10-2006

# Phylogeny of recent billfishes (Xiphioidei)

BB Collette

Jan McDowell
*Virginia Institute of Marine Science*

John Graves
*Virginia Institute of Marine Science*

# PHYLOGENY OF RECENT BILLFISHES (XIPHIOIDEI)

*Bruce B. Collette, Jan R. McDowell, and John E. Graves*

## ABSTRACT

Billfishes are genetically and morphologically distinct enough from scombroids to merit placement in a separate suborder, Xiphioidei. Two extant families are usually recognized: Xiphiidae (swordfish, *Xiphias*) and Istiophoridae, currently containing three genera, *Istiophorus* (sailfishes), *Makaira* (marlins), and *Tetrapturus* (spearfishes, white, and striped marlins). Phylogenetic analyses of molecular data from mitochondrial and nuclear gene sequences (mitochondrial control region, ND2, 12S, and nuclear MN 32 regions) show a different picture of relationships. *Makaira* is not monophyletic: blue marlin cluster with sailfish and placement of black marlin is unstable. Accepting the molecular phylogeny gives two possible classifications: (1) two genera: blue marlin + sailfish (as *Istiophorus*) and all the rest (as *Tetrapturus*), or (2) five genera: blue marlin (*Makaira*), sailfish (*Istiophorus*), black marlin (*Istiompax*), striped and white marlin (*Kajikia*), and four spearfishes (*Tetrapturus*). We prefer the latter possibility. There is no genetic evidence to support recognition of separate species of Atlantic and Indo-Pacific sailfishes or blue marlins. Atlantic white marlin, *Kajikia albida* (Poey, 1860) is closely related to Indo-Pacific striped marlin, *Kajikia audax* (Philippi, 1887). The four spearfishes are closely related: the three Atlantic species, longbill (*Tetrapturus pfluegeri* Robins and de Sylva, 1963), Mediterranean (*Tetrapturus belone* Rafinesque, 1810), and roundscale (*Tetrapturus georgii* Lowe, 1841), and the one Indo-Pacific species, shortbill (*Tetrapturus angustirostris* Tanaka, 1915). The roundscale is the most divergent of the spearfishes. A fifth putative *Tetrapturus* sp., the "hatchet marlin" clusters with roundscale spearfish but these two "species" could not be differentiated in this analysis.

Billfishes comprise two extant families, the monotypic Xiphiidae and the Istiophoridae, which includes three genera and at least eight recognized species. Over the past several years there has been considerable interest in the relationship of istiophorid and xiphiid billfishes to other perciform fishes, and there is debate over the relationship of billfishes to other scombroids (Johnson, 1986; Finnerty and Block, 1995). Surprisingly, despite the attention to higher-level taxonomy of the billfishes, there has been relatively little interest in either relationships within the istiophorid genera or the alpha (species level) taxonomy of this group.

There is confusion regarding the relationships within istiophorid genera, and Morrow (1964) felt that the generic divisions of the istiophorids were in a state of flux. Finnerty and Block's (1995) analysis of 612 base pairs of the cytochrome *b* region of the mitochondrial DNA (mtDNA) noted the largest genetic difference among any istiophorids between the two species placed in *Makaira*, the black and blue marlin. However, they concluded that although current taxonomy does not agree with the molecular phylogeny, examination of a single genetic locus is inadequate to make a definitive revision of the genera. This is because the phylogeny of a gene is not always an accurate reflection of the phylogeny of species due to the possible effects of selection on a particular locus and, in the case of mtDNA, the possibility of past introgressive hybridization (Maddison, 1997).

There are also taxonomic problems within each of the three istiophorid genera. For example, Atlantic and Indo-Pacific populations of both blue marlin and sailfish have

been described both as separate species, and as conspecific populations. Most recently, Nakamura (1985) recognized Atlantic and Indo-Pacific blue marlin as distinct species, *Makaira nigricans* Lacépède, 1802 and *M. mazara* (Jordan and Snyder, 1901), respectively, based on differences in lateral line morphology. Nakamura (1985) also recognized separate species of Atlantic sailfish, *Istiophorus albicans* (Latreille, 1804) and Indo-Pacific sailfish, *I. platypterus* (Shaw, 1792) based on the relative length of pectoral and caudal fins and differences in scale shape and growth.

The genus *Tetrapturus* is also problematic. This genus is currently comprised of the white marlin [*Tetrapturus albidus* (Poey, 1860)], the striped marlin [*Tetrapturus audax* (Philippi, 1887)], and the four spearfishes, the Mediterranean [*Tetrapturus belone* (Rafinesque, 1810)], longbill [*Tetrapturus pfleugeri* (Robins and de Sylva, 1963)], shortbill [*Tetrapturus angustirostris* (Tanaka, 1915)], and roundscale [*Tetrapturus. georgii* (Lowe, 1841)](Nakamura, 1985). However there are several uncertainties, including disagreement about the total number of species described as spearfishes. Spearfishes are the scarcest of the world's istiophorids, and because of their relative rarity, their taxonomic relationships have not been thoroughly examined. Robins (1974) resurrected the roundscale spearfish, *T. georgii*, and it was considered a valid species by Nakamura (1985). Although Robins (1974) suggested that *T. georgii* might be a hybrid between *T. albidus* and *T. belone*, he ultimately rejected that hypothesis based on available data. Validity of the roundscale spearfish is addressed by Shivji et al. (2006).

Another enigmatic Atlantic Ocean *Tetrapturus*, the so-called "hatchet marlin," has yet to be formally described, although its possible existence is mentioned in Nakamura (1985). In addition, other researchers (Pristas, 1980) have noted several morphological features of this putative species which are distinct from both white marlin and longbill spearfish. Similarly, the specific relationship of white marlin (*T. albidus*) and striped marlin (*T. audax*) has been called into question. Molecular data (Finnerty and Block, 1995; Graves and McDowell, 1995) examined thus far have failed to find fixed genetic differences between the white and striped marlin. This suggests that either there is a low level of contemporaneous gene flow or white and striped marlin have only become separate species very recently and the molecular markers examined to date have not reached reciprocal monophyly (lineage sorting is not complete).

The problems outlined above, combined with the relative lack of morphological and meristic characters that can be used to discriminate billfish species, suggest that resolution of these questions requires examination of both nuclear and mitochondrial loci. In this study, we use analyses of mitochondrial and nuclear gene sequences to investigate both the alpha and higher level relationships of Istiophoridae. To arrive at a phylogeny of Recent billfishes, four questions need to be answered: (1) are billfishes scombroid fishes or do they belong in a separate suborder?, (2) are the currently accepted three genera of Istiophoridae valid?, (3) are the currently accepted species of billfishes valid?, and (4) are Atlantic and Indo-Pacific populations of sailfish and blue marlin separate species?

## Materials and Methods

Biological Materials.—Specimens were collected from commercial, artisanal, and recreational fisheries. All currently recognized species of billfish were sampled. In addition,

samples were taken from two putative species, the roundscale spearfish (*T. georgii*) and the hatchet marlin (*Tetrapturus* sp.) Originally, four samples were sequenced at all loci for each species restricted to a single ocean basin and eight samples (four Atlantic and four Pacific) for globally distributed billfishes. Inclusion of all individuals did not affect the final phylogeny obtained in preliminary analyses and was not tractable due to memory constraints, therefore two samples from each species were included in analyses presented in this study (Table 1). Sequences from the wahoo, *Acanthocybium solandri* (Cuvier, 1832) and Atlantic bluefin tuna, *Thunnus thynnus* (Linnaeus, 1758) were used as outgroups. Samples consisted of either heart tissue removed after capture and stored at –80 °C until isolation, or white muscle preserved in 0.25mM EDTA pH 8.0, 20% DMSO, and saturated NaCl (Seutin et al., 1991) at room temperature until isolation. DNA was isolated using either a phenol-chloroform (Sambrook and Russell, 2001), or a proteinase K-chelex extraction (Estoup et al., 1996).

Three mitochondrial loci, control region, ND2, and 12 SrRNA, and an anonymous nuclear locus MN32-2 were amplified using the primers and conditions listed in Table 2. For mitochondrial loci, amplified products were direct-sequenced using the original primers on a Li-cor 4200 Global IR$^2$ system using IRD-800-labelled forward primer and IRD-700-labelled reverse primer (Li-cor, Lincoln, NE). Amplified nuclear fragments were cloned using a TOPO-TA plasmid cloning kit (Invitrogen Corp., San Diego, CA). Cloned fragments were purified using QIAprep Spin Miniprep reagents (Qiagen Corp., Valencia, CA) following the manufacturer's specifications. Concentration of purified products was measured using a Biomate-3 UV spectrophotometer (Thermo Spectronic, Rochester, NY) prior to sequencing on the Global IR$^2$ system.

Data Analysis.—Standard chromatographic curves (SCF) of forward and reverse sequences were aligned, and edited using the program Sequencher 4.2.2 (Gene Codes Corp., Ann Arbor, MI). A consensus of forward and reverse sequences was created and exported to the program MacVector 8.0 (Oxford Molecular LTD, Madison, WI) and an alignment was created using the ClustalW algorithm (Thompson et al., 1994) and adjusted by eye. Saturation was examined by plotting the number of transitions and transversions against uncorrected percent nucleotide sequence divergence (p-distances) for all loci. In addition, first, second, and third codon positions were plotted against p-distances for the protein-coding ND2 region. Pairwise comparisons were generated in the program JADIS (Goncalves et al., 1999). Compatibility of gene partitions was examined using the incongruence length difference tests (ILD; Mickevich and Farris, 1981; Farris et al., 1994) implemented in PAUP* 4.0b10 (Swofford, 2003) with a heuristic search of 1000 replicates and 100 random sequence additions. This tests whether sequences from the different loci can be combined in a single analysis. If trees generated from the combined data are not significantly longer than trees from randomly generated partitions of equal length, the data are considered to have a consistent phylogenetic signal across loci.

Phylogenetic inference was conducted using maximum parsimony, maximum likelihood, and Bayesian analyses. Parsimony analyses were conducted on each individual locus, combined mitochondrial regions, and all regions (mitochondrial + nuclear) combined. All parsimony analyses used equal weighting schemes and were carried out using heuristic searches of 100 random taxon addition sequences with 10 trees held at each stepwise addition and tree bisection-reconnection (TBR) branch swapping. Gaps were treated as missing data. Bootstrap analysis (Felsenstein, 1985) using 100 random addition sequences with TBR per replicate and 10,000 pseudoreplicates was carried to evaluate support for each node in all parsimony analyses.

ModelTest version 3.7 (Posada and Crandall, 1998) was used to infer the most appropriate model of molecular evolution for each locus based on the Akaike Information Criterion (AIC) by comparing successively more complex models. The chosen models were used in all subsequent maximum likelihood and Bayesian analyses (Table 3). Maximum likelihood analyses were performed using a heuristic search algorithm in PAUP* with 10 random taxon addition sequences. Bayesian analyses were carried out using MrBayes version 3.1 (Ronquist and Huelsenbeck, 2003). Program defaults were used for estimation of priors. Analyses were run

Table 1. Species included in the phylogenetic analysis with number of specimens (n), collection locations, and GenBank accession numbers.

| Common name | n | Collection location | Accession numbers | | | |
|---|---|---|---|---|---|---|
| | | | 12S | Control region | ND2 | Mn32-2 |
| *Xiphias gladius* | 1 | Central Pacific | DQ854646 | DQ855112 | DQ854689 | ---------- |
| *Istiophorus platypterus* | 2 | Eastern Atlantic | DQ854626 | DQ855092 | DQ854669 | DQ854649 |
| | | Eastern Atlantic | DQ854627 | DQ855093 | DQ854670 | DQ854650 |
| *Istiompax indica* | 2 | Eastern Pacific | DQ854630 | DQ855096 | DQ854673 | DQ854653 |
| | | Eastern Pacific | DQ854631 | DQ855097 | DQ854674 | DQ854654 |
| *Makaira nigricans* | 2 | Eastern Atlantic | DQ854628 | DQ855094 | DQ854671 | DQ854651 |
| | | Western Pacific | DQ854629 | DQ855095 | DQ854672 | DQ854652 |
| *Kajikia albida* | 2 | Western Atlantic | DQ854632 | DQ855098 | DQ854675 | DQ854655 |
| | | Western Atlantic | DQ854633 | DQ855099 | DQ854676 | DQ854655 |
| *Kajikia audax* | 2 | Western Pacific | DQ854634 | DQ855100 | DQ854677 | DQ854657 |
| | | Western Pacific | DQ854635 | DQ855101 | DQ854678 | DQ854658 |
| *Tetrapturus angustirostris* | 2 | Central Pacific | DQ854636 | DQ855102 | DQ854679 | DQ854659 |
| | | Central Pacific | DQ854637 | DQ855103 | DQ854680 | DQ854660 |
| *Tetrapturus belone* | 2 | Mediterranean | DQ854640 | DQ855106 | DQ854683 | DQ854663 |
| | | Mediterranean | DQ854641 | DQ855107 | DQ854684 | DQ854664 |
| *Tetrapturus georgii* | 2 | Central Atlantic | DQ854642 | DQ855108 | DQ854685 | DQ854665 |
| | | Central Atlantic | DQ854643 | DQ855109 | DQ854686 | DQ854666 |
| *Tetrapturus pfluegeri* | 2 | Central Atlantic | DQ854638 | DQ855104 | DQ854681 | DQ854661 |
| | | Central Atlantic | DQ854639 | DQ855105 | DQ854682 | DQ854662 |
| Hatchet marlin | 2 | Western Atlantic | DQ854644 | DQ855110 | DQ854687 | DQ854667 |
| | | Western Atlantic | DQ854645 | DQ855111 | DQ854688 | DQ854668 |
| *Acanthocybium solandri* | 1 | Eastern Pacific | DQ854648 | DQ855114 | DQ854691 | ---------- |
| *Thunnus thynnus* | 1 | Mediterranean | DQ854647 | DQ855113 | DQ85469 | ---------- |

Table 2. Primers used for amplification and sequencing of loci. ND2: the mitochondrial NADH dehydrogenase subunit 2; Dloop: the mitochondrial control region; 12S: the mitochondrial 12S ribosomal RNA; and MN32: a single copy anonymous nuclear locus. F and R designate forward and reverse primers.

| Locus | Name | Anneal | Sequence |
|---|---|---|---|
| ND2 F | ND2B-LM13F | 55 °C | CAC GAC GTT GTA AAA CGA CTA AGC TTT YGG GCC CAT AC |
| ND2 R | ND2E-HM13R | | GGA TAA CAA TTT CAC ACA GGC RRT TAG GRC TTT GAA GGC |
| Dloop F | Pro-5M13F | 54 °C | CAC GAC GTT GTA AAA CGA CCT ACC YCY AAC TCC CAA AGC |
| Dloop R | dloopi | | CCA TCT TAA CAT CTT CAG TG |
| 12S F | Phe-5M13F | 52 °C | CAC GAC GTT GTA AAA CGA CAA AGC ATA ACA CTG AAG ATG T |
| 12S R | 16S– 3M13R | | GGA TAA CAA TTT CAC ACA GGA CCA GCT ATM ACY AGG TTC G |
| MN32F | BM32F-2 | 58 °C | GTA GCA AGG GGC TGT TGC ATA G |
| MN32R | BM32-2R | | GAG TCA GTG GTT CGG GAT TTT ATC |

Table 3. Locus, number of base pairs used in this study (Bp), number of variable sites, number of parsimony informative sites (PI), number of equally parsimonious trees (EPT), retention index (RI), rescaled consistency index (RC), length of shortest tree found in parsimony analysis (length), best model estimated from Akaike Information Criterion in Modeltest (model), and likelihood of best tree found in maximum likelihood analysis (–Ln).

| | | | | | Parsimony | Maximum likelihood | | |
|---|---|---|---|---|---|---|---|---|
| Locus | Bp | Variable sites | PI | EPT | RI /(RC) | Length | Model | (–Ln) |
| ND2 | 995 | 388 | 254 | 8 | 0.802 (0.628) | 652 | TrN+I+G | 4,124.51367 |
| Dloop | 920 | 649 | 535 | 2 | 0.793 (0.532) | 1,554 | HKY+I+G | 7,539.56897 |
| 12S | 953 | 163 | 113 | 784 | 0.931 (0.878) | 191 | GTR+G | 2,257.97050 |
| MN32 | 919 | 121 | 62 | 84 | 0.886 (0.758) | 145 | HKY+G | 2,266.9240 |
| $mtDNA_{TOTAL}$ | 2,868 | 1,200 | 902 | 3 | 0.801 (0.577) | 2,402 | | |
| All Loci | 3,787 | 1,321 | 964 | 3 | 0.803 | 2,557 | | |

using a random starting tree with three heated chains and one cold chain over 1 million generations, with sampling every 100 generations. The burn-in period was removed by plotting generation time against the log likelihood (–ln) score as well as against gamma shape, proportion of invariant sites, nucleotide frequency, and other generated parameters. Stationarity was inferred when values reached an asymptote. Remaining trees were used to construct a 50% majority-rule consensus tree and the frequency of all observed bipartitions was used to assess the level of support for each node.

## Results

Parsimony analysis of each individual gene region resulted in between 2 (control region, 1554 steps long, retention index, RI = 0.79) and 784 (12s rRNA, 191 steps long, RI = 0.913) equally parsimonious trees (EPTs). The mitochondrial control region had the largest number of parsimony informative sites (535) while the anonymous nuclear locus, MN32, had the fewest (62, Table 3). Partition homogeneity tests were used to evaluate congruence both within the combined mitochondrial data set and within the mitochondrial + nuclear data set. Results indicated that all data sets were congruent (P = 0.751 and P = 0.549, respectively), so concatenated data sets partitioned as mitochondrial only and as mitochondrial + nuclear were used for subsequent parsimony and Bayesian analyses.

For parsimony analysis using the combined mtDNA data, 902 out of 2868 sites were parsimony informative and analysis resulted in 3 EPTS of length 2402, RI = 0.801. Addition of the nuclear MN32 locus resulted in 3 EPTs of length 2557, RI = 0.803. The topology of the strict consensus trees obtained using either the combined mtDNA data or mtDNA + nuclear data were identical. The presence of three equally parsimonious trees in each of the analyses resulted from the uncertain arrangement of the roundscale spearfish (*T. georgii*) and the hatchet marlin (*Tetrapturus* sp.) relative to each other (Fig. 1A). Bootstrap support values for both analyses were relatively high for all recovered groupings (> 70%) with the exception of the aforementioned arrangement of the roundscale spearfish and hatchet marlin relative to each other. All other billfishes examined clustered within species (conspecifics clustered together) with the exception of two of the *Tetrapturus* species, the white marlin (Atlantic), and striped marlin (Pacific), which clustered together (100% bootstrap support, both analyses) but were not completely resolved at the species level. *Tetrapturus audax* 34 did not cluster with *T. audax* 12, but is a sister grouping of (*T. audax* 12, *T. albidus* 15, *T. albidus* 35; Fig. 1A,B).

Within genera, *Makaira* was not monophyletic in either analysis; a blue marlin + sailfish clade was recovered in all EPTs although bootstrap support was not particularly high (68% in the mitochondrial analysis and 71% in the combined analysis). Black marlin clustered outside the white and striped marlin, members of *Tetrapturus,* in all EPTs with fairly high bootstrap support (88% and 90%). Placement of the black marlin also rendered *Tetrapturus* polyphyletic. Although they did not cluster most closely with the other *Tetrapturus* species, the spearfishes were all most closely related to each other. The three commonly accepted spearfishes, the longbill spearfish (Atlantic), the Mediterranean spearfish (Atlantic and Mediterranean), and the shortbill spearfish (Pacific) clustered together with 100% bootstrap support and the two Atlantic species, the longbill spearfish and the Mediterranean spearfish, were the most closely related (99% and 95% bootstrap support, respectively). The two other putative spearfishes, the hatchet marlin and the roundscale spearfish, clustered

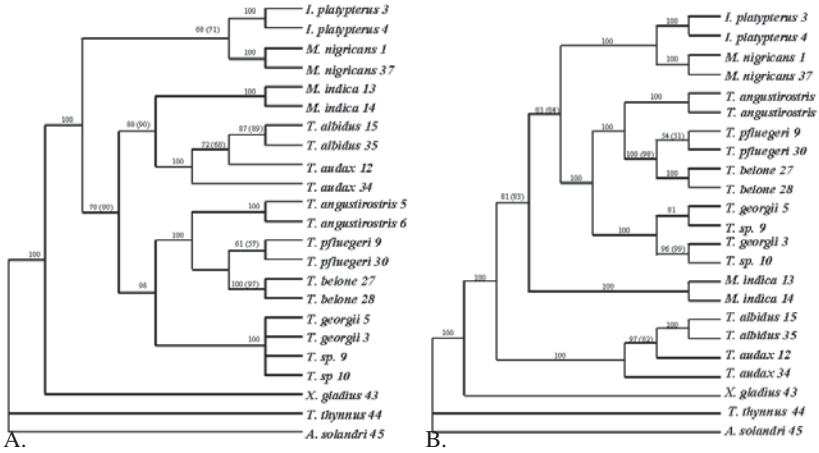


Figure 1. (A) Topology obtained in maximum parsimony analysis of both all mitochondrial loci combined and mitochondrial + nuclear data. Bootstrap support values (10,000 replicates) resulting from both analyses are shown on nodes. When support values differed, values for (mitochondrial + nuclear) data set are in parentheses. (B) Topology obtained in a Bayesian analysis of mitochondrial data and mitochondrial + nuclear data. Values shown are for 50% majority rule consensus of all trees. When support values differed between analyses, values for (mitochondrial + nuclear) data set are in parentheses. Numbers refer to specimen numbers.

outside the three commonly accepted spearfishes (this arrangement was supported by 98% of bootstrap replicates in both analyses. As noted before, *T. georgii* and the hatchet marlin were unresolved in these analyses (Fig. 1A,B).

The Bayesian analysis resulted in a 95% credible set of 4400 trees for the mitochondrial data set and 9000 trees for the mitochondrial + nuclear data set. A 50% majority rule consensus of each of these analyses was generated in PAUP* (Fig. 1B). The resulting topology resembles that of the parsimony tree with minor exceptions. While black marlin fell outside white marlin + striped marlin in the parsimony analysis, they fell outside blue marlin + sailfish + spearfishes in the Bayesian analyses, although support for this arrangement was poor (83% in the mitochondrial, 84% in mitochondrial + nuclear). The white marlin + striped marlin group was the most basal of the istiophorid billfishes in the Bayesian analysis although this was, again, poorly supported (81% and 83%), while sailfish + blue marlin were the most basal in the parsimony analysis (100% bootstrap support, both parsimony data sets).

## Discussion

Are Billfishes Scombroid Fishes?—There has been a debate in the literature as to the relationships among billfishes, tunas, and other scombroids (Carpenter et al., 1995; Orrell et al., 2006). Competing morphological cladistic hypotheses of the Scombroidei by Collette et al. (1984) and Johnson (1986) position the swordfish + billfishes as either sister to the Scombridae, or sister to the wahoo, *Acanthocybium*, within an expanded Scombridae. Two additional hypotheses have also been proposed: Finnerty and Block's (1995), based on molecular data, that billfishes form a separate suborder, Xiphioidei, sister to the Scombroidei, and that of Nakamura (1983, 1985) that billfishes are a separate suborder much more distantly related to Scombroidei.

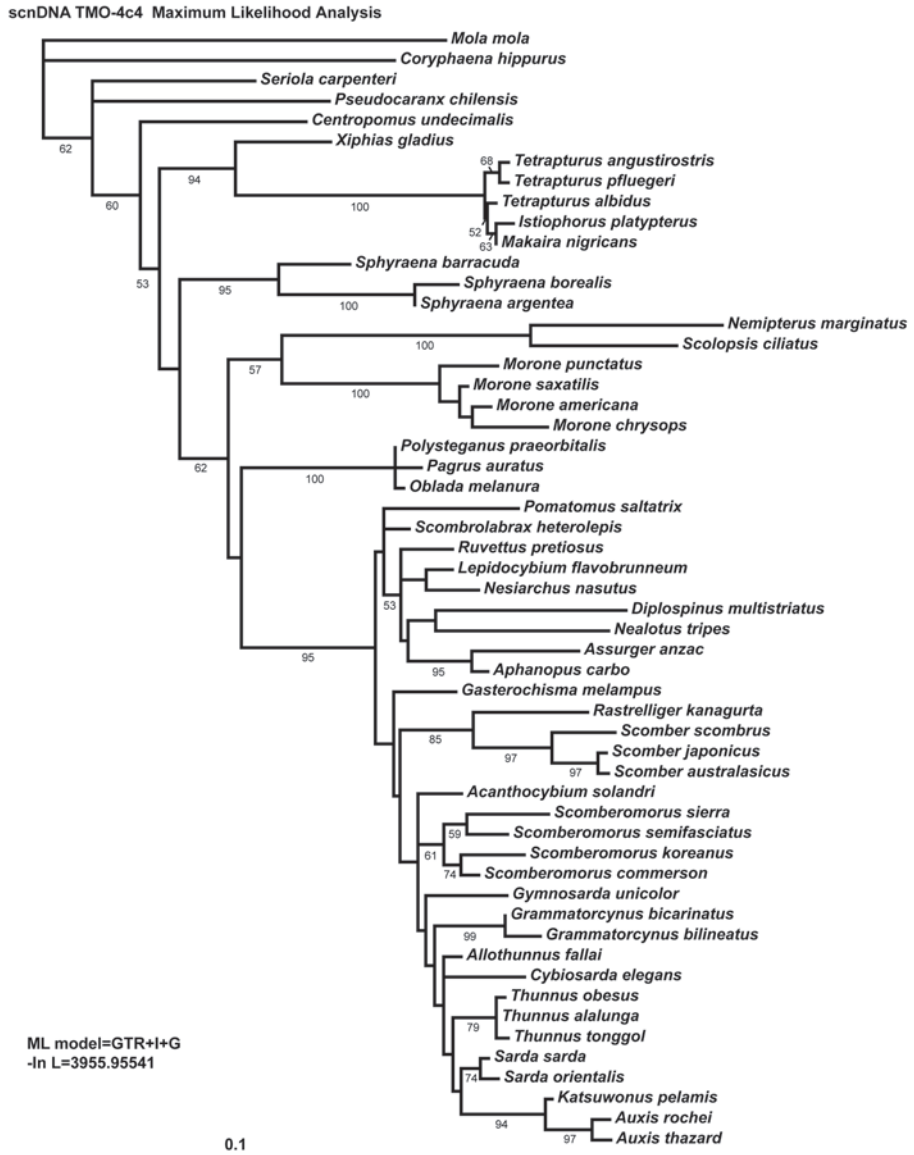


Figure 2. Maximum likelihood analysis of scombroids and billfishes based on Tmo-4C4 (reproduced from Orrell et al., 2006: fig. 2)

Recent results by Orrell et al. (2006; Fig. 2) based on 511 bp of the single copy nuclear locus Tmo-4C4 and a combined gene analysis (Tmo-4C4 plus cyt *b*) do not support the Scombroidei of either Collette et al. (1984) or that of Johnson (1986). Both the Tmo-4C4 and combined gene phylogenies recover separate Xiphioidei and Scombroidei clades. Thus, molecular data provide a clear phylogenetic signal supporting monophyly of billfishes and placement of the wahoo in the Scombridae. Parsimony analysis of the mitochondrial ATPase 6 gene (using both nucleotide and amino acid sequences) confirms monophyly of the billfishes (Alvarado-Bremer, 1994). Billfishes are also morphologically distinct from the scombroids based on possession of a

unique thermogenic organ associated with the superior rectus eye muscle (Block, 1991). Billfishes are genetically and morphologically distinct enough from scombroids to be placed in a separate suborder, Xiphioidei.

Families.—Fierstine (2006) recognizes five families of billfishes. Three are extinct, Hemingwayidae, Palaeorhynchidae, and Blochiidae, with their first occurrences in the Paleocene (56 million yrs ago [Ma], early Eocene [53 Ma], and middle Miocene [40 Ma], respectively). The extant Istiophoridae dates back to the middle Miocene (15 Ma), possibly to the late Eocene (34 Ma). The Xiphiidae has its first occurrence in the early Eocene (53 Ma), but *Xiphias* dates no further back than the middle Miocene (15 Ma). Nakamura (1985) placed the two Recent families in the suborder Xiphioidei: Xiphiidae, the monotypic family containing the swordfish, *Xiphias gladius* Linnaeus, 1758; and Istiophoridae containing three genera, *Istiophorus* (one or two species of sailfishes), *Makaira* (two or three species of marlins), and *Tetrapturus* (approximately six species of spearfishes and the white and striped marlins).

No one questions the family status of the swordfish. It differs from the Istiophoridae in having a depressed sword-like bill instead of a rounded bill, it lacks pelvic fins which are present in other billfishes, the two dorsal fins are well separated from each other instead of adjacent, there is a large median keel on the caudal peduncle instead of two small keels at the base of the caudal fin, and the swimbladder is a large single structure not composed of many small bubbles as in Istiophoridae.

Genera.—Most researchers recognize the generic distinctiveness of sailfish (*Istiophorus*) but there are problems with the common names "marlin" and "spearfish". Morrow (1964) felt that the generic divisions of the istiophorids were in a state of flux. Historically, the two large species, the black (*Makaira indica*) and the blue marlin (*M. nigricans*) have frequently been grouped with two smaller species, the Atlantic white marlin (*T. albidus*) and the Indo-Pacific striped marlin (*T. audax*) in the genus *Makaira*. However, Nakamura (1983, 1985) and others placed both small marlin species in the spearfish genus *Tetrapturus* along with four (or five) other species.

Both maximum parsimony and Bayesian analyses of the molecular data show a very different picture of relationships. *Makaira* does not appear to be monophyletic. Blue marlin cluster with sailfish and black marlin cluster either with white and striped marlin (parsimony) or outside blue marlin + sailfish + spearfishes (Bayesian). There is little morphological data to support this conclusion but the available data do not support the current arrangement of genera very well either. The high dorsal fin separates sailfish from other members of the family. Characters like a vertebral count of 11 + 13 group the blue and black marlin together apart from other istiophorids with a vertebral count of 12 + 12.

If we accept the molecular picture of phylogeny, two taxonomic arrangements would result. One could recognize genera for the two major clades: blue marlin + sailfish (as *Istiophorus*) and all the rest (as *Tetrapturus*). Alternatively, five genera could be recognized: within the first clade blue marlin (*Makaira*) are separated from sailfish (*Istiophorus*). In the second clade, there are three groups: black marlin (*Istiompax*), striped and white marlin (*Kajikia*), and three, four, or five spearfishes (*Tetrapturus*) (Fig. 3).

While the generic names *Kajikia* and *Istiompax* are not currently in use, Robins and de Sylva (1960) referred to all five generic names although they put them in quotes and stated that "names in quotes are not employed by the present writers". *Kajikia* Hirasaka and Nakamura (1947) may not be technically available according

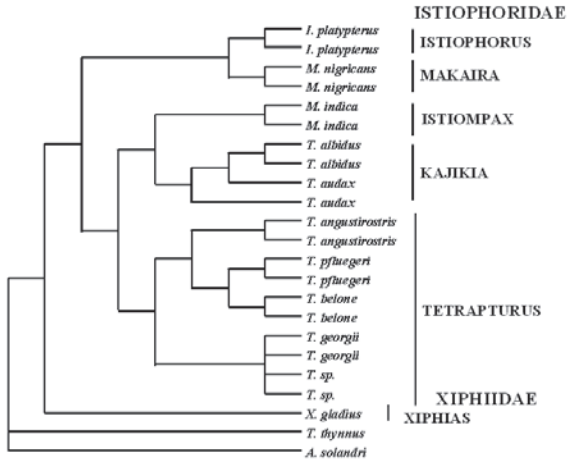


Figure 3. Parsimony analyses of mtDNA and nuclear loci with revised billfish genera.

to Article 13.3 of the International Code of Zoological Nomenclature, because the authors did not designate a type species in the original description of their new genus. However, as noted by Eschmeyer (2006), the name may be available from the treatment in the Zoological Record for 1947 where *Kajikia formosana* Hirasaka and Nakamura, 1947 (a junior synonym of *T. audax*) is listed as the type species. With regard to *Istiompax*, Morrow (1964) felt that the differences between black and blue marlins in flexibility of the pectoral fins and pattern of the lateral line were great enough to place the two species in separate genera, *Makaira* (blue) and *Istiompax* (black). Also, the bill of the blue marlin does not begin to lengthen much beyond the lower jaw until the fish reaches a length of a meter or more whereas in black marlin, the bill is well formed and extended by that size.

The molecular data of Finnerty and Block (1995) and Orrell et al. (2006) showed the same division of *Tetrapturus* species and some morphological data supports this division. The anus is far anterior to the origin of the first anal fin in all species of *Tetrapturus*, close to the anal fin in *Kajikia* (and other istiophorids).

Sailfish and Blue Marlin Populations.—There has been continuous controversy over whether Atlantic and Indo-Pacific sailfishes and blue marlins are separate species or not. Nakamura (1983, 1985) separated Atlantic from Indo-Pacific populations of both species but there is no genetic evidence to support this in either species. Sailfish were separated based on whether the pectoral and caudal fins are comparatively short, as in Indo-Pacific *I. platypterus,* or long as in Atlantic *I. albicans* (immature specimens up to about 90 cm body length). However, Morrow and Harbo (1969) failed to find any differences in pectoral fin length between populations of sailfish, or in any other morphometric or meristic characters. Indo-Pacific sailfish do attain a greater size (maximum about 100 kg) than Atlantic sailfish (60 kg).

There is no genetic evidence in the sailfish mtDNA control region to indicate that Atlantic and Indo-Pacific sailfishes are separate species; no fixed nucleotide differences were seen in control region sequences of 58 sailfish taken from throughout their range (McDowell, 2002). However, both restriction fragment length polymorphism (RFLP) analysis of whole molecule mtDNA and sequencing of the mitochon-

drial control region show two distinct mtDNA clades. Both clades are evident in the Atlantic, while only one is found in the Indo-Pacific (Graves and McDowell, 1995; McDowell, 2002).

Nakamura (1983, 1985) separated Atlantic *M. nigricans* from Indo-Pacific *M. mazara* blue marlins based on the pattern of the lateral line; Atlantic blue marlin have a reticulate pattern, Indo-Pacific blue marlin have only simple loops. As with the sailfish, there is no evidence from analysis of blue marlin mtDNA genotypes to indicate that the Atlantic and Indo-Pacific blue marlins are separate species; there are no fixed differences in RFLP haplotypes (Buonaccorsi et al., 2001). However, as with the sailfish, there are two distinct mtDNA clades in the Atlantic, only one of which occurs in the Pacific.

Striped and White Marlins.—There are two species groups within the current genus *Tetrapturus*. Atlantic white marlin, *T. albidus*, are closely related to Indo-Pacific striped marlin, *T. audax* forming the genus *Kajikia*. However, white and striped marlin were not resolved relative to each other in either these analyses or in analysis of RFLP haplotypes (Graves and McDowell, 2003), so further study is needed.

Spearfishes.—The four or five species of spearfishes are closely related, three or four Atlantic species, longbill (*T. pfluegeri*), Mediterranean (*T. belone*), roundscale (*T. georgii*), and perhaps the so-called "hatchet marlin"; and one Indo-Pacific species, the shortbill (*T. angustirostris*). The Mediterranean and longbill spearfishes are most closely related to each other (Robins and de Sylva, 1963). The shortbill spearfish is particularly divergent from the Mediterranean spearfish; an analysis of 27 control region sequences found fixed nucleotide differences at 17 positions (McDowell, unpubl. data). The validity of *T. georgii*, the roundscale spearfish, has been in question since its resurrection by Robins (1974) but appears to be valid based on morphological and molecular data (Shivji et al., 2006). Molecular data indicate that the so-called hatchet marlin (Pristas, 1980) may be an additional valid species of spearfish. These two putative species of spearfishes are more closely related to each other than to the three commonly accepted spearfish species. The fact that they could not be resolved relative to each other in this study suggests the need for further morphological and genetic analyses. Suggested names for the species of billfishes are contained in Appendix A.

## Acknowledgments

This study was funded by NOAA CMER program grant no. NA17FE2839. Permission for BBC to participate in leg 3 of the Shoyo Maru long-line cruise across the Atlantic and to collect billfish tissue was received from H. Kinoshita (Japanese Fisheries Agency), and H. Okamoto and K. Yokawa (Far Seas Fisheries, Shimizu). We are indebted to K. Gray and S. Boles for technical support. Contribution #2781 of the Virginia Institute of Marine Science.

## Literature Cited

Alvarado Bremer, J. R. 1994. Assessment of morphological and genetic variation of the swordfish (*Xiphias gladius* L.): Evolutionary implications of allometric growth and of the patterns of nucleotide substitution in the mitochondrial genome. Ph.D. Diss., University of Toronto, Toronto.

Block, B. A. 1991. Endothermy in fish: thermogenesis, ecology, and evolution. *In* P. W. Hochachka and T. Mommsen, eds. Biochemistry and molecular biology of fishes. 1: 269–311.

Buonaccorsi, V. P., J. R. McDowell, and J. E. Graves. 2001. Reconciling patterns of interocean-molecular variance from four classes of molecular markers in blue marlin (*Makaira nigricans*). Mol. Ecol. 10: 1179–1196.

Carpenter, K. E., B. B. Collette, and J. L. Russo. 1995. Unstable and stable classifications of scombroid fishes. Bull. Mar. Sci. 56: 379–405.

Collette, B. B., T. Potthoff, W. J. Richards, S. Ueyanagi, J. L. Russo, and Y. Nishikawa. 1984. Scombroidei: development and relationships. Pages 591–620 *in* H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall, Jr., and S. L. Richardson, eds. Ontogeny and systematics of fishes. Amer. Soc. Ichthyol. Herp. Spec. Publ. 1.

Eschmeyer, W. N. 2006. Catalog of fishes. Calif. Acad. Sci. Available from: http://www.calacademy.org/research/ichthyology/catalog via the Internet. Accessed 17 April 2006.

Estoup, A., C. R. Largiader, E. Perrot, and D. Chourrout. 1996. Rapid one-tube DNA extraction for reliable PCR detection of fish polymorphic markers and transgenes. Mol. Mar. Biol. Biotech. 5: 295–298.

Farris, J. S., M. Källersjö, A. G. Kluge, and C. Bult. 1994. Testing significance of incongruence. Cladistics 10: 315–319.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

Fierstine, H. L. 2006. Fossil history of billfishes (Xiphioidei). Bull. Mar. Sci. 79: 433–453.

Finnerty, J. R. and B. A. Block. 1995. Evolution of cytochrome *b* in the Scombroidei (Teleostei): molecular insights into billfish (Istiophoridae and Xiphiidae) relationships. Fish. Bull. 93: 78–96.

Goncalves, I., M. Robinson, G. Perrière, and D. Mouchiroud. 1999. JaDis: computing distances between nucleic acid sequences. Bioinformatics 15: 424–425.

Graves, J. E. and J. R. McDowell. 1995. Inter-ocean genetic divergence of istiophorid billfishes. Mar. Biol. 122: 193–204.

_____ and _____. 2003. Population structure of the world's billfishes: a genetic perspective. Mar. Freshw. Res. 54: 1–11.

Hirasaka, K. and H. Nakamura. 1947. On the Formosan spear-fishes. Bull. Oceanogr. Inst. 3: 9–24.

Johnson, G. D. 1986. Scombroid phylogeny: an alternative hypothesis. Bull. Mar. Sci. 39: 1–41.

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

McDowell, J. R. 2002. Genetic stock structure of the sailfish, *Istiophorus platypterus*, based on nuclear and mitochondrial DNA. Ph.D. Diss., Virginia Institute of Marine Science: College of William and Mary.

Mickevich, M. F. and J. S. Farris. 1981. The implications of congruence in *Menidia*. Syst. Zool. 30: 351–369.

Morrow, J. E. 1964. Marlins, sailfish, and spearfish of the Indian Ocean. Proc. Symp. Scombroid Fishes, Mar. Biol. Assoc. India 1: 429–440.

_____ and S. J. Harbo. 1969. A revision of the sailfish genus *Istiophorus*. Copeia 1969: 34–44.

Nakamura, I. 1983. Systematics of the billfishes (Xiphiidae and Istiophoridae). Publ. Seto Mar. Biol. Lab. 28: 255–396.

_____. 1985. Billfishes of the world. FAO Species Catalogue, vol. 5, FAO Fish. Synop. No. 125, vol. 5, 65 p.

Orrell, T. M., B. B. Collette, and G. D. Johnson. 2006. Molecular data support separate clades for Scombroidei (tunas and relatives) and Xiphioidei (billfishes). Bull. Mar. Sci. 79: 505–519.

Posada, D. and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817–818.

Pristas, P. J. 1980. A possible hatchet marlin (*Tetrapturus* sp.) from the Gulf of Mexico. Northeast Gulf Sci. 4: 51–56.

Robins, C. R. 1974. The validity and status of the roundscale spearfish, *Tetrapturus georgei*. NOAA Tech. Rep. NMFS SSRF-675, part 2: 54–61.

Robins, C. R. and D. P. de Sylva. 1960. Description and relationships of the longbill spearfish, *Tetrapturus belone*, based on western North Atlantic specimens. Bull. Mar. Sci. Gulf Carib. 10: 383–413.

_____ and _____. 1963. A new western Atlantic spearfish *Tetrapturus pfluegeri*, with a redescription of the Mediterranean spearfish, *Tetrapturus belone*. Bull. Mar. Sci. Gulf Carib. 13: 84–122.

Ronquist, F. and J. P. Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.

Sambrook, J. and D. W. Russell. 2001. Molecular cloning: a laboratory manual, 3rd. ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

Seutin, G., B. N. White, and P. T. Boag. 1991. Preservation of avian blood and tissue samples for DNA analysis. Can. J. Zool. 69: 82–90.

Shivji, M. S., J. E. Magnussen, L. R. Beerkircher, G. Hintegger, D. W. Lee, J. E. Serafy, and E. D. Prince. 2006. Validity, identification, and distribution of the roundscale spearfish, *Tetrapturus georgii*: morphological and molecular evidence. Bull. Mar. Sci. 79: 483–491.

Swofford, D. L. 2003. PAUP*. Phylogenetic analysis using parsimony (*and other methods), Version 4. Sinauer Associates, Sunderland, MA.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. ClustalW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22: 4673–4680.

Addresses: (B.B.C.) *National Marine Fisheries Service Systematics Laboratory, National Museum of Natural History MRC-0153, Washington D.C. 20560.* (B.B.C., J.R.D., J.E.G.) *School of Marine Science, Virginia Institute of Marine Science, Gloucester Point, Virginia 20362.* Corresponding Author: (B.B.C.) *E-mail: <collettb@si.edu>.*

APPENDIX A

| Suggested names for Recent billfishes (Xiphioidei): | |
|---|---|
| Xiphiidae | |
| *Xiphias gladius* Linnaeus, 1758 | Swordfish |
| Istiophoridae | |
| *Istiophorus platypterus* (Shaw in Shaw and Nodder, 1792) | Sailfish |
| *Istiompax indica* (Cuvier, 1832) | Black marlin |
| *Makaira nigricans* Lacépède, 1802 | Blue marlin |
| *Kajikia albida* (Poey, 1860) | White marlin |
| *Kajikia audax* (Philippi, 1887) | Striped marlin |
| *Tetrapturus angustirostris* Tanaka, 1915 | Shortbill spearfish |
| *Tetrapturus belone* Rafinesque, 1810 | Mediterranean spearfish |
| *Tetrapturus georgii* Lowe, 1841 | Roundscale spearfish |
| *Tetrapturus pfluegeri* Robins and de Sylva, 1963 | Longbill spearfish |