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Validity, Identification, and Distribution of the Roundscale Spearfish, *Tetrapturus georgii* (Teleostei: Istiophoridae): Morphological and Molecular Evidence

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VALIDITY, IDENTIFICATION, AND DISTRIBUTION
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GEORGII (TELEOSTEI: ISTIOPHORIDAE):
MORPHOLOGICAL AND MOLECULAR EVIDENCE

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ABSTRACT

The roundscale spearfish, *Tetrapturus georgii* Lowe, 1840, is known only from four specimens from the Mediterranean and eastern North Atlantic. Additional specimens have not been identified since 1961, making the validity and distribution of this species unclear. Analysis of 16 billfish specimens from the western North Atlantic on the basis of scale morphology, anus position, and mitochondrial DNA confirms the validity of this species and extends its distribution. Mid-lateral scales are soft, notably rounded anteriorly, and bear 2–3 points distinct from those of the sympatric longbill spearfish (*Tetrapturus pfluegeri* Robins and de Sylva, 1963) and white marlin (*Tetrapturus albidus* Poey, 1860). Position of anus relative to first anal fin and a related morphometric ratio (distance from anus to first anal fin origin: height of first anal fin) are intermediate between *T. pfluegeri* and *T. albidus*. These characteristics match those described by Robins (1974) from the four eastern North Atlantic specimens of *T. georgii*. The mitochondrial ND4L, ND4, and cyt *b* gene sequences strongly support reciprocal monophyly of the western North Atlantic specimens relative to other Atlantic istiophorids. The difficulty in distinguishing between morphologically similar *T. georgii* and *T. albidus* in the field and the previously unrecognized presence of *T. georgii* in the western North Atlantic has implications for stock assessments of *T. albidus*, a species that is severely overfished.

The number of species in the istiophorid genus *Tetrapturus* is uncertain. Nakamura (1985) lists four species in the Atlantic Ocean and Mediterranean Sea: *Tetrapturus albidus* Poey, 1860, white marlin; *Tetrapturus pfluegeri* Robins and de Sylva, 1963, longbill spearfish; *Tetrapturus belone* Rafinesque 1810, Mediterranean spearfish; and *Tetrapturus georgii* Lowe, 1840, roundscale spearfish. Pristas (1980) suggested the possible occurrence of a fifth *Tetrapturus* species dubbed the “hatchet marlin”. Validity of the white marlin, longbill spearfish, and Mediterranean spearfish as distinct species is uncontroversial (Nakamura, 1985). However, description of the roundscale spearfish is based on only four specimens (Robins, 1974), and the validity and distribution of this species has long been in need of clarification (Nakamura, 1985).

The roundscale spearfish was first described by Lowe (1840) as *Tetrapturus georgii*, with a cursory diagnosis consisting only of the location of one specimen captured (Madeira), and comments on the greater length of the specimen’s pectoral fins relative to that of the Mediterranean spearfish and the “peculiar shape and nature” of its large scales. With only this cursory description, the lack of a type specimen and absence of additional specimens led Robins and de Sylva (1960) to conclude that the identity of Lowe’s *T. georgii* was unlikely to ever be solved.

The next re-examination of this issue was reported by Robins (1974) who provides the most extensive morphological description of the roundscale spearfish to date. This analysis was based on assessment of four specimens from the Mediterranean

and northeastern Atlantic, and included modification of the name to *T. georgii* (Note: Robins (1974) uses "*T. georgei*"). Because of the small number of specimens examined (one of which was damaged, preventing robust morphometric analysis) and concomitant uncertainty about the validity and distribution of this species, we report here a genetic and morphological analysis of additional specimens of an istiophorid billfish from the western North Atlantic. These specimens superficially resemble the eastern Atlantic roundscale spearfish described by Robins (1974) as well as the white marlin. The additional analyses confirm *T. georgii* as a distinct evolutionary lineage and extend its distribution to the western North Atlantic. This finding may have important ramifications as it pertains to the stock status of the severely overfished white marlin.

METHODS

Whole billfish specimens ($n = 16$) were obtained by fishery observers with the National Marine Fisheries Service Pelagic Observer Program from 1996–2005 working in western North Atlantic waters (Fig. 1). The specimens (hereafter referred to as "roundscale spearfish") generally resembled either white marlin or longbill spearfish, but subjectively were deemed to "look a bit different" based on scale morphology. Photographs were taken of the whole animal plus ventral side views to clearly show the relative position of the anal opening to the first anal fin. To collect data for subsequent morphometric analyses, the observers also measured (to the

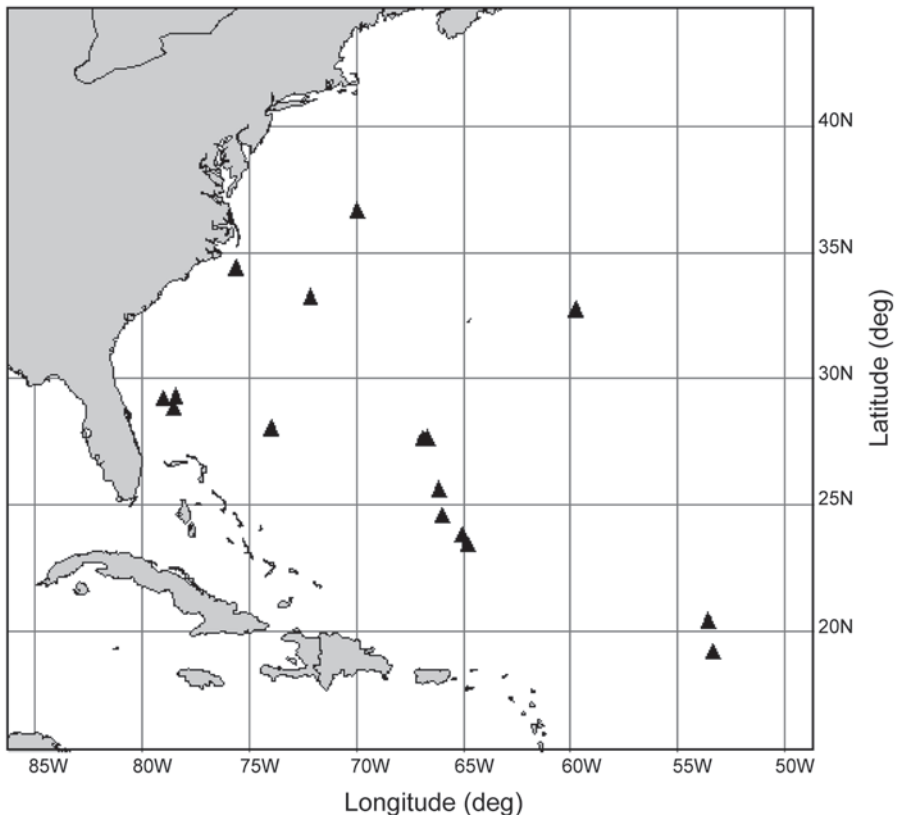


Figure 1. Capture locations of the 16 roundscale spearfish individuals analyzed in this study.

nearest cm): (1) the distance between the anal opening and the origin of the first anal fin; and (2) the greatest height of the first anal fin. Skin patches with attached muscle were taken from the mid-lateral side of each animal a few centimeters behind the pectoral fin, and stored on ice or frozen for subsequent scale morphology and DNA analyses. For comparative analysis, the above sampling and measurement protocols were also applied to the sympatric white marlin ($n = 13$) and longbill spearfish ($n = 9$) landed in the same fishery.

Genomic DNA was extracted from approximately 25 mg of tissue from the roundscale spearfish ($n = 10$), white marlin ($n = 4$), longbill spearfish ($n = 4$), Mediterranean spearfish ($n = 4$), sailfish [*Istiophorus platypterus* (Shaw in Shaw and Nodder, 1792), $n = 4$], blue marlin (*Makaira nigricans* Lacépède, 1802; $n = 3$), black marlin (*Makaira indica* Cuvier, 1832; $n = 1$) and striped marlin (*Tetrapturus audax* Philippi, 1887; $n = 1$) using the DNeasy kit (Qiagen Inc., Valencia, CA). The Indo-Pacific endemic black and striped marlins were included in the genetic analyses to obtain an additional, comparative perspective on evolutionary distances between the billfishes.

Mitochondrial cytochrome *b* (*cyt b*) and NADH dehydrogenase subunits 4L-4 (ND4L-ND4) genes were amplified by the polymerase chain reaction (PCR). Total amplification reaction volumes were 50 μ L, and contained 1 μ L of the extracted genomic DNA, 12.5 pmol of each primer, 40 μ M dNTPs, 1X PCR buffer, and 1 unit of HotStar Taq[™] DNA Polymerase (Qiagen Inc.). The primer pairs and PCR thermal cycling conditions used for each locus were: *Cyt b* primers: 40F 5'GACATCGCAACAGCCTTCACATCCG3' and 942R: 5'AAATACAGGAATGAGGCYACTGTCC3'; thermal profile: 94 °C initial heating for 15 min to activate the hot start DNA polymerase, followed by 35 cycles of 94 °C for 1 min, 55 °C for 2 min, 65 °C for 3 min and a 7 min final extension step at 65 °C. ND4L-ND4 primers: 61F 5'GACCCCTGTTCACTTCGCCTTC3' and 1837R 5'GCAGTCTTCGCTGCTAACGAGC3'; thermal profile same as for *cyt b*. All amplifications were performed in an MJ Research PTC-200 thermal cycler. Following amplification, the *cyt b* and ND4L-ND4 amplicons were purified using the QIAquick PCR purification kit (Qiagen Inc.) and both strands were sequenced completely using standard protocols on an Applied Biosystems 3100 genetic analyzer.

Forward and reverse sequences (number of individuals sequenced for each locus shown in Fig. 3A, B) were assembled, aligned, and edited using GeneDoc 2.6.002 (Nicholas and Nicholas, 1997). Maximum likelihood (ML), neighbor joining (NJ), and maximum parsimony (MP) phylogenetic reconstructions were performed using PAUP version 4.0b10 (Swofford, 2002). ML and NJ analyses employed the TrN+I model of sequence evolution, which was determined to be the best-fit model using the Akaike Information Criterion (AIC) in MODELTEST version 3.06 (Posada and Crandall, 1998). Equal weights were assigned to all character changes in MP analysis. Heuristic searches were performed using random stepwise addition with ten replicates to obtain starting trees. Tree bisection-reconnection was used as the branch swapping algorithm. Statistical support for branch nodes was acquired using nonparametric bootstrap analyses (200 replicates for ML, 1000 replicates each for NJ and MP trees; Felsenstein, 1985). To assess the evolutionary distance between species, average, pairwise sequence divergences (as uncorrected *p*-distances) were determined using MEGA3 (Kumar et al., 2004).

RESULTS AND DISCUSSION

SCALE MORPHOLOGY AND MORPHOMETRICS.—Morphology of the mid-lateral scales from western Atlantic roundscale spearfish matched exactly the description of scales reported for the four eastern Atlantic and Mediterranean roundscale spearfish specimens by Robins (1974). Furthermore, the roundscale spearfish scales were distinct, being notably rounded at the anterior end and soft with two to three posterior points present. In comparison, scales of the congener white marlin and longbill spearfish are pointed at the anterior end and stiffer with one to two (white marlin) or two to five (longbill spearfish) posterior points (Fig. 2 and Nakamura, 1985). The

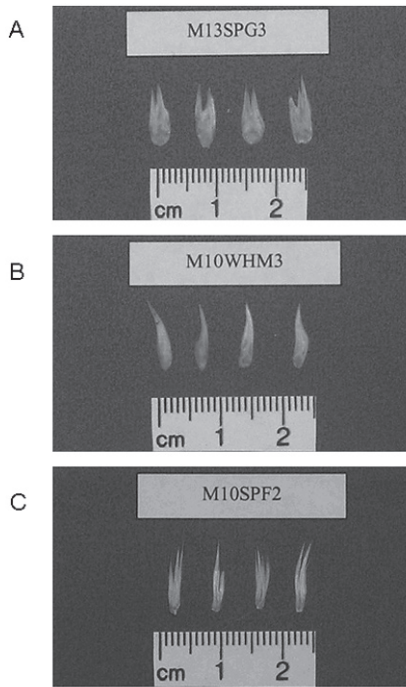


Figure 2. Comparative, mid-body scale morphology of three western North Atlantic istiophorid species: (A) study roundscale spearfish, (B) white marlin, and (C) longbill spearfish.

morphometric ratio of the distance from the anus to the first anal fin origin to the maximum height of the first anal fin for the roundscale spearfish was slightly higher than reported by Robins (1974), but did not overlap with the same ratio for specimens of the two sympatric congeners, white marlin and longbill spearfish (Table 1). Roundscale spearfish scale morphology and relative position of anus were also distinct from the same characters reported for the Mediterranean spearfish by Robins (1974, page 57) (i.e., scales pointed at anterior end and pungent; distance of anus anterior to first anal fin equal to or exceeding height of first anal fin). These two morphological characters for the 16 roundscale spearfish specimens are consistent with those described by Robins (1974) for the four roundscale specimens he analyzed. The caveat of relatively small sample sizes examined for the white marlin and longbill spearfish notwithstanding, these two morphological characters appear to robustly distinguish the roundscale spearfish from its three Atlantic congeners. However, we note that from a field-identification perspective, these species identification characters may be too subtle and impractical for use by untrained recreational and commercial fish-

Table 1. Relationship between distance of anus to first anal fin: height of first anal fin in three species of *Tetrapturus* billfish.

Species	Source	Mean	Range	N
<i>T. georgei</i>	Robins (1974)	~0.5		3
<i>T. georgii</i>	This study	0.67	0.53–0.77	16
<i>T. pfluegeri</i>	This study	1.12	0.93–1.36	9
<i>T. albidus</i>	This study	0.30	0.18–0.39	13

ers, especially for identifying live fish in the water. Misidentifications of roundscale spearfish as white marlin may have significant implications for management and conservation of the latter highly depleted species (see Conclusions).

GENETIC ANALYSIS.—Maximum likelihood, neighbor joining and maximum parsimony analyses of 1268 bp of the ND4L-ND4 (60 bp ND4L, 1208 bp ND4) and 782 bp of the *cyt b* genes revealed highly structured trees, with most billfish species including roundscale spearfish displaying reciprocal monophyly with very high bootstrap support for most nodes (Fig. 3A,B). Intraspecific sequence diversity at both loci in the 10 roundscale spearfish was about the same as that seen in other billfish species (however, note smaller sample sizes for other billfish species). Pairwise sequence divergence at both loci between roundscale spearfish and all other billfishes (3.1%–6.8%) fell well within the range seen between all other billfish species pairs (0.3%–7.3%; Table 2). Furthermore, sequence divergence between roundscale spearfish and its closest congeners, the longbill and Mediterranean spearfishes, (Fig. 3A, B) was notably higher (~ 12 times at ND4L-ND4 and ~ 6 times at *cyt b*) than between two other congener species pairs (Mediterranean spearfish vs longbill spearfish; white marlin vs striped marlin; Table 2). Sequence divergence between roundscale spearfish and white marlin was 5.5% at ND4L-ND4 and 3.4% at *cyt b*. Robins (1974) considered the white marlin to be morphologically most similar to roundscale spearfish, yet the sequence divergence between these two species was similar to that observed between blue marlin and sailfish (i.e., 4.8% at ND4L-ND4; 4.4% at *cyt b*), billfish species placed in different genera. All mitochondrial DNA sequence haplotypes obtained in this study have been deposited in GenBank under the following accession numbers: *T. georgii*: *cyt b*: DQ882011–DQ882013; ND4L-ND4: DQ872424–DQ872429; *T. audax*: *cyt b*: DQ882017; ND4L-ND4: DQ872421; *T. albidus*: *cyt b*: DQ882009; ND4L-ND4: DQ872419–DQ872420; *T. belone*: DQ882010; ND4L-ND4: DQ872422–DQ872423; *T. pfluegeri*: *cyt b*: DQ882014–DQ882016; ND4L-ND4: DQ872430–DQ872433; *M. nigricans*: *cyt b*: DQ882007–DQ882008; ND4L-ND4: DQ872417–DQ872418; *M. indica*: *cyt b*: DQ882018; ND4L-ND4: DQ872416; *I. platypterus*: *cyt b*: DQ882006; ND4L-ND4: DQ872414–DQ872415.

In his description of the roundscale spearfish, Robins (1974) considered, but rejected on morphological grounds, the possibility of its phenotype resulting from hybridization between other Atlantic *Tetrapturus* species. Our results demonstrating the unambiguous, reciprocal monophyly of roundscale spearfish relative to other Atlantic billfish species based on three mitochondrial genes supports it as a distinct evolutionary lineage, also discounting the notion that the roundscale spearfish results from contemporary, ongoing hybridization between other sympatric billfish species. Furthermore, results from screening larger sample sizes of reference congeners (white marlin $n = 105$; longbill spearfish $n = 73$; Mediterranean spearfish $n = 10$ and roundscale spearfish $n = 22$) with species-specific PCR primers are consistent with roundscale spearfish possessing unique ND4L-ND4 sequences (JEM and MSS, unpubl. data).

CONCLUSIONS

Based on the unequivocal mitochondrial genome monophyly of the roundscale spearfish, coupled with its unique morphological characteristics of distinct squamation and non-overlapping anus position morphometrics (compared to the other

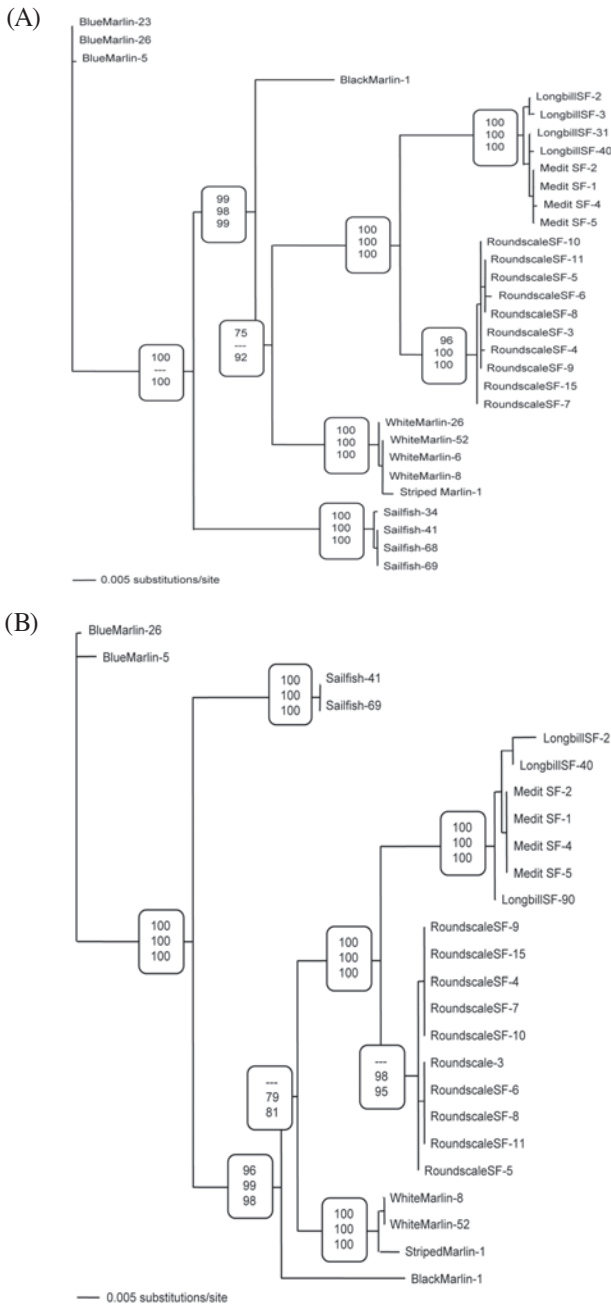


Figure 3. (A) Maximum likelihood tree depicting major billfish clades inferred from the ND4L-ND4 genes. (B) Maximum likelihood tree depicting major billfish clades inferred from the *cyt b* gene. Bootstrap values from top to bottom are for maximum likelihood, neighbor-joining, and maximum parsimony analyses. Bootstrap values 75% and higher are shown. SF = spearfish; Medit SF = Mediterranean spearfish. Numbers after each OTU represent different billfish individuals analyzed.

Table 2. Pairwise sequence divergences (p-distances) for ND4L-ND4 (normal font) and *cyt b* (italicized font) between the roundscale spearfish and other billfishes. SF = spearfish.

	Blue marlin	Black marlin	Longbill SF	Roundscale SF	Medit. SF	White marlin	Striped marlin
Black marlin	0.045 <i>0.054</i>						
Longbill SF	0.061 <i>0.072</i>	0.052 <i>0.060</i>					
Roundscale SF	0.060 <i>0.062</i>	0.051 <i>0.043</i>	0.038 <i>0.031</i>				
Medit. SF	0.062 <i>0.072</i>	0.053 <i>0.060</i>	0.003 <i>0.005</i>	0.038 <i>0.032</i>			
White marlin	0.054 <i>0.056</i>	0.036 <i>0.042</i>	0.055 <i>0.050</i>	0.055 <i>0.034</i>	0.058 <i>0.051</i>		
Striped marlin	0.055 <i>0.059</i>	0.037 <i>0.042</i>	0.054 <i>0.053</i>	0.054 <i>0.037</i>	0.055 <i>0.054</i>	0.003 <i>0.005</i>	
Sailfish	0.048 <i>0.044</i>	0.051 <i>0.055</i>	0.071 <i>0.068</i>	0.067 <i>0.068</i>	0.073 <i>0.068</i>	0.058 <i>0.045</i>	0.061 <i>0.050</i>

recognized Atlantic *Tetrapturus* species) consistent with the morphological description of roundscale spearfish by Robins (1974), we propose the roundscale spearfish is indeed *T. georgii*, and confirm the validity of this species. Furthermore, we extend the distribution of *T. georgii* to western North Atlantic waters, where it is sympatric with the longbill spearfish and white marlin.

The strong morphological similarity of roundscale spearfish to white marlin (Fig. 4; Robins, 1974; Nakamura, 1985), the difficulty of using scale morphology and anus position as practical identifying characteristics for live fish in the water, and the

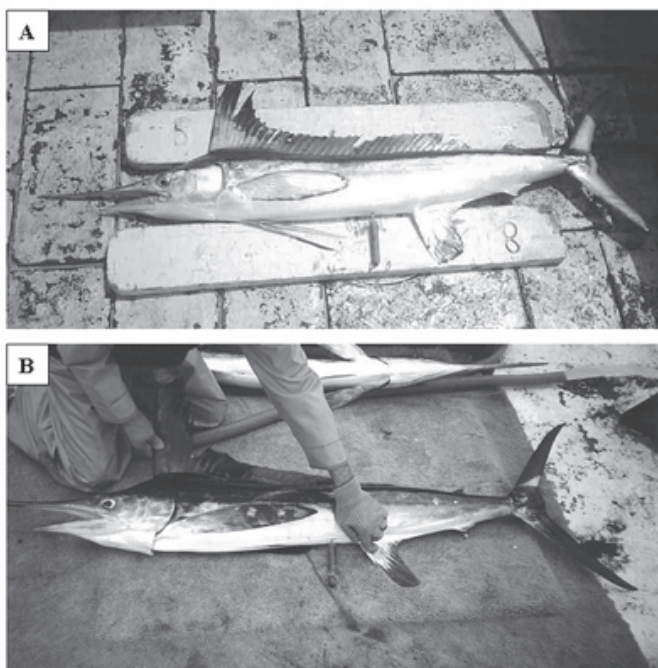


Figure 4. Photographs of a roundscale spearfish (A) and white marlin specimen (B). Probe marks position of the anus.

heretofore unrecognized presence of the roundscale spearfish in the western North Atlantic suggest that some unknown proportion of billfish captured in commercial and recreational fishing and recorded as "white marlin" may actually be roundscale spearfish. Depending on roundscale spearfish population size, this type of field misidentification could result in overestimates of white marlin landings, potentially contributing uncertainty to white marlin stock assessments.

White marlin are considered severely overfished, with their 2002 stock biomass at only about 12% of the level required to produce maximum sustainable yield (ICCAT 2002; Restrepo et al., 2003). As a result, an Atlantic-wide plan to rebuild the white marlin stock has been in place since 2002 under the auspices of ICCAT, and the species was petitioned for listing as threatened or endangered under the U.S. Endangered Species Act [White Marlin Status Review Team. 2002. Atlantic White Marlin Status Review Document, 49 p. Report to National Marine Fisheries Service, Southeast Regional Office, 263 13th Avenue, St. Petersburg, FL 33701-5511]. To facilitate rebuilding of the white marlin stock, further research is clearly necessary to determine the spatio-temporal distribution and abundance of roundscale spearfish and the extent of possible misidentifications with white marlin, throughout the Atlantic Ocean. Application of both genetic and conventional morphometric techniques will likely play an important role in this endeavor.

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