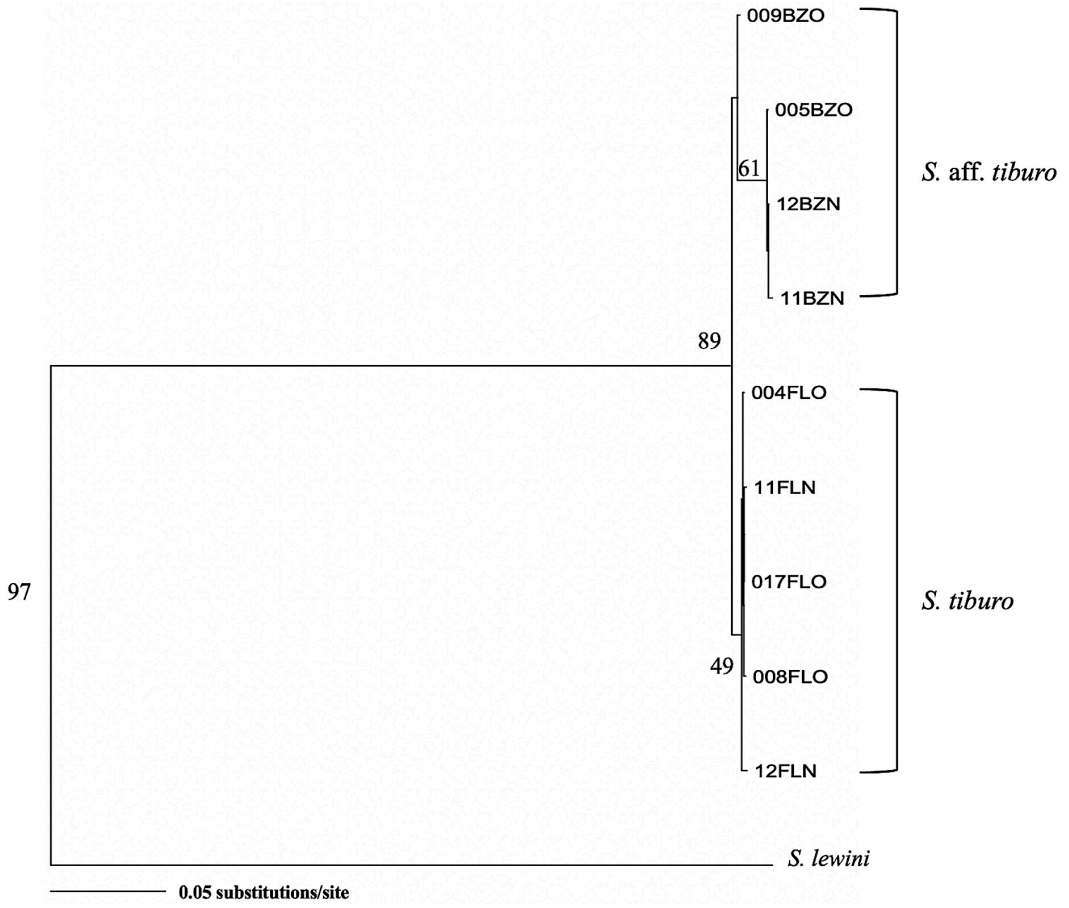

FIGURE 5. Neighbor joining concatenated tree (COI + mtCR) for *S.* aff. *tiburo* and *S. tiburo*. Branch lengths and scale represent the proportion of polymorphic sites between haplotypes, and bootstrap values given in percentage (%).

The K values calculated by STRUCTURE using the Evanno method identified $K = 2$ as the most likely number of clusters in the data, clearly separating the *S. tiburo* and *S.* aff. *tiburo* as distinct gene pools (Figure 7). Pairwise F_{ST} values and *Jost's D* values were calculated, and significant population differentiation was detected in all cases except between NC and FL (F_{ST}) and BZ and BDT (*Jost's D*) (Table 5).

TABLE 3. Genetic diversity per population across all loci. N= total sample size, *Ho* = observed and H_E expected heterozygosities, F_{IS}= inbreeding coefficient.

Species	Locality	N	H_O	H_E	F_{IS}
S. tiburo	FL	38	0.594	0.765	**0.177*****
	NC	13	0.652	0.766	**0.085***
	SC	12	0.613	0.760	**0.157****
S.* aff. *tiburo	BDT	15	0.658	0.718	0.058
	BR	7	0.597	0.747	**0.167****
	BZ	32	0.688	0.755	0.005

H_O, H_E, and F_{IS} were calculated keeping one representative per MLG and per sampling site. With the F_{IS} is indicated the test significance for deviation to Hardy–Weinberg equilibrium: $p < 0.05$*; $p < 0.01$**; and $p < 0.001$***. Localities: Florida, U.S (FL), North Carolina, U.S (NC), South Carolina, U.S (SC), Bocas del Toro, Panama (BDT), Brazil (BR), Belize (BZ).

TABLE 4. Genetic diversity for 12 microsatellite loci for *S. tiburo* and *S.* aff. *tiburo*.

		S. tiburo (n=63)									*S.* aff. *tiburo* (n=54)								
		FL (n=38)			NC (n=13)			SC (n=12)			BDT (n=15)			BR (n=7)			BZ (n=31)		
Locus	***A***	***nA***	H_O	H_E	***nA***	H_O	H_E	***nA***	H_O	H_E	***nA***	H_O	H_E	***nA***	H_O	H_E	***nA***	H_O	H_E
Spti2	10	10	0.71	0.87	9	0.92	0.89	6	0.80	0.80	8	**0.73**	**0.88**	8	**0.57**	**0.90**	10	0.87	0.88
Spti4	4	3	0.11	0.13	2	0.08	0.08	1	0	0	5	**0.85**	**0.73**	3	**0.20**	**0.64**	6	**0.60**	**0.77**
Spti9	6	5	0.63	0.70	5	0.77	0.79	5	0.83	0.81	3	0.13	0.13	1	0	0	3	0.22	0.23
Spti3	7	7	0.79	0.77	5	0.77	0.82	5	0.67	0.67	7	0.71	0.72	7	0.86	0.90	8	0.74	0.84
Spti10	14	13	0.84	0.87	12	1.00	0.92	7	0.75	0.74	5	**0.80**	**0.78**	5	0.80	0.87	8	**0.64**	**0.85**
Spti34	10	8	**0.55**	**0.84**	8	**0.45**	**0.90**	4	0.75	0.74	4	0.53	0.61	3	0.43	0.38	5	0.79	0.63
Spti44	7	7	**0.36**	**0.78**	5	**0.50**	**0.80**	4	**0.08**	**0.58**	2	0.50	0.49	2	0.00	0.26	3	0.45	0.52
Spti48	17	15	**0.69**	**0.92**	12	**0.67**	**0.95**	9	**0.67**	**0.90**	10	**0.57**	**0.89**	10	0.86	0.95	16	**0.58**	**0.94**
Spti42	11	11	0.89	0.89	7	0.91	0.88	7	0.88	0.82	9	0.80	0.82	7	0.86	0.89	10	0.87	0.87
Spti46	5	5	**0.35**	**0.75**	3	0.46	0.55	4	**0.08**	**0.75**	7	0.93	0.84	5	0.71	0.82	7	0.81	0.81
Spti26	8	8	0.65	0.77	5	0.69	0.73	6	0.64	0.69	6	**0.47**	**0.84**	4	0.57	0.70	8	0.69	0.81
Spti41	16	16	**0.56**	**0.90**	9	**0.60**	**0.89**	8	**0.60**	**0.88**	9	0.87	0.88	8	0.71	0.89	13	1.00	0.90

U.S locations: FL: Florida; NC: North Carolina; SC: South Carolina; Caribbean: BDT: Bocas del Toro, Panama; BR: Maranhao, Brazil; BZ: Riversdale, Belize. *n*: samples per location; *A*: number of alleles across all populations; *n*A: number of alleles at each locus; H_O and H_E observed and expected heterozygosity. Loci out of Hardy Weinberg equilibrium shown in bold before Bonferroni correction ($P<0.05$), and in bold highlighted in grey for loci out of HW equilibrium after Bonferroni correction ($P<0.001$) for *S. tiburo* and ($P<0.001$) for *S.* aff. *tiburo*.

TABLE 5. Pairwise FST values (above diagonal) and Jost's D values (below diagonal) for the microsatellites analyzed. Western Atlantic, U.S: Florida (FL), North Carolina (NC), South Carolina (SC). Caribbean and Southwestern Atlantic: Bocas del Toro, Panama (BDT), Brazil (BR), and Belize (BZ).

FST Jost's D	FL (n=38)	NC (n=13)	SC (n=12)	BDT (n=15)	BR (n=7)	BZ (n=31)
FL	AGD=0.6884 +/- 0.3575	0.0116	0.0344***	0.1572***	0.1553***	0.1366***
NC	0.0204	AGD=0.6943 +/-0.3794	0.0322**	0.1701***	0.1579***	0.1547***
SC	0.0997***	0.0708	AGD=0.6393 +/- 0.3535	0.2176***	0.20389**	0.1931***
BDT	0.5287***	0.5689***	0.6546***	AGD=0.6709 +/- 0.3566	0.0724***	0.0055
BR	0.4805***	0.4709***	0.5727***	0.1922***	AGD=0.6703 +/- 0.3777	0.0621***
BZ	0.4886***	0.5363***	0.6089***	0.0117	0.1695**	AGD=0.6405 +/- 0.3378

Significant P values at <0.005*, <0.002** and < 0.001***. Probability values based on 10,000 permutations. Significant P values (P <0.002 after Bonferroni correction) in bold. Average genetic diversity values (AGD) are shown in the diagonal for each locality. Numbers of samples of each locality in parentheses.

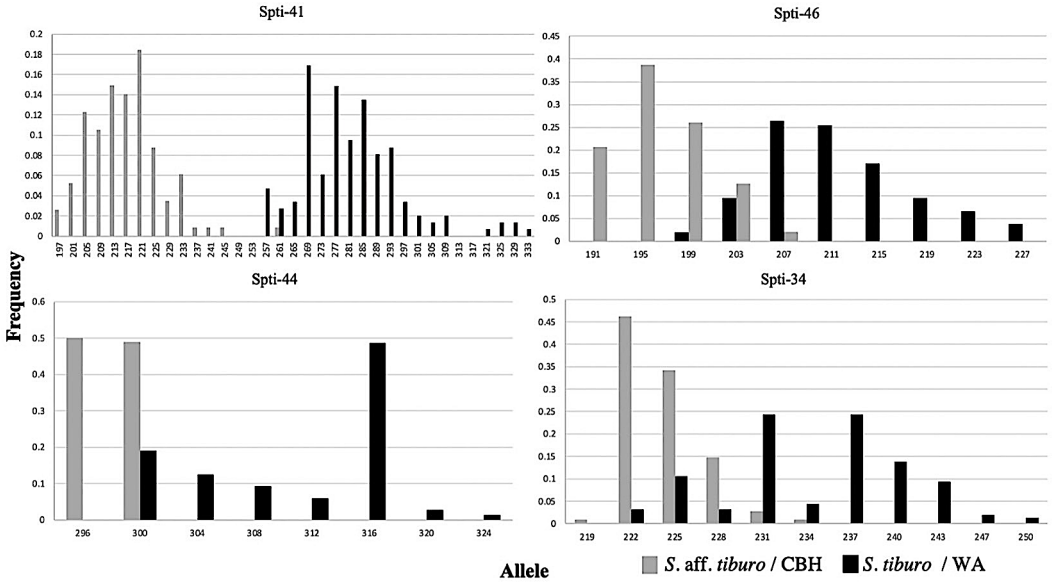

FIGURE 6. Allele frequencies for four microsatellite loci: Spti 41, Spti 44, Spti 46, and Spti 34. Grey bars represent *S.* aff. *tiburo* and black bars represent *S. tiburo* specimens analyzed (See supplementary materials: S2 for the other eight loci analyzed).

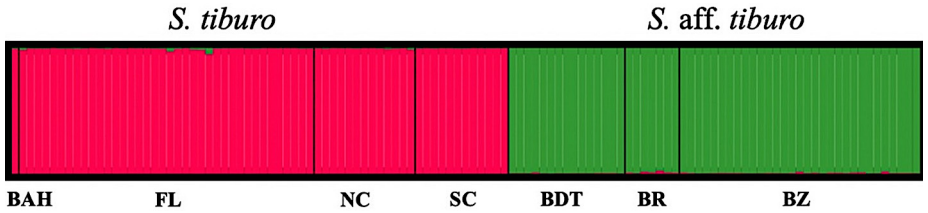

FIGURE 7. STRUCTURE bar plot showing the assignment probabilities (*K*=2) of each genotyped individual of *S. tiburo* and *S.* aff. *tiburo* from seven different localities. BAH: Bahamas; FL: Florida, U.S; NC: North Carolina, U.S; SC: South Carolina, U.S; BDT: Bocas del Toro, Panama; BR: Maranhão State, Brazil; BZ: Riversdale, Belize. *K*-groupings correspond to: Red: Western Atlantic (Bahamas and U.S locations: North Carolina South Carolina, U.S); and Green: Southwestern Atlantic and the Caribbean (Bocas del Toro, Panama; Maranhão State, Brazil; and Riversdale, Belize).

***Sphyrna alleni* sp. nov.**

Family: Sphyrnidae, Carcharhiniformes; Genus *Sphyrna,* Gilbert 1967.
Sphyrna tiburo Bigelow & Schroeder, 1948; Gilbert, 1967; Compagno, 1973, 1979, 1988.
Sphyrna cf. *tiburo* Naylor, 2012; Fields, 2016; Gonzalez, 2019; Gonzalez, 2021.
Sphyrna aff. *tiburo* Gonzalez, 2021; Aroca, 2022.

Proposed common name. Shovelbill Shark (EN), Requin-marteau pelle (FR), Tiburón Cabeza de Pala (SP) (Figure 8).

Materials examined. *Sphyrna alleni* sp. nov. specimens were collected at Robinson Point and Riversdale Village, Belize, and were provided by local fishermen in 2016 and 2019. *S. tiburo* specimens were collected in Panama City, Terra Ceia Bay, and Sarasota Bay, FL between 2015–2021 and provided by Dr. J. Carlson from NOAA and Dr. J Gardiner from New College of Florida. Type specimens were placed in the collection of the Florida Museum of Natural History (see Table 1 for details).

Holotype. UF 245705, female, 103 cm TL. Riversdale, Riversdale, Belize; Collected: February 2019 by C. Gonzalez (Figure 9).

Paratypes. All from Riversdale, Riversdale, Belize., and collected by C. Gonzalez in February 2019. UF 245723, male, TL 60.5 cm; UF 245724, female, 83 cm; UF245725, female, TL 71.5 cm; UF245726, male, TL 63 cm, UF245727, male, 64.5 cm.

Non-Types. Six *S. alleni* sp. nov., sharks collected by D. Chapman and local fishermen in 2016 at Robinson Point, Riversdale, Belize, decayed after fixing them with 10% formalin, probably because the meat was decomposed after shipping delays. After taking the measurements and correspondent genetic samples, the vertebrae and the jaws were kept frozen at the Mote Marine Laboratory & Aquarium, Sarasota, FL.

Diagnosis. Small hammerhead shark (<150 cm at maturity) with a flat, shovel shaped head that lacks indentations on its anterior edge. Cephalofoil anterior margin is pointed (like a tringle) in both sexes and the posterior margins are lobule shaped. The anterior margin of males exhibits a pronounced bulge. Enlarged, molariform posterior teeth, first dorsal rear tip in front of pelvic origins, and shallowly concave posterior anal margin. It is distinct from *S. tiburo* because in this species the anterior margin of the head is more rounded and the lobules on the posterior margin are not present. Precaudal vertebral counts for *S. alleni* sp. nov., are between 80-83 (~10 more vertebrae than *S. tiburo*).

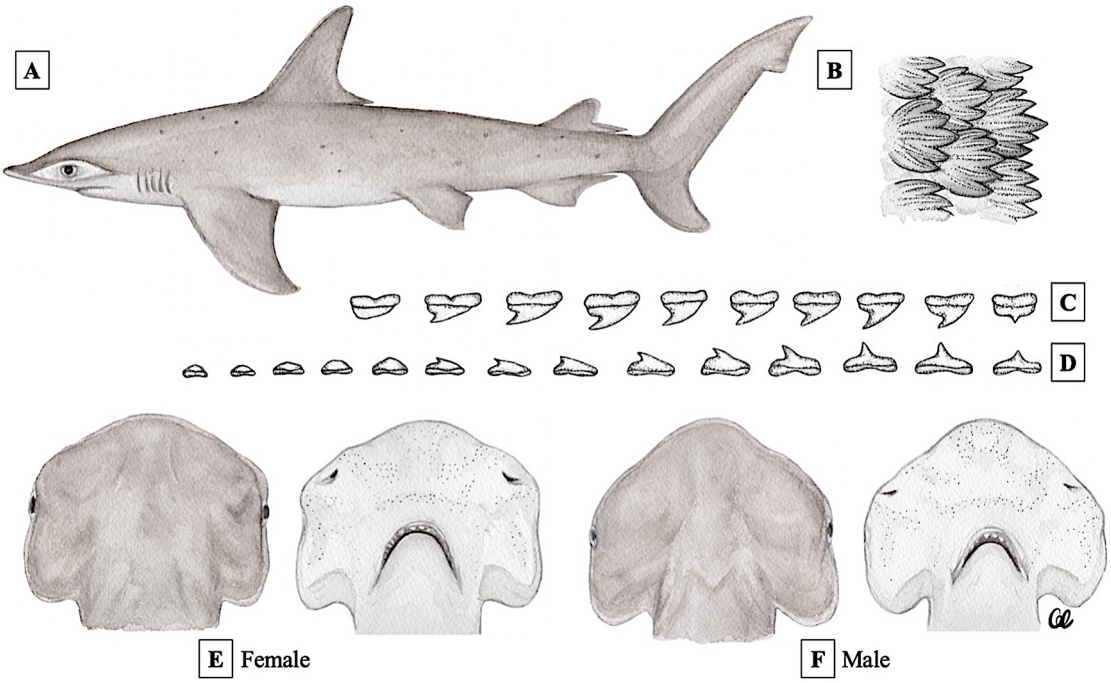

FIGURE 8. *Sphyrna alleni* sp. nov. A) Horizontal view of the shark; B) dermal denticles; C) upper and D) lower teeth; Dorsal and ventral representation of the head shape of E) Female and F) Male. Illustrations: Gina Clementi.

TABLE 6. Proportional measurements for *S. alleni*. sp. nov., and *S. tiburo*. ANOVAs values are followed by p-values in parentheses. Values not reported when the data was non normal distributed. When the data was non normally distributed a Kruskal-Wallis (K-W) test was performed and p-values are reported in parentheses.

	Morphological Character		*n*	Mean (cm)	(S.D)	Range	ANOVA	K-W test
1	**Head length**	*S. alleni*	12	16.48	2.13	13.4–19.71	0.488 (0.49)	
		S. tiburo	11	16.15	1.53	12.5–18		
2	**Pre-first dorsal length**	*S. alleni*	12	24.81	4.31	19.5–35	3.673 (0.07)	
		S. tiburo	11	23.75	2.41	18.4–27.3		
3	**Pre-second dorsal length**	*S. alleni*	12	50.02	8.92	38–68.5	3.717 (0.07)	
		S. tiburo	11	48.4	5.19	36–54.5		
4	**Pre-pectoral length**	*S. alleni*	12	16.31	2.24	13.5–21	0.805 (0.38)	
		S. tiburo	11	15.9	1.49	12.2–17		
5	**Pre-pelvic length**	*S. alleni*	12	36.58	6.65	28–51	3.289 (0.08)	
		S. tiburo	11	35.12	3.69	26.7–40.9		
6	**Preanal length**	*S. alleni*	12	47.25	8.51	36–65	0.359 (0.55)	
		S. tiburo	11	46.59	4.99	35.3–54.2		
7	**Snout-vent length**	*S. alleni*	12	38.85	7.51	28.8–54.5		6.367 (0.012)*
		S. tiburo	11	36.33	4.69	27.5–45.2		
8	**Inter-dorsal space**	*S. alleni*	12	19.08	3.53	14.2–26.2	0.877 (0.36)	
		S. tiburo	11	18.40	2.46	12.5–21.5		
9	**Second dorsal-caudal space**	*S. alleni*	12	6.06	1.16	4.5–8.2	24.73 (6.39e-05) ***	
		S. tiburo	11	6.55	0.66	5.1–7.7		
10	**Pectoral-pelvic space**	*S. alleni*	12	17.18	3.53	12–23.6		7.670 (0.005)
		S. tiburo	11	15.82	2.06	12–18		
11	**Pelvic-anal space**	*S. alleni*	12	7.02	1.69	5–10.1	0.635 (0.43)	
		S. tiburo	11	7.30	1.40	5–9.5		
12	**Anal-caudal space**	*S. alleni*	12	4.60	0.86	3.5–6	31.54 (1.42e-05) ***	
		S. tiburo	11	5.63	0.66	3.9–6.36		
13	**Pelvic-caudal space**	*S. alleni*	12	16.80	2.68	13.2–21.2	8.093 (0.009) **	
		S. tiburo	11	17.75	1.99	13.8–20.4		
14	**Vent-caudal length**	*S. alleni*	12	40.91	6.17	32.1–53		6.368 (0.012)*
		S. tiburo	11	35.54	6.00	24.7–43.8		
15	**Head width**	*S. alleni*	12	13.17	1.44	10.97–15.3	14.9 (0.000) ***	
		S. tiburo	11	12.31	1.01	10.45–14.1		
16	**Snout length**	*S. alleni*	12	5.63	0.75	4.68–7.2	5.188 (0.033) *	
		S. tiburo	11	5.31	0.44	4.42–6		
17	**Eye length**	*S. alleni*	12	1.40	0.21	1.1–1.74		0.640 (0.428)
		S. tiburo	11	1.38	0.08	1.25–1.55		

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TABLE 6. (Continued)

	Morphological Character		*n*	Mean (cm)	(S.D)	Range	ANOVA	K-W test
18	**Eye height**	*S. alleni*	12	1.01	0.14	0.85–1.35	0.066 (0.8)	
		S. tiburo	11	1.02	0.13	0.7–1.15		
19	**Internarial space**	*S. alleni*	12	9.05	1.13	7.61–11.5	46.17 (1.02e-06) ***	
		S. tiburo	11	8.32	0.65	6.8–9.3		
20	**Nostril length**	*S. alleni*	12	1.28	0.72	0.78–3.53	19.64 (0.000) ***	
		S. tiburo	11	1.05	0.06	1–1.2		
21	**Mouth width**	*S. alleni*	12	5.26	1.09	3.79–7.68	0.002 (0.962)	
		S. tiburo	11	5.13	0.54	4.1–6		
22	**Mouth length**	*S. alleni*	12	3.33	0.55	2.55–4.32	0.493 (0.49)	
		S. tiburo	11	3.20	0.34	2.7–3.75		
23	**Intergill length**	*S. alleni*	12	3.99	0.93	2.85–6.55		0.0038 (0.951)
		S. tiburo	11	3.82	0.50	3.2–4.6		
24	**First gill slit height**	*S. alleni*	12	2.24	0.44	1.54–2.96	2.968 (0.09)	
		S. tiburo	11	2.05	0.28	1.5–2.67		
25	**Second gill slit length**	*S. alleni*	12	2.45	0.41	1.78–3.12		3.640 (0.06)
		S. tiburo	11	2.27	0.29	1.8–2.9		
26	**Third gill slit length**	*S. alleni*	12	2.53	0.42	1.97–3.57	0.053 (0.82)	
		S. tiburo	11	2.48	0.23	2.13–3		
27	**Forth gill slit length**	*S. alleni*	12	2.54	0.48	1.85–3.34	0.221 (0.64)	
		S. tiburo	11	2.55	0.27	2.23–3.15		
28	**Fifth gill slit length**	*S. alleni*	12	2.27	0.49	1.6–3.22	0.078 (0.78)	
		S. tiburo	11	2.19	0.33	1.85–2.82		
29	**Pectoral anterior margin**	*S. alleni*	12	11.55	2.06	8.6–15.3	4.563 (0.04) *	
		S. tiburo	11	11.67	0.98	9.62–12.7		
30	**Pectoral base**	*S. alleni*	12	4.35	0.97	2.95–6.3		1.670 (0.19)
		S. tiburo	11	4.32	0.40	3.5–4.9		
31	**Pectoral inner margin**	*S. alleni*	12	4.20	0.73	3.05–5.35	0.073 (0.79)	
		S. tiburo	11	4.15	0.53	3–4.8		
32	**Pectoral posterior margin**	*S. alleni*	12	9.80	2.08	7.07–13.44	4.071 (0.05)	
		S. tiburo	11	9.02	0.90	7.67–10.4		
33	**Pectoral height**	*S. alleni*	12	10.44	2.08	7.45–14.69		3.409 (0.06)
		S. tiburo	11	9.74	0.97	8–11.2		
34	**First dorsal anterior margin**	*S. alleni*	12	12.93	2.12	10.12–17.2	7.188 (0.01) *	
		S. tiburo	11	12.19	1.07	9.8–14		
35	**First dorsal base**	*S. alleni*	12	6.52	1.25	4.83–9.15	0.001 (0.97)	
		S. tiburo	11	6.38	0.85	4.8–7.56		
36	**First dorsal inner margin**	*S. alleni*	12	4.65	2.69	2.65–12.45	0.192 (0.66)	
		S. tiburo	11	4.02	0.87	2.83–5.55		

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TABLE 6. (Continued)

	Morphological Character		*n*	Mean (cm)	(S.D)	Range	ANOVA	K-W test
37	**First dorsal posterior margin**	*S. alleni*	12	9.12	1.74	6.5–12.2	0.058 (0.81)	
		S. tiburo	11	9.01	1.01	6.7–10.3		
38	**First dorsal height**	*S. alleni*	12	9.84	2.17	6.32–12.33	0.254 (0.62)	
		S. tiburo	11	9.38	1.08	7.1–10.71		
39	**Second dorsal anterior margin**	*S. alleni*	12	4.89	1.40	3–8.75		0.969 (0.325)
		S. tiburo	11	4.87	0.55	3.87–5.83		
40	**Second dorsal base**	*S. alleni*	12	2.80	0.44	1.95–3.44	2.337 (0.14)	
		S. tiburo	11	3.03	0.58	2–4		
41	**Second dorsal inner margin**	*S. alleni*	12	4.18	0.69	3.03–5.35	4.316 (0.050)	
		S. tiburo	11	4.37	0.47	3.35–4.95		
42	**Second dorsal posterior margin**	*S. alleni*	12	4.61	0.81	3.35–6.2	2.514 (0.13)	
		S. tiburo	11	4.81	0.72	3.6–5.8		
43	**Second dorsal height**	*S. alleni*	12	3.45	0.77	2.25–4.44	0.238 (0.63)	
		S. tiburo	11	3.47	0.55	2.4–4.2		
44	**Pelvic anterior margin**	*S. alleni*	12	5.52	0.93	4–7	0.219 (0.64)	
		S. tiburo	11	5.51	0.74	4.3–6.48		
45	**Pelvic base**	*S. alleni*	12	4.44	0.91	2.66–6.13	0.058 (0.81)	
		S. tiburo	11	4.39	0.67	3.43–5.52		
46	**Pelvic inner margin**	*S. alleni*	12	2.99	0.47	2.13–4	2.41 (0.14)	
		S. tiburo	11	3.21	0.42	2.45–3.8		
47	**Pelvic posterior margin**	*S. alleni*	12	4.93	0.94	3.49–6.88	1.737 (0.20)	
		S. tiburo	11	4.60	0.48	3.56–5.14		
48	**Pelvic height**	*S. alleni*	12	4.43	1.13	2.52–6	0.957 (0.34)	
		S. tiburo	11	4.57	0.73	3.5–5.66		
49	**Anal anterior margin**	*S. alleni*	12	3.41	0.72	2.38–5.05	2.993 (0.09)	
		S. tiburo	11	3.61	0.43	2.86–4.3		
50	**Anal base**	*S. alleni*	12	5.38	0.95	3.85–7.07	1.947 (0.18)	
		S. tiburo	11	5.06	0.64	4–5.8		
51	**Anal inner margin**	*S. alleni*	12	3.05	0.48	2.23–3.9		2.004 (0.157)
		S. tiburo	11	2.81	0.56	1.6–3.42		
52	**Anal posterior margin**	*S. alleni*	12	5.96	1.04	4.32–7.75	3.894 (0.062)	
		S. tiburo	11	5.46	0.79	4.22–6.57		
53	**Anal height**	*S. alleni*	12	2.32	0.67	1.41–3.5	0.112 (0.742)	
		S. tiburo	11	2.34	0.70	1.46–3.85		
54	**Dorsal caudal margin**	*S. alleni*	12	20.73	2.73	17.2–26.3	3.787 (0.06)	
		S. tiburo	11	19.94	1.62	16.3–21.5		
55	**Pre-ventral caudal margin**	*S. alleni*	12	8.28	1.20	6.57–10.54	9.828 (0.005) **	
		S. tiburo	11	7.59	0.91	6.05–9.33		

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TABLE 6. (Continued)

	Morphological Character		*n*	Mean (cm)	(S.D)	Range	ANOVA	K-W test
56	**Upper post-ventral caudal margin**	*S. alleni*	12	12.72	1.88	10.27–16.3	24.87 (6.18e-05) ***	
		S. tiburo	11	11.18	1.27	9–12.5		
57	**Lower post-ventral caudal margin**	*S. alleni*	12	3.78	0.75	3–5.55	0.732 (0.40)	
		S. tiburo	11	3.57	0.58	2.7–4.9		
58	**Caudal fork length**	*S. alleni*	12	5.83	0.72	4.72–6.66	4.737 (0.04) *	
		S. tiburo	11	5.50	0.58	4.3–6.4		
59	**Caudal fork width**	*S. alleni*	12	5.48	0.83	4.35–7.25	0.136 (0.72)	
		S. tiburo	11	5.47	0.50	4.7–6.5		
60	**Subterminal caudal margin**	*S. alleni*	12	2.42	0.42	1.97–3.4		0.136 (0.712)
		S. tiburo	11	2.15	0.36	1.35–2.52		
61	**Terminal caudal margin**	*S. alleni*	12	4.11	0.79	2.15–5.22		0.852 (0.356)
		S. tiburo	11	4.23	0.62	3.1–4.85		

Description. Proportional measurements expressed as a percentage of the total length (TL) are given for the specimens analyzed (Table 6).

Body slender; expanded and narrow pre-branchial head that is shovel-shaped, no indentation on the anterior margin, tip of the head with a triangular contour shape (Figure 8). Head dorsoventrally flattened and narrow but longitudinally elongated, measuring from 19 to 22% of total length (TL) in the specimens analyzed. Distance from tip of snout to mouth about a 34-36% of the head length; posterior margins of head short, forming a lobule shape with a pronounced angular termination; pre-narial grooves not present; mucous pores on the ventral side and on the top of the head distributed in a diffuse pattern, pores highly concentrated in the tip of snout, and surrounding the eyes. There is sexual dimorphism in the individuals, so males have a more pronounced triangular termination of the head than females that have a slightly more rounded head (Figure 3). Eyes oval, small, located at the lateral sides of the head with a diameter of about 1.2 cm (1.2- 1.5% of TL). Nostrils small, apertures transversely oval to tear shaped; internarial space about 66% of the total head width (11% of TL). Prominent rostral cartilage on the top of the head. Mouth about a 40% of the head width, broadly arched; symphysis of mouth; 25 teeth in average in the upper and lower rows of functional teeth; anterior teeth not serrated with short, smooth-edged cusps; posterior molar teeth with a broader basis, flattened, cusp-less, keeled, expanded, and rounded; lower teeth shorter than uppers. Labial furrow around corner of mouth on the lower jaw, but none on the upper jaw (Figure 3).

First dorsal moderately hooked or curved like a sickle on its anterior margin, its origin over the end of the inner margins of pectoral fins, vertical height of the dorsal fin about 60% and base about a 40% of the length of head; posterior margin slightly curved with very small serrations on the end, its free rear tip usually somewhat anterior to pelvic origins. Interdorsal space about 25% of the total length. Second dorsal fin small, moderately high, height is about 35% of the dorsal fin height, one centimeter less than anal fin, concave posterior margin like an “L” shape; inner margin moderately long, longer than fin height. Five gill openings extended after the posterior head lobules, evenly spaced; each slit increases a little bit after the first one, each slit is about 2.2-2.5 cm in length. Pectoral fins originate just after the gill openings, about 74% of the head length and proportional to 14% of the total length. Pelvic fins slightly curved at the corners, with posterior margins almost straight to slightly curved. Anal fins larger and longer than second dorsal fin; base of anal fin is 6.8 to 7.8% of the total length, its origin well in front of second dorsal origin; posterior margin shallowly concave. Caudal fin long about 25%–28% of the total length; well defined precaudal pit; lower lobule of the fork about 1/3 (36–40%) of the upper lobule; upper margin and subterminal caudal margin straight, fork width about ¼ (26%) of total caudal fin length. Precaudal vertebrae counts between 80 to 83, ten or nine vertebrae more than *S. tiburo* (72-74).

Teeth counts of the functional rows for the upper and lower jaw were 25 in average for each row. When compared to *S. tiburo* no significant differences in shape or structure of the jaws were found. The *S. alleni* sp. nov., sharks have anterior teeth that are pointier, narrower, and more sharpened than *S. tiburo,* that have wider teeth. Also, *S. alleni* sp. nov., the anterior and posterior teeth seem to be bigger than *S. tiburo*. Lower jaw: *S. alleni* sp. nov., have fewer posterior teeth without cusps with a flatter crown foot, fewer rows of teeth exposed (3-2 rows), and are more spaced than *S. tiburo*. Contrary, the arrangement of posterior teeth in *S. tiburo* is a clumped pattern and more rows of teeth are exposed (5-3 rows). Upper jaw: *S. alleni* sp. nov., have only one column of posterior teeth without cusps and flattened crown foots. The second column of teeth have a small cusp developed, that starts to get more prominent until the anterior teeth with well-defined cusp ends. In comparison, the upper jaws of *S. tiburo* have 3 columns of posterior teeth that are cusp-less and flattened. Only in the 4th column of posterior teeth the cusps start to develop, but they are not as pointed and sharp as the new species. Dermal denticles with 5 ridges imbricated or loosely spaced in the body (Figure 8).

Coloration. Color after preservation varies from light brown to dark gray; the ventral side tends to become "yellower" with formalin and keeps its original color if preserved in ethanol (Figure 9). In life color varies between gray or gray-brown on the dorsal side; white, or light beige on the ventral side, often with small dark spots on lateral sides of the body (Figure 10).

FIGURE 9. Holotype of *Sphyrna alleni* sp. nov. collected in Riversdale, Belize. UF 245705, Female, 103 cm TL. Photo: Zachary Randall, Digital Imaging Division, Florida Museum.

Distribution range. *S. alleni* sp. nov. is distributed in coastal waters, estuaries, coral reefs, seagrass beds, and sand bottoms from Belize to Brazil. Presence has been confirmed in the Caribbean in Belize, Panama, Colombia, Trinidad and Tobago, and in the southwestern Atlantic in Brazil. *S. tiburo* is distributed from North Carolina, U.S to Belize where a potential contact zone for the two species could occur between Mexico and Belize (Yucatan Peninsula).

Etymology. *S. alleni* sp. nov., is named after Paul G. Allen (1953-2018) who was an investor, co-founder of Microsoft, and philanthropist who, through the Paul G. Allen Family Foundation, has generously supported shark research and conservation.

Comparative material. Eleven *S. tiburo* specimens from Sarasota Bay, FL, U.S were used. From those, six specimens were fixed and kept at the Florida Museum of Natural History. UF 247324 (n=3): female, TL 86; female, TL 74.5; female, TL 82, Collected 07/20/20; UF 247325 (n=2): male, TL 80.5; male, TL 73.5, Collected 10/13/20; UF 247326 (n=1): male (Figure 10), TL 59.5 Collected 08/18/20, all specimens collected by J. Gardiner. The other five specimens were measured, genetic samples were taken, and the vertebrae and teeth were cleaned and counted by hand. The vertebrae and jaws were kept frozen at the Predator Ecology and Conservation Lab at Florida International University. They correspond to the following individuals: FLO-017, male, TL 82.3 cm; FLO-023, female, TL 71.6; FLO-004, male TL 83.3; FLO- 008, female TL 82cm; FLO-006, male, TL 76 cm, collected by J. Carlson from NOAA in 2015 in Panama City, FL.

FIGURE 10. Paratype. External morphology and coloration of a fresh specimen of *Sphyrna alleni sp. nov.,* collected in Riversdale, Belize. UF: 247326, Male, 64.5 cm TL. Photos: Cindy Gonzalez.

Discussion

Here, we present multiple lines of evidence confirming that *S. tiburo* and *S. alleni* sp. nov., are distinct species. We reconfirm that they are reciprocally monophyletic when examining mitochondrial DNA, and they also separate into two gene pools when examining 12 nuclear microsatellites, with numerous private alleles observed (i.e., alleles unique to one species or the other). Although none of the morphometric measurements were able to separate them, visual inspection reveals a more pointed anterior margin of the cephalofoil in *S. alleni* sp. nov., which is aligned with previous geometric morphometric analysis (Aroca *et al.* 2022). This species is also separated from *S. tiburo* by non-overlapping precaudal vertebral counts, which is a common meristic trait used to classify closely related and cryptic shark species (Ebert & Compagno 2009; Quattro *et al.* 2013; Springer & Garrick 1964). We propose the new species: *Sphyrna alleni* sp. nov., with the common name 'Shovelbill', to acknowledge the name it is referred to by the people of Belize where the holotype was collected.

Sphyrna tiburo is distributed from North Carolina, U.S to Belize, while *S. alleni* sp. nov., is distributed from Belize to Brazil (Gonzalez *et al.* 2021). Major geological changes took place in Belize during the late Pliocene to the Holocene including sea-level fluctuations and changes in the carbonate platform (Mazzullo 2006). These events could have separated bonnetheads in this region and enabled speciation to occur. Indeed, vicariant events caused by sea-level changes during this geological time have been recognized as responsible for speciation in several plant and animal lineages in the Mesoamerican reef (Briggs & Bowen 2013), including sharks (Domingues *et al.* 2018, 2019). There is no contemporary geophysical barrier preventing contact between the two lineages and more sampling between Belize and Mexico (Yucatan Peninsula) could reveal the extent of sympatry and possibly hybridization between these species, as has been seen in other shark species complexes (Barker *et al.* 2019; Morgan *et al.* 2012). However, according to a relaxed molecular clock calculated by Fields *et al.*, (2016) these species diverged between 3·61 and 5·62 Mya., which means they could have diverged prior to the uplifting of the Isthmus of Panama (Montes *et al.* 2015; O'Dea *et al.* 2016). Given some similarity in cephalofoil shape in *S. alleni* sp. nov. and *S. vespertina* it is possible that they are sister lineages and *S. tiburo* diverged from them as it expanded into the subtropical and temperate Atlantic, with a later separation of *S. vespertina* and the incipient *S. alleni* sp. nov. by the Isthmus closure.

The phylogenetic and head morphology reconstruction from Aroca *et al.* (2022) also indicates that *S. tiburo* is a species complex composed of at least three distinguishable entities: two in the Western Atlantic: a southern central lineage (Caribbean), and a north- western one (i.e., Gulf of Mexico, Florida, South and North Carolina), and one in the eastern Pacific (Aroca *et al.* 2022). Detailed phylogenetic analysis of this complex is required to better understand their evolutionary history. We also highlight the need for further meristic and genetic investigation into *S. vespertina* to resurrect its species status and provide a fuller description.

Bonnetheads are currently assessed as Globally 'Endangered' by the International Union for the Conservation of Nature (IUCN) but they have been assessed as one amphi-American species. The assessment highlights that the species is well managed in higher latitude parts of its Northern Hemisphere Atlantic range (U.S., Bahamas) but heavily fished and poorly managed elsewhere, with evidence of population collapse in Brazil and throughout much of the Tropical Eastern Pacific (Cardeñosa *et al.* 2020; Harper *et al.* 2014; Pérez-Jiménez 2014; Reis-Filho *et al.* 2014). Reevaluating this assessment considering the geographic distribution of *S. tiburo* and *S. alleni* sp. nov. is now warranted. Given how fishing and management is distributed it is likely that the IUCN status of *S. tiburo* would improve and *S. alleni sp. nov.* would warrant a highly threatened status. Greater management attention is necessary to rebuild populations of *S. alleni* sp. nov., which could take the form of restrictions on gillnets and trawls as these gear types are responsible for most catches of this coastal species (Pollom *et al.*, 2021).

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Conflict of Interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Institutional Animal Care and Use Committee (IACUC)

All the samples were collected under the Belize fisheries department marine scientific research permit (Most recent permit for this work #0065-19). The methodology for sample collection and animal research was approved by the Wildlife Research group—Florida International University under the IACUC (Lab Animal Research -Record ID 30291259) and the Smithsonian Institute IACUC (Animal Care and Use—Record ID 20676904).

Author Contributions

Study concept and design: Cindy Gonzalez and Demian Chapman. Analysis and interpretation of data: Cindy Gonzalez, Bautisse Postaire, Susana Caballero, William Driggers, and Demian Chapman. Statistical Analysis: Cindy Gonzalez, Bautisse Postaire, William Driggers. Critical revision of the manuscript for important intellectual content: Demian Chapman, Susana Caballero. Study supervision: Demian Chapman, Susana Caballero.

Data Availability Statement

All relevant data are within the manuscript and its Supporting Information files.

Supporting Information

All relevant data are within the manuscript and its Supporting Information files (which can be downloaded at the DOI landing page of this paper). Data from this study are also available in GenBank NCBI (https://www.ncbi.nlm.nih.gov/genbank/). **Table S1** Microsatellite primer and multiplexing details. **Table S2** Allele frequencies for eight microsatellite loci: Spti 2, Spti 3, Spti 4, Spti 9, Spti 10, Spti 48, Spti 26, Spti 42.

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