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Cryptic hammerhead shark lineage occurrence in the western South Atlantic revealed by DNA analysis

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Abstract A cryptic lineage of hammerhead shark closely related to but evolutionarily distinct from the scalloped hammerhead (*Sphyrna lewini*) was recently documented in the western North Atlantic Ocean. Here, we demonstrate using nuclear and mitochondrial DNA sequences that this cryptic lineage also occurs in the western South Atlantic Ocean, extending its distribution >7,000 km from its only previously reported location. Our results also further validate the existence of this evolutionarily distinct hammerhead shark lineage. The southern hemisphere cryptic individuals were 1.6 and 5.8% divergent from *S. lewini* (sensu *stricto*) for the nuclear internal transcribed spacer 2 (ITS2) and mitochondrial control region loci, respectively, and formed a strongly supported, reciprocally monophyletic sister group to sympatric *S. lewini*. Coalescent analysis (ITS2 locus) yielded a divergence estimate of ~4.5 million years between *S. lewini* and the cryptic lineage. Given expanding concerns about overfishing of the large-bodied hammerhead sharks, this cryptic lineage needs to be formally recognized and incorporated into shark management and conservation planning to avoid the inadvertent, potential extirpation of a unique hammerhead lineage.

Introduction

One offshoot of the burgeoning use of DNA for species identification has been a surge in the discovery of cryptic species, with attendant ramifications for biodiversity assessment and wildlife management (Bickford et al. 2007; Richards et al. 2009). Recently, two independent studies have reported the unexpected existence in western North

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Atlantic waters of a cryptic lineage of large hammerhead shark (Sphyrnidae) closely related and morphologically very similar to the cosmopolitan and highly exploited scalloped hammerhead *Sphyrna lewini*. Abercrombie et al. (2005) first suggested the existence of a putatively cryptic hammerhead lineage based on the unanticipated discovery of fixed nucleotide differences in the nuclear ribosomal DNA internal transcribed spacer 2 (ITS2) locus of three individual sharks in a survey of 143 sharks morphologically identified as *S. lewini* and originating from globally widespread locations. Quattro et al. (2006) independently reported discovery of this cryptic hammerhead lineage, providing more extensive genetic and morphological (vertebral counts) analyses that confirmed its existence in the western North Atlantic. These authors found 18 specimens of the cryptic lineage (hereafter "*Sphyrna* sp.") within a set of 76 nominal *S. lewini* samples collected from globally distributed areas. Both mitochondrial (control region [CR] and cytochrome oxidase I [COI]) and nuclear (lactate dehydrogenase A intron 6 [LDHA6]) locus sequences demonstrated deep phylogenetic separation and reciprocal monophyly of the *Sphyrna* sp. and *S. lewini* (sensu *stricto*) lineages, despite their sympatric distribution in the US western North Atlantic (Quattro et al. 2006). Furthermore, there was a higher sequence divergence between these two lineages than between clearly differentiated populations of *S. lewini* (sensu *stricto*) from the Atlantic and Indo-Pacific oceans (Duncan et al. 2006; Quattro et al. 2006).

However, other studies examining the population genetics of nominal *S. lewini* from global distributions (Duncan et al. 2006; $n = 271$), Indonesia/Australia (Ovenden et al. 2009; $n = 47$ and Ovenden et al. 2011; $n = 237$), eastern Pacific (Nance et al. 2011; $n = 221$) and Mesoamerican Caribbean/Brazil in the western Atlantic (Chapman et al. 2009; $n = 140$), did not report finding *Sphyrna* sp. among their samples. Thus, based on a large global dataset of nominal *S. lewini* samples (>900 sharks) examined by genetic analyses, *Sphyrna* sp. has not been reported from outside the western North Atlantic.

Of note is that despite compelling genetic evidence and concordant preliminary morphological support (i.e., four specimens of each lineage showing non-overlapping vertebral counts; Quattro et al. 2006) for its existence, the *Sphyrna* sp. lineage remains enigmatic, taxonomically undescribed and overlooked in hammerhead shark fishery management and conservation planning. The apparently low-frequency occurrence of this lineage, reported based on identification of only 21 individuals out of 194 nominal *S. lewini* genetically investigated from the western Atlantic (total n compiled from Quattro et al. 2006; $n = 54$ and Chapman et al. 2009; $n = 140$), and its thus far detection only off the USA Atlantic coast ranging from North Carolina to Florida, may have contributed to a hesitancy to accept its existence and subsequent neglect in management and conservation efforts. However, with heavy fishing pressure on hammerhead sharks to supply the international fin trade and updated listings of the three known large hammerhead species as vulnerable or endangered (Casper et al. 2005; Baum et al. 2007; Denham et al. 2007), further evaluation of this cryptic lineage is prudent.

Here, we report on the unexpected discovery of this cryptic lineage in the western South Atlantic, demonstrating its occurrence over a broad geographic range that includes the southern hemisphere.

Materials and methods

Sampling and molecular identification

Two hundred and three nominal *S. lewini* landed in Brazilian fisheries between coordinates 0 28.0639 S, 46 47.2220 W and 32 23.9101 S, 52 03.4209 W from May 2005 to June 2009 were sampled for tissues (muscle, fin or gill) as part of a broader population genetics study. Sharks were preliminarily identified in the field using the diagnostic shape of the cephalofoil (hammer-shaped head). To exclude field species misidentification with two other large hammerhead species (*S. mokarran* and *S. zygaena*) known in Brazilian fisheries, we checked the species identity of our samples using the DNA isolation and ribosomal ITS2-based, species-specific primer methods of Abercrombie et al. (2005). Unexpectedly, the PCR screening failed to diagnose three of the 203 samples as originating from either *S. lewini*, *S. mokarran* or *S. zygaena*. A check of the landings records revealed that two of these "variant" individuals were caught off the south coast of São Paulo State and one off the coast of Santa Catarina State close to southern Brazil (i.e., approximately between 29 20.2477 S, 48 42.4378 W and 24 21.0856 S, 44 59.1282 W). None of the whole shark specimens were available to investigate their morphology. We therefore decided to further genetically investigate these three variant animals to elucidate their identity.

Mitochondrial and nuclear DNA sequencing

We PCR-amplified and sequenced the entire 655–675 bp of the nuclear ribosomal ITS2 locus of the hammerhead sharks using the shark universal primers FISH5.8SF and FISH28SR (Pank et al. 2001) and methods of Abercrombie et al. (2005). We also sequenced ~550 bp fragment of the initial portion of the mitochondrial control region (mtCR) using the primers CRF6 (forward primer 5′-AAGCGTCGA CTTTGTAAGTC-3′) and CRR10 (reverse primer 5′-CT TAGAGGACTGGAAATCTTGATCGAG-3′) using amplification and sequencing conditions outlined in Duncan et al. (2006). All sequence reactions were carried out using the

Table 1 List of shark species and nuclear and mitochondrial sequences analyzed

Species	Locus	GenBank acess. nos.	Reference
Carcharhinus plumbeus	CR	GU724583	Portnoy et al. (2010)
	ITS2	AY033820	Pank et al. (2001)
Eusphyra blochii	CR	GU385320	Lim et al. (2010)
	ITS2	GU385344	Lim et al. (2010)
	ITS2	JF899236	Present study
Sphyrna tiburo	CR	GU385313	Lim et al. (2010)
	CR	DQ168923; DQ168924	Quattro et al. (2006)
	ITS2	GU385340	Lim et al. (2010)
	ITS2	JF899242	Present study
Sphyrna corona	CR	GU385319	Lim et al. (2010)
	ITS2	GU385343	Lim et al. (2010)
Sphyrna media	CR	GU385317	Lim et al. (2010)
	ITS2	GU385342	Lim et al. (2010)
Sphyrna tudes	CR	GU385316	Lim et al. (2010)
	ITS2	GU385341	Lim et al. (2010)
	ITS2	JF899243	Present study
Sphyrna zygaena	CR	GU385314	Lim et al. (2010)
	ITS2	AY860839; AY860840	Abercrombie et al. (2005)
Sphyrna mokarran	CR	GU385315	Lim et al. (2010)
	CR	DQ168925	Quattro et al. (2006)
	ITS2	AY860837; AY860838	Abercrombie et al. (2005)
Sphyrna lewini	CR	GU014384-GU014391	Chapman et al. (2009)
	CR	GU385318	Lim et al. (2010)
	CR	DQ168917-DQ168920	Quattro et al. (2006)
	CR	DQ438149-DQ438172	Duncan et al. (2006)
	ITS2	AY858051; AY858052	Abercrombie et al. (2005)
	ITS2	JF899237-JF899241	Present study
Sphyrna sp.	CR	DQ168921; DQ168922	Quattro et al. (2006)
	CR	DQ438148	Duncan et al. (2006)
	CR	JF899230-JF899232	Present study
	ITS2	AY864857	Abercrombie et al. (2005)
	ITS2	JF899233-JF899235	Present study

Applied Biosystems BigDye Terminator v3.1 Cycle Sequencing Kit and resolved on the 3130 Genetic Analyzer (Applied Biosystems, www.appliedbiosystems.com) according to manufacturer's instructions. All sequences evaluated were aligned using Muscle (Edgar 2004) and checked with Bioedit (Hall 1999).

Sequence and phylogenetic analysis

We combined sequences generated in this study for ITS2 and mtCR with sequence data available in the literature for all eight known hammerhead species. Sequences from *Carcharhinus plumbeus* (GenBank accession numbers in Table 1) were used as outgroups for reconstructing ITS2 and mtCR gene trees. A total of 23 ITS2 and 53 mtCR sequences were gathered for all hammerheads analyzed (the complete alignments used in the final analyses are available upon request to D. Pinhal). FaBox (Villesen 2007) online was used for collapsing ITS2 and mtCR sequences into sequence types and haplotypes, respectively. The evolutionary model that best fit each dataset was selected by using the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) tests implemented in jModeltest (Posada 2008). Uncorrected and corrected genetic distances calculated in PAUP*4.0b10 (Swofford 2002) were used for estimating the genetic divergence between and within hammerhead species.

Maximum likelihood (ML) and Bayesian inference (BI) were applied to nuclear and mitochondrial sequences to evaluate phylogenetic relationships. Bayesian analyses were conducted using MrBayes v3.0b4 (Huelsenbeck et al. 2001) with four heated chains, each with five million generations, sampled every 100 generations, with the application of the stop rule command. Runs were checked for convergence

Table 2 Distinct ITS2 sequence types of *Sphyrna lewini* (sensu *stricto*) and *Sphyrna* sp. sharks showing number of individuals, their geographic origin and GenBank accession numbers

	ITS2 Sequence type	2 1 7	2 2 5	2 9 8	3 0 2	3 6 5	4 2 5	4 3 7	4 6 0	5 1 1	5 1 6	5 4 5	5 7 9	6 4 8	Atlantic	GenBank acess. nos.
S. lewini	1	C	C	C	A	C	G	A	C	G	C	C	C	C	6	AY858051; AY858052; 4 in present study
	2	.	.	.	.	.	.	.	.	.	.	.	.	T	1	Present study
Sphyrna sp.	3	G	T	T	C	–	A	T	T	T	T	–	T	C	4	AY864857; 3 in present study

Numbers on the top correspond to positions of polymorphic nucleotides for ITS2

using Tracer 1.4 (Rambaut and Drummond 2007). Burn-in was set up to discard the first 200 trees and the remaining trees used to generate the final consensus tree for each run. Maximum likelihood analyses were carried out in PAUP*4.0b10 (heuristic search, tree bisection-reconnection) and statistical support for nodes estimated by bootstrapping with 1,000 pseudo-replicates (Felsenstein 1985).

Estimating divergence time

The divergence time between *S. lewini* (sensu *stricto*) and the *Sphyrna cryptic* lineage was estimated using the nuclear ribosomal ITS2 sequences following divergence time estimates for nuclear loci inferred by Lim et al. (2010). Calibration points were assumed to be 45 million years ago (Ma) for the separation between Carcharhinidae and Sphyrnidae (Cappetta 1987), 15–20 Ma for the split between *Eusphrya* and *Sphyrna* and 10 Ma for the earliest divergence within the genus *Sphyrna* (Lim et al. 2010). These divergence times were applied to the Bayesian coalescent analysis implemented in BEAST v1.4.8 (Drummond and Rambaut 2007). The relaxed (uncorrelated lognormal) molecular clock method was employed when estimating time to the most recent common ancestor (TMRCA) and the 95% credibility intervals (CI) for various clades. We conducted two independent runs (chain length of 2,500,000; sampled every 1,000 iterations; Yule speciation process; 10% burn-in). We checked results for convergence and the posterior age distributions in Tracer 1.4 (Rambaut and Drummond 2007). A GTR+I+G model of nucleotide substitution was used, based on jModelTest. Tree topologies were assessed using TreeAnnotator v.1.4.6 (distributed as part of the BEAST package) and FigTree v.1.1.2 (Rambaut 2008).

Results

Both ITS2 and mtCR sequences of the three *Sphyrna* sp. hammerhead individuals discovered in the western South Atlantic (GenBank accession numbers in Table 1) exhibited several nucleotide polymorphisms distinguishing them from the sympatric *S. lewini* (sensu *stricto*) (Tables 2, 3) and from all other described hammerhead species (not shown). The ITS2 sequences of the three cryptic western South Atlantic animals were an exact match to the *Sphyrna* sp. sequences reported by Abercrombie et al. 2005 (GenBank Accession AY864857). The ITS2 sequence divergence (HKY corrected) between the *Sphyrna* sp. lineage and each of the eight taxonomically described hammerhead species ranged from 1.6 to 6.9% (Table 4), being lowest between *Sphyrna* sp. and *S. lewini* (sensu *stricto*). Mitochondrial control region sequences from the three western South Atlantic *Sphyrna* sp. hammerheads were identical to each other and differed from *S. lewini* (sensu *stricto*) by 5.3–5.8% sequence divergence (p- and K81+G distances, respectively). This level was consistent with the 5.3 and 5.6–7.5% divergence reported between these two lineages from the North Atlantic for the same locus (Quattro et al. 2006; Duncan et al. 2006, respectively).

The ITS2 sequences and mtCR haplotypes were unique for each of the eight described hammerhead species and *Sphyrna* sp. lineage. Comparison of genetic distances among all hammerhead species analyzed (Tables 4, 5) showed lower overall polymorphism within the ITS2 locus compared to the mtCR. Both ITS2 and mtCR sequences of the three western South Atlantic *Sphyrna* sp. specimens formed strongly supported monophyletic groups with their respective western North Atlantic cryptic lineage sequences (Abercrombie et al. 2005; Duncan et al. 2006; Quattro et al. 2006) (Figs. 1, 2). Both loci were also congruent in their support of the western South Atlantic *Sphyrna* sp. individuals as a sister group to *S. lewini* (sensu *stricto*) (Figs. 1, 2). The Bayesian coalescent analysis using ITS2 sequences estimated a divergence time between these two lineages of around 4.5 Ma (95% confidence intervals ~2–10 Ma).

Discussion

We show here using nuclear and mitochondrial DNA sequence analyses that a cryptic hammerhead shark lineage

Table 3 Distinct mtCR haplotypes of *Sphyrna lewini* (sensu *stricto*) and *Sphyrna* sp. sharks showing number of individuals, their geographic origin and GenBank accession numbers

	mtCR Haplotype	1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 4 4 4 4 4 4 4 4 4 4 4 5 5 5 5 0 1 4 4 4 5 2 3 3 3 4 4 7 7 1 2 3 3 5 5 6 6 6 8 8 8 8 8 8 1 4 4 5 5 6 9 2 2 4 4 4 5 7 7 7 7 9 9 1 2 3 3 5 6 6 7 9 0 0 3 7 8 1 4 2 9 8 5 3 7 7 9 2 3 5 0 1 5 7 9 0 3 6 4 7 2 3 3 5 0 1 2 2 5 6 7 8 3 4 5 9 0 1	Atlantic	Indo-Pacific	GenBank Acess. Nos.
S. lewini	1	T C T A T A A C T A T T A T A C C A T G T C C T C A C A C T A A C G A T A T A A A C C T A T A C C A G		2	DQ438152; DQ168920
	2	. . . . . T . . .		1	DQ438153
	3	. . . . . C		1	DQ438160
	4	. . . . . T T		1	DQ438170
	5	. . . . . T		2	DQ438171; DQ168919
	6	. . . . . C . . . . . T		1	DQ438157
	7	. . . . . T . . . . . T . . .		1	DQ438159
	8	. . . . . T . . . . . G		1	DQ438158
	9	. . . . . T		1	DQ438155
	10	. G . . . . . A . . A . . . T . . G C A - . . C T . . . T . . . . . C G . . . . A . . G		1	DQ438167
	11	. . . . . A . . A . . . T . . G C A - . . C T . . . T . . . . . C G . . . . A . . G		1	DQ438168
	12	. . . . . A . . A . . . T . . G C A - . . C T . . . . . C G		1	DQ438161
	13	. . . . . A . . A . . . . . G C A - . . C T . . . . . C G		1	DQ438169
	14	. . . . . A . . . C . . . . T . . . . . C G		1	DQ438162
	15	. . . . . A . . T C . . . . T . . . . . C G C G . . . . . C G		1	DQ438163
	16	. . . . . A . . T C . . . . T . . . . . G C G C G . . . . . C G		2	DQ438156; DQ168918
	17	. G . . . . . A . . A . . . T . . G C A - . . C T . . . T . . . . . C G		1	DQ438172
	18	. . . . . A . . A . T . T T . G C A - . . C T . . . . . C G . . . . A . . G		2	GU014389; DQ168917
	19	. . . . . A . . A . T . T T T G C A - . . C T . . . . . C G . . . . A . . G		1	GU014390
	20	. . . . . A . . A . T . T . . G C A - . . C T . . . . . C G . . . T A . . G . . T . . .		1	GU014391
	21	. . . . . A . . . . . G T		1	GU014384
	22	. . . . . A . . A . T . T T . G C A - . . C T . . . . . C G . . . . A . . T . . T . . .		1	GU014385
	23	. . . . . A		1	GU014386
	24	. . . . . T G . .		1	GU014387
	25	. . . . . A . . A . T . T T . G C A - . . C T . . . . . C G . . . . A . . T		1	GU014388
	26	G . . . . . A . . A . . . T . . G C A - . . C T . . . . G . . . . . C G		1	GU385318
	27	. . . . . A . . A . T . T T . G C A - . . C T . . . . . C G . . . . A . . G . . T . . .	1		DQ438150
	28	. . . . . A . . A . T . T T . G C A - . . C T . . . . . C G . . . . . T . . .	1		DQ438151
	29	. . . . . A . . A . T . T . . G C A - . . C T . . . . . C G . . . . A . . G . . T . . .	1		DQ438154
	30	. . . . . A . . A . T . T . . G C A - . . C T . . . . . C G . . . . A . . C	1		DQ438165
	31	. . . . . A . . A . T . T . . G C A - . . C T . . . . . C G . . . . A . . T	1		DQ438166
	32	. . . . . A . . A . T . T . . G C A - . . C T . . . . . C G . . . . . T . . .	1		DQ438149
	33	. . . . . A . . A . T . T . . G C A - . . C T . . . . . C G	1		DQ438164
Sphyrna sp.	34	. . A T G T C . A T C . . C . T . . . A C T . C T T . . . . T G T A . C T C G G . A T C T C G	5		DQ438148; DQ168921; this study
	35	. . A T G T C . A T C . . C . . . . . A C T . C T T . . . . T G T A . ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ?	1		DQ168922

Numbers on the top correspond to positions of polymorphic nucleotides for mtCR. Positions indicated by question marks refer to nucleotides missing in the mtCR sequence retrieved from GenBank

Table 4 Genetic distances for the nuclear ITS2 locus within (bolded diagonal) and between hammerhead shark species calculated as HKY+G-corrected distance (above diagonal) and pairwise p-distance (below diagonal)

	Eusphyra ($n = 2$)	*S. tiburo* ($n = 2$)	*S. corona* ($n = 1$)	*S. media* ($n = 1$)	*S. tudes* ($n = 2$)	*S. zygaena* ($n = 2$)	*S. mokarran* ($n = 2$)	*S. lewini* ($n = 7$)	*Sphyrna* sp. ($n = 4$)
Eusphyra	**0.00000**	0.06951	0.06951	0.06770	0.06279	0.06124	0.06489	0.07616	0.06939
S. tiburo	0.06625	**0.00000**	0.00000	0.01822	0.01517	0.03764	0.05161	0.03825	0.02892
S. corona	0.06625	0.00000	–	0.01822	0.01517	0.03764	0.05161	0.03825	0.02892
S. media	0.06465	0.01798	0.01798	–	0.00601	0.03507	0.04826	0.02999	0.02230
S. tudes	0.06014	0.01501	0.01501	0.00598	**0.00000**	0.03284	0.04355	0.02523	0.01748
S. zygaena	0.05870	0.03663	0.03663	0.03507	0.03209	**0.00000**	0.03727	0.03782	0.03337
S. mokarran	0.06205	0.04967	0.04967	0.04662	0.04224	0.03627	**0.00000**	0.05522	0.04931
S. lewini	0.07226	0.03713	0.03713	0.02929	0.02473	0.03681	0.05305	**0.00043**	0.01557
Sphyrna sp.	0.06614	0.02828	0.02828	0.02190	0.01723	0.03260	0.04757	0.01539	**0.00000**

–, intraspecific genetic distances not calculated due to availability of a single sequence type. The number of individuals analyzed for each species is shown (n)

recently discovered in the western North Atlantic (Abercrombie et al. 2005; Quattro et al. 2006) and morphologically resembling *Sphyrna lewini* also occurs in the western South Atlantic. These southern cryptic specimens also form a strongly supported, reciprocally monophyletic sister lineage to sympatric *S. lewini* (sensu *stricto*), as has been reported for its northern counterpart. This unexpected finding further validates the existence of this *Sphyrna* sp. lineage and extends its range into the southern hemisphere >7,000 km from its only previously reported location in the western North Atlantic. Interestingly, all specimens of the *Sphyrna* sp. lineage have thus far been captured at relatively high latitudes only (Southern Brazil and South Carolina and Florida, USA).

Table 5 Genetic distances for the mitochondrial CR within (bolded diagonal) and between hammerhead shark species calculated as pairwise K81+G-corrected distance (above diagonal) and p-distance (below diagonal)

	Eusphyra ($n = 1$)	*S. tiburo* ($n = 3$)	*S. corona* ($n = 1$)	*S. media* ($n = 1$)	*S. tudes* ($n = 1$)	*S. zygaena* ($n = 1$)	*S.mokarran* ($n = 2$)	*S. lewini* ($n = 37$)[a]	*Sphyrna* sp. ($n = 6$)
Eusphyra	–	0.15394	0.13338	0.17470	0.18028	0.16003	0.20238	0.21854	0.25091
S. tiburo	0.12566	**0.04025**	0.11524	0.12649	0.12473	0.17447	0.21327	0.16017	0.16446
S. corona	0.11134	0.09755	–	0.12464	0.11518	0.16145	0.23468	0.22477	0.22195
S. media	0.13765	0.10415	0.10256	–	0.11095	0.16100	0.20319	0.18870	0.18597
S. tudes	0.14112	0.10266	0.09670	0.09430	–	0.15391	0.21588	0.17685	0.17247
S. zygaena	0.12903	0.13762	0.13004	0.12821	0.12308	–	0.16017	0.22008	0.25023
S.mokarran	0.15235	0.15896	0.16989	0.15282	0.15162	0.12671	**0.00500**	0.22054	0.22058
S. lewini	0.16489	0.12917	0.16868	0.14776	0.13967	0.16329	0.16235	**0.01696**	0.05823
Sphyrna sp.	0.18220	0.13202	0.16670	0.14519	0.13617	0.17960	0.16151	0.05339	**0.00067**

–, intraspecific genetic distances not calculated due to availability of a single sequence type. The number of individuals analyzed for each species is shown (n)

[a] *S. lewini* (sensu *stricto*) sequences selected for genetic divergence estimates are from Table 3

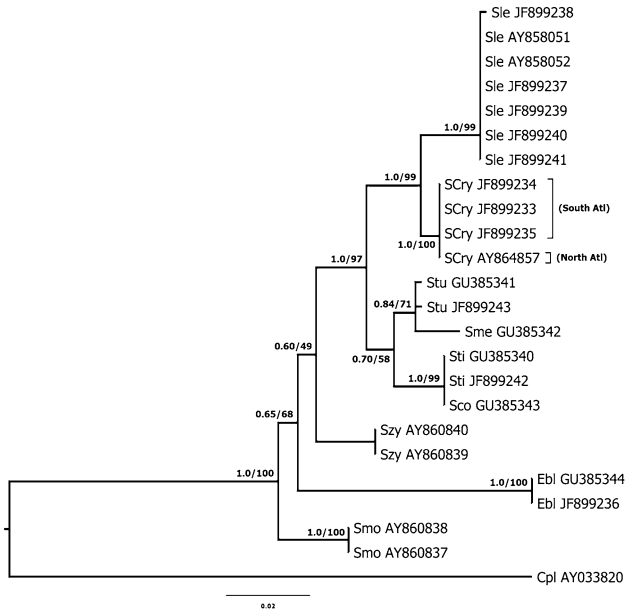

Fig. 1 Phylogenetic relationships among hammerhead species based on the BI and ML analysis of ITS2 sequences. Bayesian posterior probabilities/maximum likelihood bootstrap values are indicated at nodes. *SCry, Sphyrna* sp.; *Sle, Sphyrna lewini; Sti, S. tiburo; Szy, S. zygaena; Smo, S. mokarran; Sco, S. corona; Stu, S. tudes*; *Sme, S. media; Ebl, Eusphyra blochii; Cpl, Carcharhinus plumbeus*

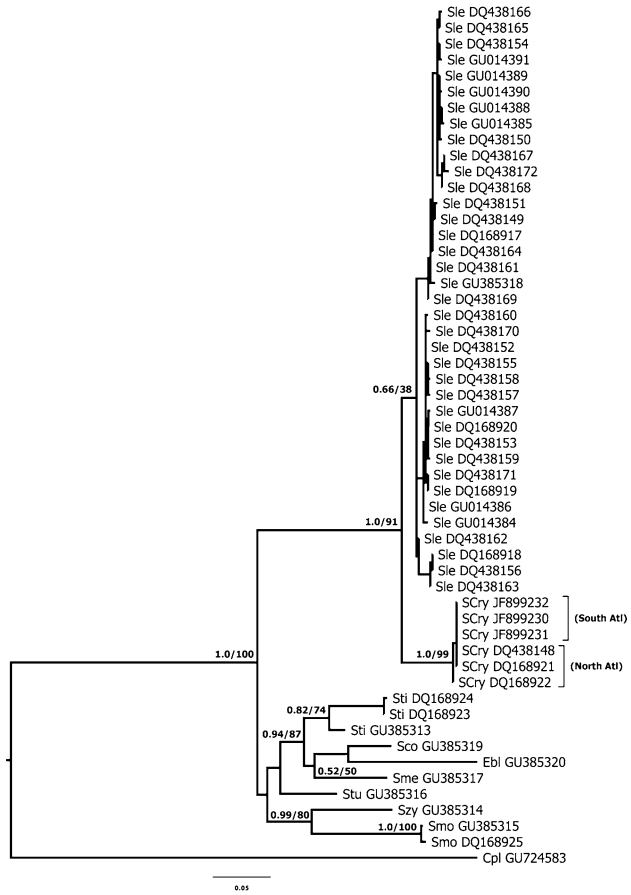

Fig. 2 Phylogenetic relationships among hammerhead species based on the BI and ML analysis of mtCR sequences. Posterior probabilities and bootstrap values are indicated. *SCry, Sphyrna* sp.*; Sle, Sphyrna lewini; Sti, S. tiburo; Szy, S. zygaena; Smo, S. mokarran; Sco, S. corona; Stu, S. tudes*; *Sme, S. media; Ebl, Eusphyra blochii; Cpl, Carcharhinus plumbeus*

The genetic screening of now ~1,100 individuals a priori identified as *S. lewini* (i.e., 203 individuals from the present study and ~900 individuals from previous studies) suggests that the cryptic lineage is enigmatically rare globally. However, the present study also suggests a cautionary note that this lineage may well be present in other areas where nominal *S. lewini* has not been extensively sampled

and genetically tested. We suggest that genetic surveys of nominal *S. lewini* should continue all around the world, especially in the eastern Atlantic and western Indian Ocean where they remain minimally examined, to continue to assess the distribution of this cryptic hammerhead lineage.

Sphyrna lewini (sensu *stricto*) in the western Atlantic form highly structured populations based on mtCR sequences (Chapman et al. 2009). We note, however, that the three *Sphyrna* sp. animals discovered in Brazilian fisheries exhibited identical mtCR haplotypes as cryptic animals from U.S.A. waters. While hinting at historical or recent gene flow across the equator, it is premature to draw inferences about inter-hemispheric population connectivity given the small mtCR sequence sample sizes (i.e., 21 from the northern hemisphere; 3 from the southern hemisphere) available for examination. More extensive sampling for this lineage across its western Atlantic range (including Caribbean) will be necessary to elucidate the extent to which this hammerhead inhabits the tropics and degree of gene flow between its southern and northern populations.

The estimated ~4.5 Ma divergence time between *Sphyrna* sp. and *S. lewini* (sensu *stricto*) derived from the ITS2 locus is surprising given the highly similar gross morphologies of *Sphyrna* sp. and *S. lewini,* and in view of divergence estimates for other hammerhead species. This estimate is close to the estimated divergence between the two morphologically more differentiated large hammerheads, *S. zygaena* and *S. mokarran*, and potentially predates the estimated divergence between two morphologically quite distinct, smaller hammerhead species, *S. tiburo* and *S. corona* (Lim et al. 2010). Reasons for retention of strong morphological similarity between *Sphyrna* sp. and *S. lewini* over an apparent, relatively ancient divergence are unclear. Both ITS2 and mtCR loci also place *Sphyrna* sp. as a sister lineage to *S. lewini* (sensu *stricto*), suggesting that the cryptic lineage is more closely related to the small hammerheads of the eastern Pacific and western Atlantic (*S. tudes*, *S. tiburo*, *S. corona* and *S. media*) than to the large, circumglobally distributed hammerheads (*S. mokarran* and *S. zygaena*) (Lim et al. 2010).

Given its only recent discovery and close morphological resemblance to *S. lewini* (sensu *stricto*), it is reasonable to postulate that the *Sphyrna* sp. lineage has been subject to similar fishing pressure as *S. lewini*, which is in the midst of a stock collapse in the western North Atlantic (Hayes et al. 2009) and listed as "Endangered" worldwide by the International Union for the Conservation of Nature (Baum et al. 2007). We therefore speculate that the population status of the *Sphyrna* sp. lineage throughout the western Atlantic might similarly be of concern, especially in light of its presence in fisheries landings and apparent low-frequency occurrence in the wild. However, comprehensive studies of its life history (i.e., fecundity, breeding regularity, age and growth) and population status are needed to evaluate its population status. Since none of these studies will likely occur until after this cryptic lineage is formally described and named, we hope that the further unequivocal validation of its existence as a distinct hammerhead lineage with its own evolutionary history and a broad geographic distribution as demonstrated here will spur such taxonomic efforts. In the interim, we recommend that this cryptic lineage be recognized and included in fishery management and conservation planning for nominal *S. lewini* in the western Atlantic to prevent the inadvertent, potential extirpation of a unique evolutionary lineage of hammerhead shark.

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