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Original Article

Genomic data resolve long-standing uncertainty by distinguishing white marlin (*Kajikia albida*) and striped marlin (*K. audax*) as separate species

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Large pelagic fishes are often broadly distributed and capable of long-distance movements. These factors can promote gene flow that makes it difficult to disentangle intra- vs. inter-specific levels of genetic differentiation. Here, we assess the relationship of two istiophorid billfishes, white marlin (*Kajikia albida*) and striped marlin (*K. audax*), presently considered sister species inhabiting separate ocean basins. Previous studies report levels of genetic differentiation between these species that are smaller than those observed among populations of other istiophorid species. To determine whether white marlin and striped marlin comprise separate species or populations of a single globally distributed species, we surveyed 2520 single nucleotide polymorphisms (SNPs) in 62 white marlin and 242 striped marlin across the Atlantic, Pacific, and Indian oceans. Multivariate analyses resolved white marlin and striped marlin as distinct groups, and a species tree composed of separate lineages was strongly supported over a single lineage tree. Genetic differentiation between white marlin and striped marlin ($F_{ST} = 0.5384$) was also substantially larger than between populations of striped marlin ($F_{ST} = 0.0192$–0.0840), and we identified SNPs that allow unambiguous species identification. Our findings indicate that white marlin and striped marlin comprise separate species, which we estimate diverged at approximately 2.38 Mya.

Keywords: billfish, genomics, highly migratory species, species delimitation, striped marlin, white marlin.

Introduction

Relatively low levels of genetic divergence have been observed within and among large pelagic fishes, including tunas and billfishes, many of which support socioeconomically valuable fisheries around the globe. Large pelagic fishes are often continuously distributed across broad geographic regions with few absolute barriers to gene flow and are capable of long-distance movements spanning hundreds to thousands of kilometres. These factors potentially facilitate persistently high levels of genetic connectivity among populations from geographically distant regions (Palumbi, 1994). Additionally, large pelagic fishes frequently exhibit large effective population sizes, slowing the rate of genetic drift and reducing heterogeneity among lineages (Martin *et al.*, 1992; Ward *et al.*, 1994; Waples, 1998). Despite these factors, population structure has been reported for several large pelagic fishes (Grewe *et al.*, 2015; Williams *et al.*, 2015; Mamoozadeh *et al.*, 2020; Vaux *et al.*, 2021), though it can be difficult to distinguish levels of genetic differentiation that correspond with separate species rather than populations of a single species (Palumbi, 1994; Waples, 1998).

Discerning population- and species-level relationships has proven challenging within the istiophorid billfishes (family Istiophoridae; marlin, spearfish, and sailfish), particularly for species with distributions that potentially span more than one ocean basin. For example, early descriptions of blue marlin (*Makaira nigricans*) recognized two species, one distributed in the Atlantic Ocean and another in the Indo-Pacific (Nakamura, 1985). However, genetic studies based on nuclear and mitochondrial markers revealed low levels of inter-oceanic divergence (Finnerty and Block, 1995; Graves and McDowell, 1995; Collette *et al.*, 2006), and blue marlin was subsequently reclassified as a single, globally distributed species (Collette *et al.*, 2006). Similarly, separate species of sailfish (*Istiophorus platypterus*) originally recognized within the Atlantic and Indo-Pacific (Nakamura, 1985) were also reclassified as a single species after genetic analyses revealed limited inter-oceanic divergence (Finnerty and Block, 1995; Graves and McDowell, 1995; Collette *et al.*, 2006). The revised relationships for blue marlin and sailfish have been further supported by more recent studies based on additional molecular markers (Hanner *et al.*, 2011; Williams *et al.*, 2018), though genetic relationships among other istiophorids remain uncertain.

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The relationship between white marlin (*Kajikia albida*) and striped marlin (*K. audax*), considered sister species inhabiting the Atlantic and Indo-Pacific, respectively, has been under scrutiny for more than three decades. White marlin and striped marlin appear morphologically similar but are distinguishable by slight differences in the shape of the dorsal and pectoral fins (Ueyanagi and Wares, 1975; Nakamura, 1985). However, given considerable intra-specific variation in fin morphology (Collette and Graves, 2019), and that fins are typically removed following capture in commercial fisheries, in practice white marlin and striped marlin are generally distinguished based on capture location (Atlantic vs. Indo-Pacific). Additionally, genetic studies have so far failed to resolve white marlin and striped marlin as separate lineages. White marlin and striped marlin are indistinguishable based on the mitochondrial cytochrome *c* oxidase subunit I "barcode" region (Hanner *et al.*, 2011). Phylogenetic studies that surveyed small numbers of mitochondrial and nuclear markers report a lack of fixed differences between white marlin and striped marlin (Finnerty and Block, 1995; Graves and McDowell, 1995; Collette *et al.*, 2006). Further, the level of genetic divergence between white marlin and striped marlin based on mitochondrial DNA is smaller than that reported for inter-oceanic comparisons within blue marlin and sailfish (Graves and McDowell, 1995; Williams *et al.*, 2018). Although separate Atlantic and Indo-Pacific species of blue marlin and sailfish have been reclassified as single, globally distributed species, this has not been extended to white marlin and striped marlin, despite a closer genetic relationship based on the molecular markers examined to date.

The contemporary evolutionary relationship of white marlin and striped marlin presumably reflects their phylogeographic history, among other factors. A previous genetic study hypothesized that during the Pleistocene, white marlin and striped marlin were either incompletely isolated or isolation occurred but was followed by gene flow (Graves and McDowell, 2003). The spatial distributions of white marlin and striped marlin extend into more temperate waters than other istiophorids (Nakamura, 1985), including blue marlin and sailfish, indicating that inter-oceanic movements may be more feasible for these species. Small numbers of striped marlin have been reported from the southeastern Atlantic Ocean off South Africa (Talbot and Penrith, 1962; Penrith and Cram, 1974), though such inter-oceanic movements may be underreported given the morphological similarity of white marlin and striped marlin. Regardless, thermal preferences of white marlin and striped marlin highlight the possibility of historical and contemporary gene flow between the Atlantic and Indo-Pacific, potentially facilitating a closer genetic relationship for white marlin and striped marlin than for other istiophorids.

Here, we assess the evolutionary relationship between white marlin and striped marlin by performing a statistically robust comparison based on genome-wide single nucleotide polymorphisms (SNPs). We leverage a sampling design that includes white marlin and striped marlin from across the full range of both species, spanning the Atlantic, Pacific, and Indian oceans. Results from this study resolve long-standing uncertainty about whether white marlin and striped marlin comprise distinct species or populations of a single globally distributed species. Our results have implications for the management of white marlin and striped marlin and offer a suite of SNPs that enable unambiguous identification of these species.

Methods

Sample collection and DNA preparation

Like many other large pelagic fishes, sampling of istiophorids is challenging because these species are relatively rare and only seasonally occur in offshore habitats that are difficult to access. These factors typically result in opportunistic sampling designs that represent only a portion of the species range. In this study, we obtained samples from 19 geographic regions that collectively span the full geographic range of both white marlin and striped marlin (Table 1). We opportunistically sampled white marlin and striped marlin from a variety of sources during the period 1992–2017. These sampling efforts included striped marlin from multiple genetically distinct populations within the Pacific and Indian oceans (Mamoozadeh *et al.*, 2020). We also collected samples of white marlin from locations across the Atlantic Ocean, though this species is thought to comprise a single Atlantic-wide population (Mamoozadeh *et al.*, 2017). Fin tissues were sampled from fish caught and released by recreational anglers or caught as bycatch on commercial pelagic longline vessels. Additional tissues were collected from striped marlin available in local markets. We also analysed two collections of white marlin larvae acquired through fisheries-independent surveys. Larval species identifications based on morphological characters were verified by sequencing a segment of the mitochondrial DNA control region following Mamoozadeh *et al.* (2017). Tissue samples and whole larvae were preserved in 95% ethanol or a 10% dimethyl sulfoxide solution and maintained at room temperature until DNA isolation. In addition to the white marlin and striped marlin described here, we also sampled five blue marlin in the Atlantic, Pacific, and Indian oceans (Table 1); these samples were used as an outgroup in the species delimitation analyses described below.

DArTseq genotyping

We used the samples collected in this study to perform DArTseq genotyping (Sansaloni *et al.*, 2011), which consists of a genomic complexity reduction step followed by high-throughput sequencing, similar to other methodologies for restriction site-associated DNA sequencing (Peterson *et al.*, 2012). DArTseq library preparation and initial data processing were performed as described in Mamoozadeh *et al.* (2020). Briefly, DNA was fragmented using a double restriction enzyme digestion with *Pst*I and *Sph*I, then ligated with custom proprietary adapters. Fragments with *Pst*I–*Sph*I overhangs were preferentially amplified by PCR, then normalized and pooled into libraries each comprising 94 samples and 2 controls. Libraries then underwent 77 bp single-end sequencing on an Illumina HiSeq 2500 (Illumina, Inc.). Resulting sequence data were analysed using a proprietary DArT analytical pipeline to produce robust SNP genotypes. Reads were demultiplexed using sample-specific barcodes then used to create a *de novo* catalogue of reduced representation loci. Polymorphic positions within reduced representation loci were distinguished as SNPs, which were then filtered to remove low quality loci and genotype calls. A proportion of genotypes were produced from the sequence data a second time to assess technical replication error.

Table 1. Sampling details for the white marlin and striped marlin analysed in this study.

Species	Population (identifier)	Region	Years	No. of samples
Striped marlin	Western Indian Ocean (WIO)	Kenya	2015–2016	27
		South Africa	2015–2017	11
	Eastern Indian Ocean (EIO)	Western Australia	2016	8
	Western South Pacific Ocean (WSPO)	Eastern Australia	1994, 2010–2012, 2015	37
		Ecuador	1992, 2016	3
		Hawaii	2015	4
		New Zealand	2017	23
	North Pacific Ocean (NPO)	California	2000, 2016	15
		Hawaii	2015	11
		Japan	2015	14
		Taiwan	2014–2016	12
	Eastern Central Pacific Ocean (ECPO)	Baja California Sur	2015	22
		California	2000	1
		Ecuador	1992, 2016	34
		Peru	2016	20
			Total	242
White marlin	White marlin (WHM)	Angola	2014, 2015	1
		Azores	2012	1
		Brazil	2006, 2015	14
		Caribbean Sea*	2016	9
		Gulf of Mexico*	2007, 2008	8
		Morocco	1995, 2016	18
		United States mid-Atlantic	2015	11
			Total	62
Blue marlin	Blue marlin (BUM)	Caribbean Sea*	2015	1
		Ghana	1998	1
		Hawaii	1994	1
		Kenya	2015	1
		United States mid-Atlantic	2016	1
			Total	5

*Denotes larval sample collection. Sample collections for each region are arranged by genetically distinct population (Mamoozadeh *et al.*, 2020). The sampling year and number of sampled individuals are also shown for each sample collection. Regions that are listed more than once (e.g. Ecuador) indicate that more than one population was detected in that region by Mamoozadeh *et al.* (2020).

We performed additional quality filtering of the dataset supplied by DArT using dartR 0.93. We removed SNPs exhibiting average reproducibility <95%, followed by those with read depths <5 or >100, and loci with a minor allele frequency <5%. These filters were applied to reduce the probability of erroneous genotypes in the final dataset. We also sequentially removed SNPs and then individuals missing >30% of genotypes. Finally, to reduce the probability of linkage disequilibrium in the final dataset, we retained only a single SNP per reduced representation locus. We selected the SNP with the highest reproducibility; in the case of ties, the SNP with the highest polymorphism information content was retained.

Lineage delimitation

Because any given approach for species delimitation encompasses only a portion of the parameter space potentially relevant to evolutionary lineages (Carstens *et al.*, 2013), we employed a variety of approaches to assess whether white marlin and striped marlin comprise separate species. These approaches included methods to assign individuals to lineages and to assess the validity of inferred lineages. We then further characterized lineages by calculating metrics to quantify their level of differentiation. These calculations were also made at the population level to facilitate comparisons between lineages and previously reported populations.

We assigned individuals to lineages using principal component analysis (PCA) and discriminant analysis of principal components (DAPC). These multivariate methods efficiently summarize complex genetic information without strong assumptions about an underlying population genetic model (Jombart *et al.*, 2009; Jombart *et al.*, 2010). We summarized overall variability among individuals by performing PCA in adegenet 2.1.1 using centred and non-scaled allele frequencies. We also assessed relationships between groups of individuals and hierarchical structure among groups by performing DAPC in adegenet. Groups evaluated by DAPC were formed using sequential *K*-means clustering, which identifies groups where variation is minimized within groups and maximized among groups. The optimal number of PCs to include in each DAPC scenario was determined by assessing cluster reassignment probabilities. We used DAPC to evaluate grouping scenarios that corresponded with $K = 2$–20. The most likely range of values for K was identified by calculating Bayesian information criterion (BIC) for each K scenario. We used results from PCA and DAPC to organize individuals into groups putatively corresponding with lineages.

We evaluated the validity of lineages inferred using multivariate analyses by performing Bayes factor species delimitation. This approach enables the efficient estimation of species trees from genomic data while concurrently evaluating competing user-defined species delimitation models (Bryant *et al.*, 2012; Grummer *et al.*, 2014; Leaché *et al.*, 2014). We estimated species trees using the SNAPP 1.3.0 framework implemented in BEAST 2 2.6.0. Path sampling for marginal likeli-

hood calculations was performed using 50 steps and a burn-in of 50000 followed by 500000 Markov Chain Monte Carlo (MCMC) iterations. Model convergence was evaluated using Tracer 1.7. We compared two species delimitation models: one model in which white marlin and striped marlin comprised a single species, and a second model in which white marlin and striped marlin comprised two species. Analyses for each model were performed twice to assess the consistency of results. For computational tractability, species trees were estimated using a reduced dataset comprising five white marlin and five striped marlin samples. To create this reduced dataset, a single individual was randomly selected from each previously reported population of striped marlin and from five geographically diverse sampling sites for white marlin. We also included five blue marlin as an outgroup for both species models. Prior to species delimitation analyses, we removed loci that appeared as monomorphic or exhibited a minor allele frequency <0.05 in the reduced dataset. The most likely species delimitation model was identified by calculating Bayes factors for each model. We visualized species trees for both models using DensiTree 2.2.7.

To assess the phylogenetic relationship of lineages identified using multivariate analyses and Bayes factor delimitation, we inferred a maximum likelihood phylogeny using RAxML-NG 1.0.1. RAxML-NG analyses were performed using a dataset comprising concatenated sequences from reduced-representation loci that corresponded with the SNPs contained in the quality filtered dataset. Heterozygous genotypes were represented by standard IUPAC codes. We used the GTR + gamma model of evolution for RAxML-NG analyses, as recommended by the programme authors, and ten randomized maximum parsimony-based starting trees. We performed 1000 bootstrap replicates to assess support for tree nodes.

Lineage characterization

We characterized the lineages identified via the analyses described above by computing metrics reflecting the degree of differentiation between lineages. We also evaluated genetic differentiation between populations. For population-level calculations, white marlin were retained as a single population and striped marlin were grouped to reflect populations previously reported in the western Indian Ocean, eastern Indian Ocean, western South Pacific Ocean, North Pacific Ocean, and eastern central Pacific Ocean (Table 1; Mamoozadeh *et al.*, 2020). We used hierfstat 0.04–22 to calculate Nei's standard genetic distance (D_S; Nei, 1972) between lineages and populations. We also calculated F_{ST} (Weir and Cockerham, 1984) between lineages and populations using StAMPP 1.6.1. The statistical significance of F_{ST} values was assessed by performing 10000 bootstrap replicates. Finally, we used dartR to assess the presence of fixed allelic differences between lineages and populations.

Divergence time estimation

We estimated the divergence time for white marlin and striped marlin using BEAST. We implemented the fossilized birth-death (FBD) model (Heath *et al.*, 2014) in BEAST to calibrate node age estimates to real time. FBD methods have been widely used to estimate divergence times, including with datasets comprising genome-wide SNPs (see review by Wright *et al.*, 2022). We used multiple previously described fossils for calibration to real time. These comprised fossils for four extinct species of istiophorid billfishes (*Makaira belgicus*, *M. calvertensis*, *M. courcelli*, *M. purdyi*; Fierstine, 2001, 2006; Ray and Bohaska, 2001) and for one extinct species (*Hemingwaya sarissa*; Fierstine, 2006) in a closely related family of billfishes (family Hemingwayidae) known only from the fossil record. To determine an age prior for each fossil, we used the function runif in R to randomly select a value within the range of years that corresponded with the geologic time period reported for each fossil. We tested three sets of fossil age priors (Supplementary Table S1) to account for uncertainty in the exact age of each fossil and to assess their effect on divergence time estimates.

A subset of the white marlin and striped marlin in our quality filtered SNP dataset were used to estimate divergence time. Previous work indicates that the presence of population subdivision can lead to overestimates of divergence time among species (Hancock and Blackmon, 2020). Divergence times estimated using only variable sites can also contribute to overestimates of branch lengths and thus divergence times among species (Stange *et al.*, 2018). Therefore, we used SNPs plus their flanking sequences to estimate divergence time between single specimens of white marlin and striped marlin. We used our quality filtered SNP dataset and the gl2fasta function in dartR to create a concatenated alignment based on the reduced representation loci built during DArTseq genotyping. This approach has been implemented in other studies to estimate divergence times among species based on genome-wide SNPs (Longo and Bernardi, 2015; Zhou *et al.*, 2018; Vernygora *et al.*, 2022). We produced independent estimates of divergence time using six pairs of white marlin and striped marlin; these pairs represented each of the major geographic regions sampled in this study. We did this to account for potential variation in divergence time estimates due to the geographic origin of analysed specimens.

To estimate divergence time in BEAST, we used an FBD model as a prior on the tree topology and branching times. Additionally, we used a relaxed log normal clock model to allow for uncorrelated substitution rates among lineages. For the origin age, we assumed a uniform distribution with an age range of 0–70 Mya; we chose this maximum age as a conservative value based on a previously estimated divergence time for a recent ancestor of istiophorid billfishes (Santini and Sorenson, 2013). We ran independent searches for each set of fossil age priors and each pair of individuals (18 runs total). Each MCMC search was conducted using 100 million generations sampled at every 100 generations. We discarded the first 10 million runs as burn-in. We used Tracer to visually assess the convergence of each MCMC search. We then used the LogCombiner application in BEAST to thin sampled trees to 10000 trees for computational feasibility, followed by the TreeAnnotator application of BEAST to produce maximum clade credibility trees with node heights that correspond with mean divergence estimates. After evaluating results for each MCMC search, we used LogCombiner to combine trees sampled across independent runs and produce a single divergence time estimate.

Results

SNP dataset

We analysed 242 striped marlin sampled from 12 regions across the Indo-Pacific [8–37 (mean = 20) individuals per re-

gion; Table 1; Figure 1]. These individuals corresponded with five genetically distinct populations identified in a previous study (Mamoozadeh *et al*., 2020; mean = 48 individuals per population). Additionally, we analysed 62 white marlin sampled from seven regions across the Atlantic Ocean (1–18 individuals per region, mean = 9). These individuals corresponded with a single genetic population (Mamoozadeh *et al*., 2017).

DArTseq genotyping resulted in a dataset comprising genotypes for 308 individuals at 23508 SNPs (Table 2). A total of 2708 SNPs remained after filtering to remove loci with poor average reproducibility, low or excessive read depths, low minor allele frequencies, and large proportions of missing genotypes. Four individuals were missing >30% of genotypes and were therefore excluded from further analysis. A final dataset comprising 304 individuals and 2520 SNPs was produced by retaining a single SNP per reduced representation locus. This dataset was used for all subsequent analyses unless stated otherwise.

Lineage delimitation

Multivariate analyses consistently resolved white marlin and striped marlin as two distinct groups. In results from the PCA (Figure 2; Supplementary Figure S1), white marlin comprised a single group most clearly differentiated from striped marlin on PC axis one, which explained 38.45% of variation. Five genetically distinctive populations of striped marlin were also apparent in results from PCA. In DAPC results for the scenario with $K = 2$ (Figure 2), white marlin and striped marlin comprised two distinct groups where individuals within each group exhibited posterior membership probabilities of 100%. The BIC values calculated for each K scenario ranged from 1674 to 1594, with the smallest scores associated with $K = 2$–6 (Supplementary Figure S2). Admixture between white marlin and striped marlin was not observed at any value for K. Additional groups apparent at $K > 2$ corresponded with genetically distinctive populations of striped marlin (Supplementary Figure S3), therefore we focused on results based on $K = 2$.

Based on results from multivariate analyses, we assigned white marlin and striped marlin to two groups. We then evaluated the distinctiveness of these groups using Bayes factor species delimitation. Results from species delimitation analyses included strong support for the model where white marlin and striped marlin comprised separate species. Marginal likelihood values for the model with two species averaged −17302 (StDev = 0.269; Figure 3). In comparison, these values averaged −22371 (StDev = 0.024) for the model with one species. Bayes factor support for the two-species model relative to the single species model was 9191. The species tree associated with the two-species model reflected a large degree of consistency among model runs.

Results from multivariate analyses and Bayes factor species delimitation were consistent with a maximum likelihood phylogeny inferred with RAxML-NG. This phylogeny comprised two clades that corresponded with white marlin and striped marlin (Figure 4). The node basal to the clade of striped marlin exhibited 100% bootstrap support, indicating that a distinct striped marlin clade was consistently recovered in RAxML-NG analyses.

Lineage characterization

Analyses to further characterize the relationship between lineages corresponding with white marlin and striped marlin revealed large levels of genetic differentiation and the presence of loci exhibiting fixed allelic differences. D_S between white marlin and striped marlin was 0.3642 and ranged from 0.3619 to 0.3868 between white marlin and populations of striped marlin (Table 3; Supplementary Figure S4). In comparison, D_S ranged from 0.0112 to 0.0312 among populations of striped marlin. F_{ST} between white marlin and striped marlin was 0.5384 and ranged from 0.5694 to 0.6254 between white marlin and populations of striped marlin, as compared to 0.0192–0.0840 among populations of striped marlin (Table 3). All F_{ST} values were statistically significant at $p < 0.0001$. White marlin exhibited larger levels of differentiation from the two striped marlin populations in the Indian Ocean relative to the striped marlin populations in the Pacific Ocean. We identified 71 SNPs with fixed allelic differences between the white marlin and striped marlin analysed here (Table 4); DAPC performed using only these SNPs resolved white marlin and striped marlin as distinct groups with no admixture (Supplementary Figure S5). The number of loci with fixed allelic differences between white marlin and individual populations of striped marlin ranged from 96 to 131. The largest number of fixed differences was observed between white marlin and the eastern Indian Ocean population of striped marlin. This result may be an artefact of sample size given that the number of striped marlin we analysed from this population ($n = 8$ individuals) was much smaller than other populations ($n \geq 38$ individuals).

Divergence time estimation

Estimates of the time since divergence between white marlin and striped marlin indicated that these species diverged relatively recently. Mean divergence time estimates ranged from 1.39 to 3.15 Mya across the scenarios we evaluated for fossil age priors and pairs of individuals (Supplementary Figure S6). After combining the results for all 18 independent MCMC searches, we estimated that white marlin and striped marlin diverged 2.38 Mya (95% HPD = 0–12.51 Mya). This estimate indicates that white marlin and striped marlin diverged during the Pleistocene Epoch.

Discussion

The primary goal of this study was to determine whether white marlin and striped marlin comprise separate species or populations of a single globally distributed species. Our results are consistent with the presence of distinct evolutionary lineages for white marlin and striped marlin, lending support to the current classification of white marlin and striped marlin as separate species. This study offers the first comparison of white marlin and striped marlin based on genome-wide molecular markers and includes individuals from across the full distributional range of each species. Our results provide insight into events that influenced the evolutionary histories of these species, and support management efforts for white marlin and striped marlin by identifying several SNPs that can be used for unambiguous species discrimination.

Relationship of white marlin and striped marlin

Multiple lines of evidence from the range of analyses employed in this study are consistent with distinct evolutionary lineages for white marlin and striped marlin. Individual-based analyses consistently resolved white marlin and striped marlin

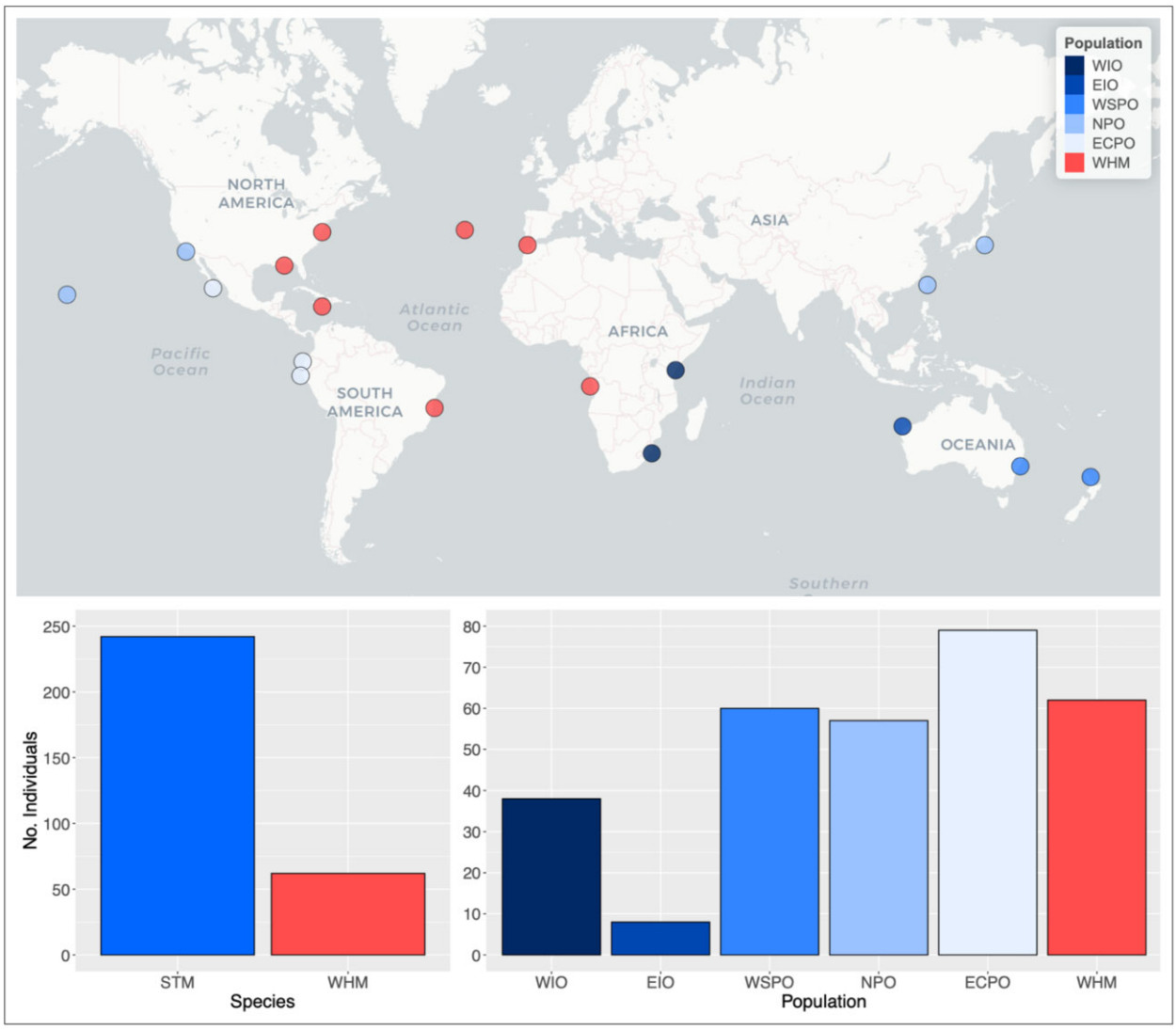

Figure 1. Sampling details for the white marlin (WHM) and striped marlin (STM) analysed in this study. Population identifiers are abbreviated as in Table 1. Top panel: Map depicting geographic regions where samples were collected. A single point per region is shown. Points are colour-coded by population (striped marlin) or species (white marlin). Bottom panel: Barplots depicting the number of individuals analysed per species (left) or population (right).

Table 2. Additional quality filters applied to the DArTseq dataset.

Quality filter	No. of individuals retained	No. of SNPs retained
Raw dataset	308	23 508
SNP average reproducibility < 95%	308	23 282
SNP read depth < 5 or > 100	308	16 222
SNP minor allele frequency < 5%	308	5 169
SNP missing > 30% of genotypes	308	2 708
Individual missing > 30% of genotypes	304	2 708
One SNP per RRL locus	304	2 520

The numbers of individuals and SNPs remaining after each filtering step are shown.

as two well-defined groups. These results were corroborated by a maximum likelihood phylogeny and strong support for a species tree composed of separate lineages for white marlin and striped marlin. F_{ST} between white marlin and striped marlin ($F_{ST} = 0.5384$) was 6.4× greater than the largest F_{ST} observed between populations of striped marlin ($F_{ST} = 0.0840$). The magnitude of difference between inter- vs. intra-specific divergence based on D_S was even larger—D_S between white marlin and striped marlin ($D_S = 0.3642$) was 11.7× greater than the largest D_S observed between populations of striped marlin ($D_S = 0.0312$). Though we included an outgroup in only a subset of analyses, we do not expect that the relationship between white marlin and striped marlin would have appeared differently had an outgroup been used in additional analyses. Collectively, these results demonstrate substantially greater genetic divergence between white marlin and striped marlin than among populations of striped marlin, indicating that white marlin and striped marlin represent distinct species.

Levels of genetic differentiation reported in genomic studies of other large pelagic fishes offer further support for recognizing white marlin and striped marlin as separate species. Based on their assessment of over 6000 SNPs, Pecoraro *et al.* (2016)

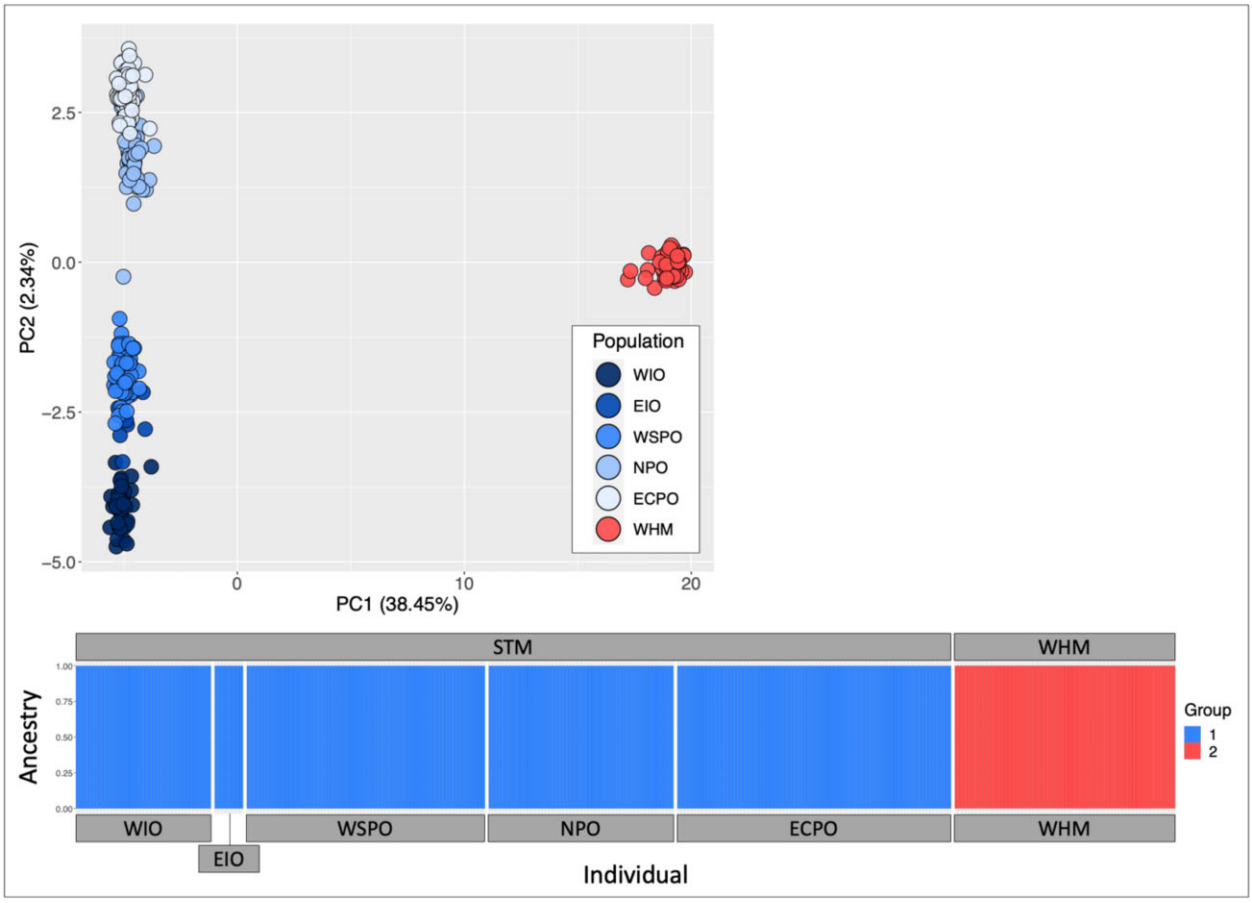

Figure 2. Top panel: Results from PCA. Axes one and two, as well as the percentage of variation explained by each axis, are shown. Individuals are colour-coded by population (striped marlin) or species (white marlin). Population identifiers are abbreviated as in Table 1. Bottom panel: Results from DAPC performed using $K = 2$. Individuals are arranged by species and population within species. STM = striped marlin, WHM = white marlin.

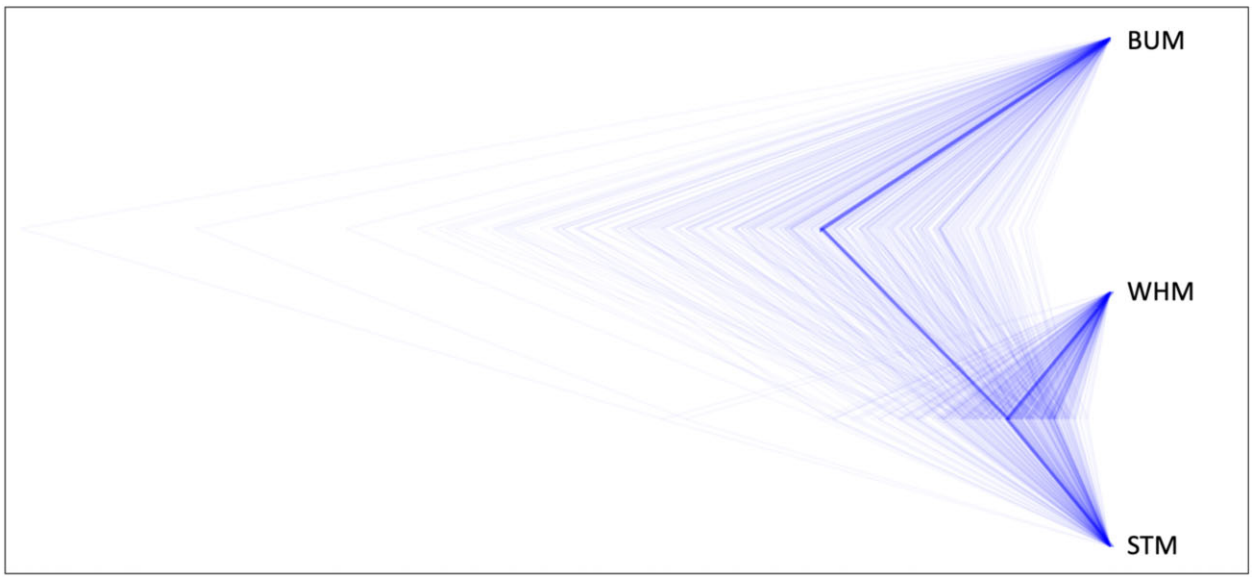

Figure 3. Species tree inferred using BEAST2. The consensus tree for the scenario where white marlin and striped marlin comprised separate lineages is shown. STM = striped marlin, WHM = white marlin, BUM = blue marlin.

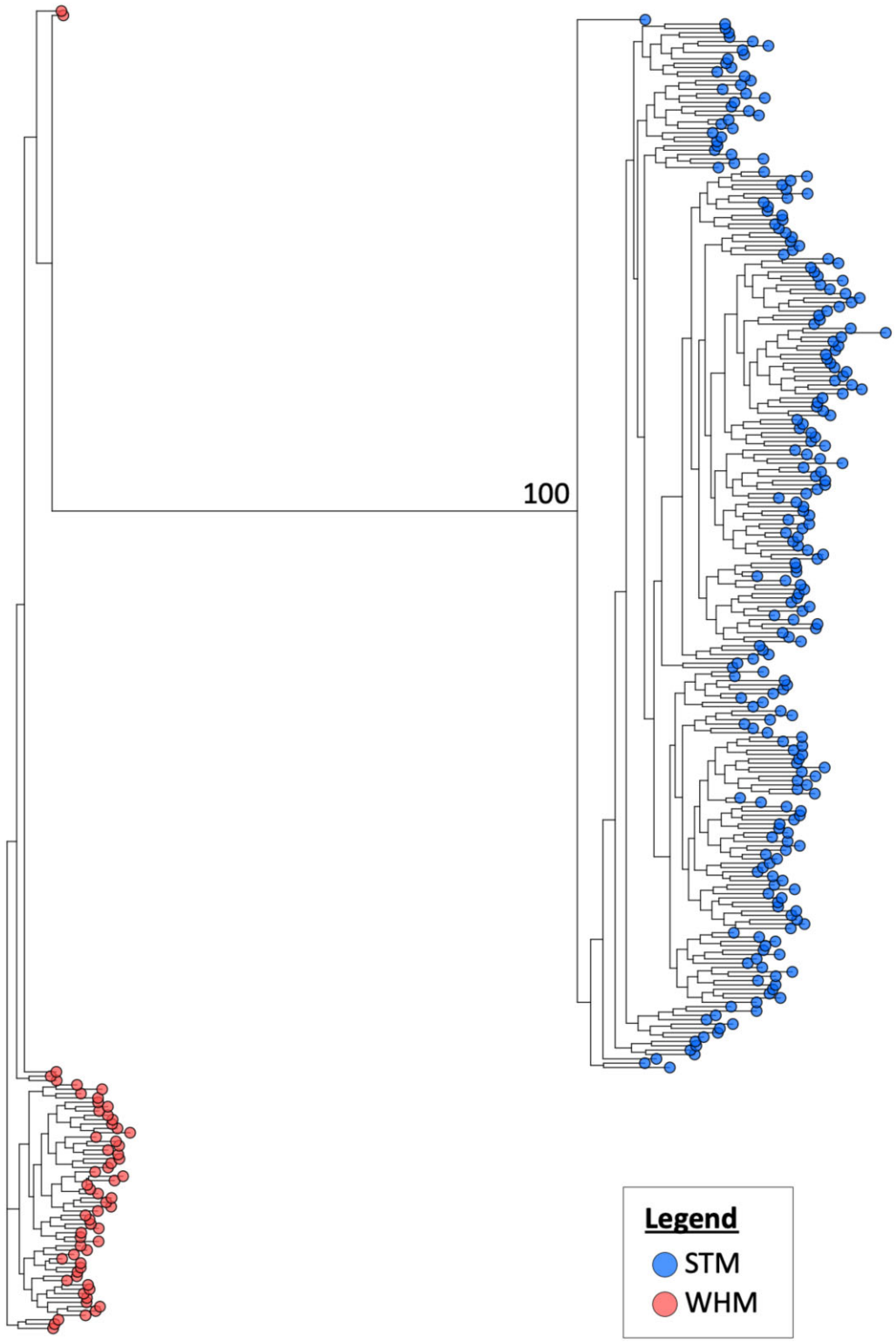

Figure 4. Unrooted maximum likelihood phylogeny inferred using *RAxML-NG*. Individuals are colour-coded by species. The node corresponding with the clade of striped marlin received 100% bootstrap support (1000 bootstrap replicates). STM = striped marlin, WHM = white marlin.

reported $F_{ST} = 0.0171–0.0474$ for inter-oceanic comparisons of yellowfin tuna (*Thunnus albacares*). Similarly, Albaina *et al*. (2013) reported $F_{ST} = 0.007–0.050$ for inter-oceanic comparisons of albacore tuna (*T. alalunga*), though these analyses were based on only a small number of SNPs. Additional inter-oceanic comparisons of large pelagic fishes based on genomic datasets are currently lacking, but comparisons within ocean basins are still informative. For example, in their analysis of nearly 13000 SNPs in albacore tuna, Vaux *et al*. (2021) reported $F_{ST} = 0.0056$ between genetically distinct populations detected within the Pacific Ocean. Collectively, the levels of differentiation reported in these studies are an order of mag-

Table 3. Pairwise genetic distances between white marlin (WHM) and genetically distinct populations of striped marlin (STM).

	STM (WIO)	STM (EIO)	STM (WSPO)	STM (NPO)	STM (ECPO)	WHM
STM (WIO)	–	0.0200	0.0138	0.0279	0.0287	0.3840
STM (EIO)	0.0260	–	0.0178	0.0295	0.0312	0.3868
STM (WSPO)	0.0365	0.0192	–	0.0138	0.0171	0.3698
STM (NPO)	0.0811	0.0550	0.0366	–	0.0112	0.3619
STM (ECPO)	0.0840	0.0627	0.0489	0.0287	–	0.3699
WHM	0.6047	0.6254	0.5718	0.5764	0.5694	–

Population identifiers shown for striped marlin are abbreviated as in Table 1. F_{ST} values (Weir and Cockerham, 1984) are shown below the diagonal and D_S values (Nei, 1972) are shown above the diagonal.

Table 4. Numbers of SNPs exhibiting fixed allelic differences between white marlin (WHM) and striped marlin (STM) based on the dataset analysed in this study.

	Population					Species
	STM (WIO)	STM (EIO)	STM (WSPO)	STM (NPO)	STM (ECPO)	STM
WHM	107	131	99	96	99	71

Numbers are shown for comparisons between white marlin and striped marlin, and between white marlin and genetically distinct populations of striped marlin. Population identifiers shown for striped marlin are abbreviated as in Table 1.

nitude lower than that calculated between white marlin and striped marlin in the present study ($F_{ST} = 0.5384$) and are instead comparable to intra-specific comparisons of striped marlin ($F_{ST} = 0.0192$–0.0840).

Intra-specific relationships

Additional findings from this study include an apparent lack of population structure among the white marlin analysed here. This result is consistent with previous analyses based on small numbers of microsatellite markers (Graves and McDowell, 2006; Mamoozadeh *et al.*, 2017). In this study, PCA and DAPC consistently resolved white marlin as a single group of individuals, including across the values for K assessed here ($K = 2$–20). A lack of population subdivision for white marlin presumably reflects the phylogeographic history of this species and is consistent with contemporary dispersal and gene flow across the Atlantic Ocean. Differences in life history between species and in environmental heterogeneity among ocean basins presumably contribute to the lack of population subdivision for white marlin in the Atlantic Ocean relative to the high degree of population subdivision for striped marlin in the Pacific and Indian oceans.

Management implications

In addition to clarifying the relationship between white marlin and striped marlin, results from this study provide information for developing molecular tools to readily identify these species. Though geographic location may be suitable for distinguishing white marlin and striped marlin in most regions, it is unreliable in regions where these species may co-occur, such as off southern Africa (Talbot and Penrith, 1962; Penrith and Cram, 1974). In these regions, an inability to verify species identity contributes uncertainty to the monitoring of catches and inference of stock status. For example, prior to 2005, there were no reports of commercial landings of striped marlin from the Atlantic Ocean; however, since 2005 annual landings as high as 75 mt have been reported from this region (ICCAT, 2021). Reports of striped marlin landings in the Atlantic Ocean coincide with management measures adopted by the International Commission for the Conservation of Atlantic Tunas and first implemented in 2001 (ICCAT Recommendation 2000–05). These measures established country-specific catch limits for white marlin, but the morphological similarity of white marlin and striped marlin and the fact that fins are typically removed after capture makes it possible for catches of white marlin to be misreported as striped marlin. The 71 SNPs exhibiting fixed allelic differences between the white marlin and striped marlin analysed in this study represent candidate loci for developing diagnostic genetic tools for readily identifying these species. Tools that increase the ability of managers to enforce management measures for white marlin are especially important given that the single Atlantic-wide stock of this species has been considered overfished for several decades (ICCAT, 2019) and this species is recognized as vulnerable by the IUCN.

Speciation in pelagic environments

Our divergence time estimate of 2.38 Mya indicates that white marlin and striped marlin diverged during the early Pleistocene. This result is consistent with a divergence time previously reported for white marlin and striped marlin based on nuclear and mitochondrial sequence data (0.4–2.6 Mya; Santini and Sorenson, 2013). Divergence between white marlin and striped marlin may have been facilitated by glaciation during the Pleistocene. Colder water temperatures likely resulted in a contracted north–south spatial distribution for the most recent common ancestor of these species. This contraction may have limited inter-oceanic connectivity, which would have been more likely to occur around present-day South Africa. However, because white marlin and striped marlin exhibit lower levels of divergence than other istiophorids that have spatial distributions spanning the Atlantic and Indo-Pacific (e.g. sailfish, blue marlin; Graves and McDowell, 1995), inter-oceanic isolation of white marlin and striped marlin may have been incomplete or persisted for a shorter period of time. Secondary contact of striped marlin and white marlin may have been facilitated by warming waters after the most recent glaciation event. This scenario may be plausible given differences in the thermal and habitat preferences of white marlin and striped marlin compared to other istiophorids (Nakamura, 1985; but see Boyce *et al.*, 2008). In particular, habitat use inferred using satellite archival tags indicate that white marlin and striped marlin have broader thermal tolerances than other istiophorids (Goodyear, 2003; Horodysky *et al.*, 2007; Kraus and Rooker, 2007; Sippel *et al.*, 2007; Su *et al.*, 2008; Hoolihan *et al.*, 2011; Lien *et al.*, 2014; Mourato *et al.*, 2014; Lam *et al.*, 2015; Carlisle *et al.*, 2017; Rohner *et al.*, 2020). Notably, in the western Indian Ocean, striped marlin exhibit broader thermal tolerance (Hoolihan and Luo, 2007; Rohner *et al.*, 2022) and habitat suitability (Thoya *et al.*, 2022) than other istiophorids in this region. Though analogous information for white marlin in neighbouring waters of the eastern South Atlantic Ocean is unavailable, these results indicate that inter-oceanic movement

around South Africa is likely at least for striped marlin and may reflect the historical habitat use of this species.

Concluding remarks

We provide evidence for the presence of distinct evolutionary lineages corresponding with white marlin and striped marlin. This evidence is consistent with the current taxonomy of these species, the status of which has remained in question for more than three decades. Additionally, the levels of intra- and inter-specific divergence reported in this study offer a useful reference for future genomic assessments of population- and species-level relationships in large pelagic fishes. Finally, results from this study expand our knowledge of the evolutionary history of white marlin and striped marlin and enable future analyses to further explore the demographic history of these species.

This study reflects the vital importance of cross-sector collaborations in facilitating studies of large pelagic fishes. Given that large pelagic fishes are distributed in offshore habitats across broad geographic regions, sampling designs that include enough spatial or temporal coverage to accurately represent focal relationships are challenging, especially within the context of a single study. Cross-sector collaborations that involve scientists at academic institutions, natural resource agencies, regional fisheries management organizations, non-governmental organizations, and other relevant entities are thus critical to implementing sampling designs that enable rigorous studies of large pelagic fishes. Equally important to these efforts are contributions from citizen scientists, who may have intrinsic socioeconomic incentives for contributing to research efforts aimed at supporting healthy sport fisheries. Future efforts to formalize such cross-sector collaborations by establishing dedicated sampling programmes and publicly accessible sample archives (e.g. the Pacific Marine Specimen Bank) may go a long way in reducing challenges to the study of large pelagic fishes, ultimately improving our ability to promote the long-term persistence of these iconic species.

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Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

Author contributions

NM, JM, and JG conceptualized the study and conducted field work. NM provided funding, performed laboratory work and data analysis, and wrote the original version of the manuscript. JM and JG provided funding, performed study supervision, and contributed revisions to the original manuscript. RB, JS, JH, and SO-G conducted field work and contributed revisions to the original manuscript.

Conflict of interest

The authors do not have any conflicts of interest to report.

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Data availability

The raw sequence data generated in this study are publicly available via the Dryad digital repository (https://doi.org/10.5061/dryad.8931zcrwd). Information for the $n = 71$ SNPs exhibiting fixed allelic differences between striped marlin and white marlin is also available via Dryad.

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