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Mitogenomic Architecture and Phylogenetic Relationship of European Barracuda, *Sphyraena sphyraena* (Teleostei: Sphyraenidae) from the Atlantic Ocean

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Abstract: The collective understanding of global-scale evolutionary trends in barracuda mitogenomes is presently limited. This ongoing research delves into the maternal evolutionary path of *Sphyraena* species, with a specific focus on the complete mitogenome of *Sphyraena sphyraena*, sourced from the Atlantic Ocean through advanced next-generation sequencing. This mitogenome spans 16,841 base pairs and encompasses 37 genes, including 13 protein-coding genes (PCGs), 2 ribosomal RNA genes (rRNAs), 22 transfer RNA genes (tRNAs), and an AT-rich control region. Notably, the mitogenome of *S. sphyraena* exhibits a preference for AT-base pairs, constituting 55.06% of the composition, a trait consistent with a similar bias found in related species. Most protein-coding genes initiate with an ATG codon, with the exception of Cytochrome c oxidase I (*COI*), which begins with a GTG codon. Additionally, six PCGs terminate with a TAA codon, *COI* with AGA, while six others exhibit incomplete termination codons. In the *S. sphyraena* mitogenome, the majority of transfer RNAs exhibit typical cloverleaf secondary structures, except for tRNA-serine, which lacks a DHU stem. Comparative analysis of conserved blocks within the *D-loop* regions of six Sphyraenidae species reveals that the CSB-I block extends to 22 base pairs, surpassing other blocks and containing highly variable sites. Both maximum-likelihood and Bayesian phylogenetic analyses, using concatenated 13 mitochondrial PCGs, distinctly separate all Sphyraenidae species. The European Barracuda, *S. sphyraena*, demonstrates a sister relationship with the '*Sphyraena barracuda*' group, including *S. barracuda* and *S. jello*. In conclusion, this study advances our understanding of the evolutionary relationship and genetic diversity within barracudas. Furthermore, it recommends comprehensive exploration of mitogenomes and broader genomic data for all existing Sphyraenidae fishes, providing invaluable insights into their systematics, genetic characterization, and maternal evolutionary history within marine environments.

Keywords: barracudas; Africa; next-generation sequencing; mitochondrial genome; evolution

Key Contribution: The current research enhances our understanding of the genetic makeup and structural attributes of the complete mitochondrial genome of the European Barracuda, *Sphyraena sphyraena*, as well as other members of the barracuda family. By delving into genetic distances and

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examining phylogenetic patterns, the researchers unveil the maternal lineage's evolution among different *Sphyraena* species and their broader context within Percomorpha. The generation of additional mitogenomes is anticipated to provide insights into the genetic diversity, evolutionary history, and conservation requisites of fish species within the Sphyraenidae family.

1. Introduction

Barracudas (Actinopterygii: Perciformes) are large, globally distributed ray-finned fishes found in tropical and temperate seas [1]. Fossil records date the presence of barracudas back to the Miocene period [2]. They belong to the single extant genus, *Sphyraena*, within the family Sphyraenidae, and currently, there are 26 recognized species worldwide [3]. Barracudas are characterized by their elongated body, pointed head, two widely separated dorsal fins, and large, sharp, conical teeth [4]. Their unique gill-raker morphology allows for their proper identification using classical methods [5,6]. Due to their popularity as a food source, barracudas have gained economic value and significant importance in aquaculture [7]. These species of fish are generally piscivorous (fish eaters) and act as top predators in reef and non-reef habitats, helping to maintain marine ecosystems (http://oceanexplorer.noaa.gov, accessed on 18 October 2023). Given their species' diversity, *Sphyraena* species has been extensively studied from various perspectives. Researchers have conducted ultrastructural investigations into their morphological features, bite mechanics, and length-weight relationships across different marine environments [8–11]. Additionally, ecological and physiological studies have been pursued to gain insights into their behavior and adaptation [12–14]. Furthermore, barracudas have been subjected to repeated examination for parasitological research [15,16] and the assessment of potential human health risks linked with the consumption of heavy metals through fish consumption [17,18]. These species have also been scrutinized to evaluate predator-prey interactions within coral reef ecosystems [19].

The European Barracuda, *Sphyraena sphyraena*, is distributed across the Atlantic Ocean, Mediterranean Sea, Tyrrhenian Sea, and Black Sea. Compounded by its sympatric co-existence with another closely related species, the Yellowmouth Barracuda (*Sphyraena viridensis*), in the northern Atlantic Ocean and Mediterranean Sea, these two species have been sources of enduring taxonomic confusion, particularly in various life stages. In recent years, DNA research has advanced rapidly, leading to numerous large-scale initiatives aimed at unraveling the phylogeny, diversification, and evolutionary relationships of teleostean fishes [20–22]. Despite these efforts, several acanthomorph lineages remain poorly understood due to inadequate sampling. Some projects have specifically sought to elucidate the phylogenetic relationships of acanthomorphs, including Sphyraenidae species, by examining partial mitochondrial (*COI*, *Cytb*, *12S rRNA*, *16S rRNA*, and *D-loop*) and nuclear genes (*28S rRNA*, *rhodopsin*, and *RNF213*) [23–25]. This genetic data has proven valuable in assessing the distribution of genetic variability among Sphyraenidae species and resolving the taxonomic dilemma of *S. sphyraena* and *S. viridensis* [26,27]. Nevertheless, data on the nuclear and mitochondrial genetics of Sphyraenidae species has been generated sporadically [28–36]. More recently, comprehensive genome-wide analyses of Sphyraenidae species have been initiated to uncover cryptic diversity, employing phylogenomic, population genomic, and coalescent-based species delimitation approaches while considering morphological and ecological data [37]. However, our understanding of barracudas based on mitochondrial genomes is still fragmentary. Prior to this study, mitogenomes for five species were generated and are available in the global GenBank database [38–40]. Furthermore, the genetic characterization of various genes and the mitochondrial genome-based assessment of evolutionary relationships remain incomplete for this group. To enhance taxonomic clarity in phylogenetic interpretation, the present study aimed to assemble the complete mitogenome of the European Barracuda, *Sphyraena sphyraena*, from the Atlantic Ocean. Additionally, this study sought to characterize the structure and variations in mitochondrial genes of *S. sphyraena* when compared to other available barracuda species.

In conclusion, this research contributes to our global understanding of the evolutionary history of the Sphyraenidae. However, investigating deeper into mitogenomes and extensive genomic data for *Sphyraena* species holds the potential to offer fresh perspectives on their thorough genetic profiling and evolutionary journey in the marine environment.

2. Materials and Methods

2.1. Sampling and Species Identification

A live European Barracuda, scientifically known as *S. sphyraena*, was captured in the Atlantic Ocean off the coast of Cameroon, Africa (2.8877N 9.7219E). Species identification was performed following established morphological criteria, as outlined in prior studies [41,42]. The specimen, measuring 380 mm in length, exhibited a dark dorsal surface and a silvery ventral surface, demarcated by the lateral line. The second dorsal fin surpassed the first in length, accompanied by the presence of 15 to 17 scales above the lateral line. The anal fin displayed a whitish-yellow hue, and an enumeration of 13 pectoral fin rays was recorded. The morphological characters observed effectively distinguish *S. sphyraena* from its closest species, *S. viridensis*. Muscle tissue was carefully excised from the ventral thoracic region and stored under sterile conditions within the Department of Marine Biology at Pukyong National University in Busan, South Korea. Voucher specimens were meticulously preserved in 10% formaldehyde at the Fisheries and Animal Industries (MINEPIA) facility in Yaoundé, Cameroon. The Institutional Animal Care and Use Committee (IACUC) granted approval under the code PKNUIACUC-2022-72, dated 15 December 2022, confirming that the biological material used in the experiments did not raise ethical concerns, as the targeted fish was not subjected to harm by the researchers. The experiments were conducted in adherence to the relevant ARRIVE 2.0 guidelines (https://arriveguidelines.org).

2.2. DNA Extraction, Sequencing, and Assembly

Total genomic DNA extraction was carried out using the AccuPrep® DNA extraction kit from Bioneer in Daejeon, Republic of Korea, following established standard protocols. The quality and quantity of genomic DNA were meticulously assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific D1000, Waltham, MA, USA). To obtain the complete mitogenome of *S. sphyraena*, sequencing procedures were conducted on the NovaSeq platform at Macrogen (https://dna.macrogen.com/) in Daejeon, Republic of Korea, provided by Illumina. Sequencing libraries were prepared according to the manufacturer's guidelines for the TruSeq Nano DNA High-Throughput Library Prep Kit (Illumina, Inc., San Diego, CA, USA). Briefly, 100 ng of genomic DNA underwent fragmentation using adaptive focused acoustic technology (Covaris, Woburn, MA, USA), resulting in double-stranded DNA molecules with blunt ends and 5′-phosphorylation. Following the end-repair step, DNA fragments were size-selected using a bead-based method, modified with the addition of a single 'A' base, and ligated with TruSeq DNA UD Indexing adapters. The products were purified and enriched through PCR to create the final DNA library. Library quantification was performed using qPCR, following the qPCR Quantification Protocol Guide (KAPA Library Quantification Kits for Illumina Sequencing Platforms), and quality assessment was performed using Agilent Technologies 4200 TapeStation D1000 screentape (Agilent Technologies, Santa Clara, CA, USA). Paired-end (2 × 150 bp) sequencing was conducted by Macrogen on the NovaSeq platform (Illumina, Inc., San Diego, CA, USA). Processing of over 20 million raw reads involved the Cutadapt tool (http://code.google.com/p/cutadapt/) to trim adapters and eliminate low-quality bases with a Phred quality score (Q score) cutoff of 20. Assembly of the targeted genome from high-quality paired-end next-generation sequencing (NGS) reads was performed using Geneious Prime version 2023.0.1, employing reference mapping with the mitogenome of a closely related species as a reference, utilizing default mapping algorithms. Additionally, for the acquisition of the full-length control region, a species-specific primer pair (5′-CTTGTCGCAGATGTCGCAATCC-3′ and 5′-GCCTGATACCAGCTCCATGTTCC-3′) was designed based on the conserved region of *Cytb* and *12S rRNA* genes. The PCR was

conducted using a TaKaRa Verity Thermal Cycler with a 1X PCR buffer, 1 U Taq polymerase, 10 pmol primers, 2.5 mM dNTPs, and 1 µL template DNA. Purification of the PCR products was carried out using the AccuPrep® PCR/Gel Purification Kit (Bioneer, Daejeon, Republic of Korea). Subsequently, the amplicons were subjected to amplification with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and sequenced in both directions utilizing the ABI PRISM 3730XL DNA analyzer available at Macrogen (https://dna.macrogen.com/), Republic of Korea. The assembly of the control region with the complete mitogenome involved ensuring alignment of overlapping regions through MEGA X, after eliminating any noisy segments via SeqScanner version 1.0 (Applied Biosystems Inc., Foster City, CA, USA). To further ensure the validity of the mitogenome assembly, we examined the alignment of overlapping regions using MEGA X [43]. The boundaries and orientations of individual genes were confirmed through the utilization of MITOS v806 (http://mitos.bioinf.uni-leipzig.de, accessed on 18 October 2023) and MitoAnnotator (http://mitofish.aori.u-tokyo.ac.jp/annotation/input/, accessed on 18 October 2023) web servers [44,45]. For the validation of protein-coding genes (PCGs), the translated putative amino acid sequences underwent analysis using the Open Reading Frame Finder web tool (https://www.ncbi.nlm.nih.gov/orffinder/, accessed on 18 October 2023), based on the vertebrate mitochondrial genetic code. The resulting *S. sphyraena* mitogenome was appropriately submitted to the global GenBank database.

2.3. Characterization and Comparative Analyses

We utilized MitoAnnotator (http://mitofish.aori.u-tokyo.ac.jp/annotation/input/, accessed on 18 October 2023) to create a spherical representation of the generated mitogenome. Our comprehensive comparative analysis aimed to evaluate the mitogenomic architecture and variations in our sequenced data in comparison to five existing mitogenomes of *Sphyraena* species. Intergenic spacers separating adjacent genes and overlapping regions were manually calculated. Nucleotide compositions within protein-coding genes (PCGs), ribosomal RNA (rRNA), transfer RNA (tRNA), and the control region (CR) were determined using MEGA X. A sliding window analysis of nucleotide diversity with a window size of 200 bp and a step size of 25 bp was conducted using DnaSP6.0 [46]. Base composition skews were computed following established formulas: AT-skew = [A − T]/[A + T], GC-skew = [G − C]/[G + C] [47]. The saturation of the transition codon of the mitochondrial PCGs based on transition (s) and transversion (v), as well as AT and GC skews, was depicted using DAMBE6 [48]. To ensure the initiation and termination codons for each PCG and compliance with the vertebrate mitochondrial genetic code, validation was performed using MEGA X. Additionally, the boundaries of rRNA and tRNA genes were confirmed through the utilization of the tRNAscan-SE Search Server 2.0 in conjunction with ARWEN 1.2 [49,50]. Structural domains within the control region were identified through CLUSTAL X alignments [51].

2.4. Genetic Distance and Phylogenetic Analyses

To prepare the dataset for phylogenetic analysis, we used the iTaxoTools 0.1 tool to concatenate all 13 PCGs [52]. Pairwise genetic distances between different *Sphyraena* species were computed by utilizing the uncorrected p-distances and Kimura 2-parameter (K2P) methods within MEGA X. For the exploration of matrilineal phylogenetic relationships, we retrieved the mitogenomes of five *Sphyraena* species from the GenBank database (accessed on 18 October 2023) [38–40] (Table S1). Additionally, the mitogenome of *Polydactylus sextarius* (KP259870) from the Polynemidae family was included as an outgroup [53]. Our model selection analysis identified the 'GTR + G + I' model as the most suitable, serving as the optimal model for all PCGs and yielding the lowest Bayesian Information Criterion (BIC) scores. This model selection process was executed through PartitionFinder 2 on the CIPRES Science Gateway v3.3 and JModelTest v2 [54–56]. The maximum-likelihood (ML) tree was constructed using MEGA X with default parameters. Subsequently, the Bayesian (BA) tree was constructed using Mr. Bayes 3.1.2, with nst = 6, involving one cold and three

hot Metropolis-coupled Markov Chain Monte Carlo (MCMC) chains. The analysis ran for 10,000,000 generations, with tree sampling at every 100th generation, and 25% of the samples were discarded as burn-in [57]. The resulting BA tree was visualized using the iTOL v4 web server (https://itol.embl.de/login.cgi, accessed on 18 October 2023) [58].

3. Results and Discussion

3.1. Mitogenome Structure and Organization

In this study, we determined the mitogenome of *S. sphyraena* (16,841 bp) and deposited it under GenBank Accession No. OQ434241. Notably, the complete mitogenome of *S. sphyraena* boasts the greatest length among the five other *Sphyraena* species, ranging from 16,620 bp (*Sphyraena pinguis*) to 16,760 bp (*Sphyraena japonica*). All six *Sphyraena* species mitogenomes, including *S. sphyraena*, encode the same set of components: 13 protein-coding genes (PCGs), 22 transfer RNAs, two ribosomal RNAs, and an AT-rich control region (Table 1, Figure 1). With the exception of *S. japonica* and *Sphyraena borealis*, the remaining four species (*S. sphyraena*, *S. jello*, *S. pinguis*, and *S. barracuda*) display strand symmetry throughout their mitogenomic organization, mirroring the typical vertebrate mitochondrial genome structure. However, in the mitogenomes of *S. japonica* and *S. borealis*, an unusual transposition was observed in the WANCY region (Figure 1). The mitogenome of *S. sphyraena* is characterized by an AT bias (55.06%), with nucleotide composition percentages of 28.63% for A, 26.43% for T, 16.29% for G, and 28.65% for C. This AT bias is also observed in the other *Sphyraena* species, ranging from 52.38% (*S. pinguis*) to 54.22% (*S. jello*) (Table 2). In the mitogenome of *S. sphyraena*, the AT skew and GC skew were 0.040 and −0.275, respectively. AT skew values range from −0.027 (*S. japonica*) to 0.068 (*S. jello* and *S. barracuda*), while GC skew values range from −0.295 (*S. jello*) to −0.196 (*S. japonica*) (Table 2). This consistent pattern of nucleotide composition and AT bias has been observed in previously documented fish mitogenomes [59,60]. In the mitogenome of *S. sphyraena*, a total of 15 intergenic spacers and six overlapping regions were identified, with a combined length of 264 bp and 28 bp, respectively. The largest intergenic spacer (127 bp) was situated between *16S rRNA* and *trnL2*, while the most extensive overlap (10 bp) was observed between *atp8* and *atp6*. Similar high intergenic spacer lengths were found between *16S rRNA* and *trnL2* in other *Sphyraena* species, except in *S. japonica* with 51 bp between *trnN* and *trnC* and *S. borealis* with 58 bp between *trnW* and *trnN* (Table S2). The longest overlapping region (10 bp) was shared between *atp8* and *atp6* in three species (*S. sphyraena*, *S. pinguis*, *S. japonica*, and *S. borealis*), while *S. jello* and *S. barracuda* exhibited the most extensive overlap (12 bp) between *COI* and *trnS2* (Table S2). The genetic variations detected in the *Sphyraena* mitogenome might provide insights into their evolutionary development and energy metabolism, in line with similar observations in other fish species [61]. This study provides valuable information about the structural features of *Sphyraena* mitogenomes, which are crucial for understanding the functions of these mitogenomes and the genes they encode.

Table 1. List of annotated mitochondrial genes of *Sphyraena sphyraena*.

Name	Start	End	Strand	Size (bp)	Intergenic Nucleotide	Anticodon	Start Codon	Stop Codon
tRNA-Phe (F)	1	70	H	70	0	AAG		
12S rRNA	71	1035	H	965	0			
tRNA-Val (V)	1036	1108	H	73	0	CAU		

Table 1. *Cont.*

Name	Start	End	Strand	Size (bp)	Intergenic Nucleotide	Anticodon	Start Codon	Stop Codon
16S rRNA	1109	2889	H	1781	127			
tRNA-Leu (L2)	3017	3090	H	74	0	AAU		
ND1	3091	4065	H	975	5		ATG	TAA
tRNA-Ile (I)	4071	4141	H	71	−1	UAG		
tRNA-Gln (Q)	4141	4211	L	71	8	GUU		
tRNA-Met (M)	4220	4289	H	70	0	UAC		
ND2	4290	5351	H	1062	30		ATG	TAA
tRNA-Trp (W)	5382	5452	H	71	3	ACU		
tRNA-Ala (A)	5456	5524	L	69	1	CGU		
tRNA-Asn (N)	5526	5598	L	73	47	UUG		
tRNA-Cys (C)	5646	5712	L	67	12	ACG		
tRNA-Tyr (Y)	5725	5794	L	70	1	AUG		
COI	5796	7352	H	1557	−5		GTG	AGA
tRNA-Ser (S2)	7348	7418	L	71	3	AGU		
tRNA-Asp (D)	7422	7493	H	72	10	CUG		
COII	7504	8194	H	691	0		ATG	T--
tRNA-Lys (K)	8195	8268	H	74	8	UUU		
ATP8	8277	8444	H	168	−10		ATG	TAA
ATP6	8435	9117	H	683	0		TTG	TA-
COIII	9118	9902	H	785	0		ATG	TA-
tRNA-Gly (G)	9903	9972	H	70	1	CCU		
ND3	9974	10,322	H	349	0		ATG	T--
tRNA-Arg (R)	10,323	10,391	H	69	0	GCU		
ND4L	10,392	10,688	H	297	−7		ATG	TAA
ND4	10,682	12,062	H	1381	0		ATG	T--
tRNA-His (H)	12,063	12,131	H	69	0	GUG		
tRNA-Ser (S1)	12,132	12,199	H	68	5	UCG		
tRNA-Leu (L1)	12,205	12,278	H	74	0	GAU		
ND5	12,279	14,117	H	1839	−4		ATG	TAA
ND6	14,114	14,635	L	522	0		ATG	TAA
tRNA-Glu (E)	14,636	14,704	L	69	3	CUU		
Cyt b	14,708	15,848	H	1141	0		ATG	T--
tRNA-Thr (T)	15,849	15,921	H	73	−1	UGU		
tRNA-Pro (P)	15,921	15,991	L	71	0	GGU		
D-loop	15,992	16,841	H	850				

Table 2. Nucleotide composition of the mitochondrial genomes of five *Sphyraena* species.

Species Name	Size (bp)	A%	T%	G%	C%	A + T%	AT-Skew	GC-Skew
Complete mitogenome								
Sphyraena sphyraena	16,841	28.63	26.43	16.29	28.65	55.06	0.040	−0.275
Sphyraena japonica	16,760	25.99	27.42	18.74	27.85	53.41	−0.027	−0.196
Sphyraena jello	16,699	28.97	25.25	16.14	29.64	54.22	0.068	−0.295
Sphyraena pinguis	16,620	26.03	26.35	18.80	28.81	52.38	−0.006	−0.210
Sphyraena barracuda	16,707	28.90	25.23	16.18	29.68	54.13	0.068	−0.294
Sphyraena borealis	16,739	26.42	26.39	18.9	28.29	52.81	0.001	−0.199
PCGs								
Sphyraena sphyraena	11,450	25.8	28.6	15.9	29.7	54.41	−0.052	−0.303
Sphyraena japonica	11,453	23.64	29.31	17.88	29.17	52.95	−0.107	−0.240
Sphyraena jello	11,445	26.67	27.12	15.38	30.83	53.79	−0.008	−0.334
Sphyraena pinguis	11,439	23.46	28.18	18.37	29.99	51.64	−0.091	−0.240
Sphyraena barracuda	11,444	26.64	27.13	15.38	30.85	53.77	−0.009	−0.335
Sphyraena borealis	11,443	23.65	28.29	18.65	29.42	51.94	−0.089	−0.224
rRNAs								
Sphyraena sphyraena	2746	31.32	22.61	20.9	25.16	53.93	0.161	−0.092
Sphyraena japonica	2772	29.94	22.87	22.22	24.96	52.81	0.134	−0.058
Sphyraena jello	2696	31.49	22.07	20.73	25.7	53.56	0.176	−0.107
Sphyraena pinguis	2699	30.57	22.23	21.9	25.31	52.80	0.158	−0.072
Sphyraena barracuda	2696	31.45	22	20.77	25.78	53.45	0.177	−0.108
Sphyraena borealis	2772	30.56	22.22	22.29	24.93	52.78	0.158	−0.056
tRNAs								
Sphyraena sphyraena	1559	28.48	27.13	23.16	21.23	55.61	0.024	0.043
Sphyraena japonica	1561	28.12	27.99	23.96	19.92	56.11	0.002	0.092
Sphyraena jello	1562	27.98	27.14	23.88	21.00	55.12	0.015	0.064
Sphyraena pinguis	1552	27.06	26.29	24.48	22.16	53.35	0.014	0.050
Sphyraena barracuda	1559	28.03	27.26	23.93	20.78	55.29	0.014	0.070
Sphyraena borealis	1551	27.92	27.08	24.24	20.76	55.00	0.015	0.077
CRs								
Sphyraena sphyraena	850	36.71	27.06	12.82	23.41	63.77	0.151	−0.292
Sphyraena japonica	859	28.21	30.42	17.6	23.78	58.63	−0.038	−0.149
Sphyraena jello	799	31.79	28.79	18.15	21.28	60.58	0.050	−0.079
Sphyraena pinguis	832	29.69	29.45	16.71	24.16	59.14	0.004	−0.182
Sphyraena barracuda	809	30.41	28.68	19.28	21.63	59.09	0.029	−0.057
Sphyraena borealis	839	32.54	29.2	16.33	21.93	61.74	0.054	−0.146

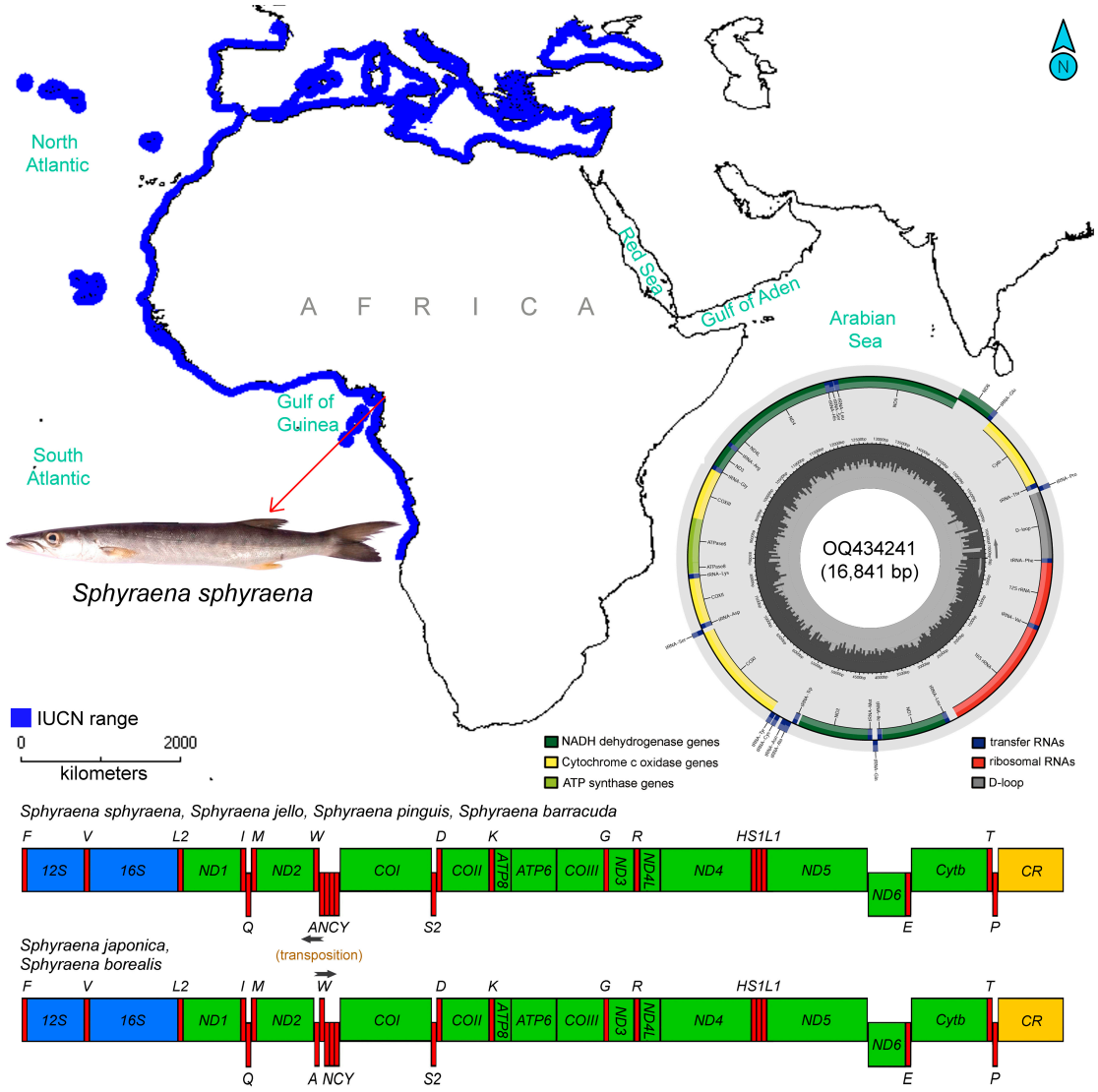

Figure 1. Distribution pattern and collection locality of the European Barracuda (*S. sphyraena*) in Cameroon. The mitochondrial genome of *S. sphyraena* has been visually represented and annotated using the MitoAnnotator online server. Different color arcs highlight the presence of protein-coding genes (PCGs), ribosomal RNA genes (rRNAs), transfer RNA genes (tRNAs), and the control region (CR). The linearized view of the complete mitochondrial genome organization reveals the gene arrangement in barracuda species, while transpositions of tRNA genes are denoted by black arrows.

3.2. Protein-Coding Genes

The mitogenomes of *S. sphyraena* encompassed 13 protein-coding genes (PCGs), with *atp8* being the shortest (168 bp) and *ND5* the longest (1839 bp). Collectively, the PCGs in *S. sphyraena* accounted for a total length of 11,450 bp, constituting 67.98% of the complete mitogenome. In comparison, the mitogenomes of other *Sphyraena* species were slightly longer, ranging from 11,439 bp (*S. pinguis*) to 11,453 bp (*S. japonica*) (Table 2). To assess nucleotide diversity, we performed a sliding window analysis on the concatenated PCGs of *Sphyraena* species. The average nucleotide diversity value (Pi) was calculated to be 0.22281, with a total of 4721 polymorphic sites observed in all *Sphyraena* species (Figure 2A). Sequence saturation analysis revealed both transitions and transversions were slightly saturated with increasing divergence values (Figure 2B). The PCGs of all *Sphyraena* species displayed an AT bias, ranging from 51.64% to 54.41%, and AT skews and GC skews varied from −0.107 (*S. japonica*) to −0.008 (*S. jello*) and from −0.335 (*S. barracuda*) to −0.224

(*S. borealis*), respectively (Figure 2C,D). Most of the PCGs initiated with the ATG start codon, except for *COI*, which initiated with GTG in all *Sphyraena* species (Table S3). The TTG start codon was observed in *atp6* of *S. sphyraena*, and GTG was observed in *COI*, *ND3*, *ND4*, and *ND5* of *S. japonica*. Among the six PCGs of *S. sphyraena*, TAA served as the termination codon for five, while *COI* used AGA, and the remaining PCGs utilized an incomplete stop codon. Similar termination codon patterns were observed in the other *Sphyraena* species, except for *S. japonica* and *S. borealis* (Table S3). It is crucial to emphasize that these incomplete stop codons may potentially be completed with TAA during RNA processing, as suggested in previous studies [62]. As observed in other fish species, the identified genetic variations could lead to the independent selection of protein-coding genes (PCGs) [63]. These PCGs play essential roles in oxidative phosphorylation, ATP synthesis, and the encoding of proteins involved in electron transport pathways. Therefore, the incorporation of mitogenomes from various *Sphyraena* species could assist in investigating variations in gene expression and energy utilization.

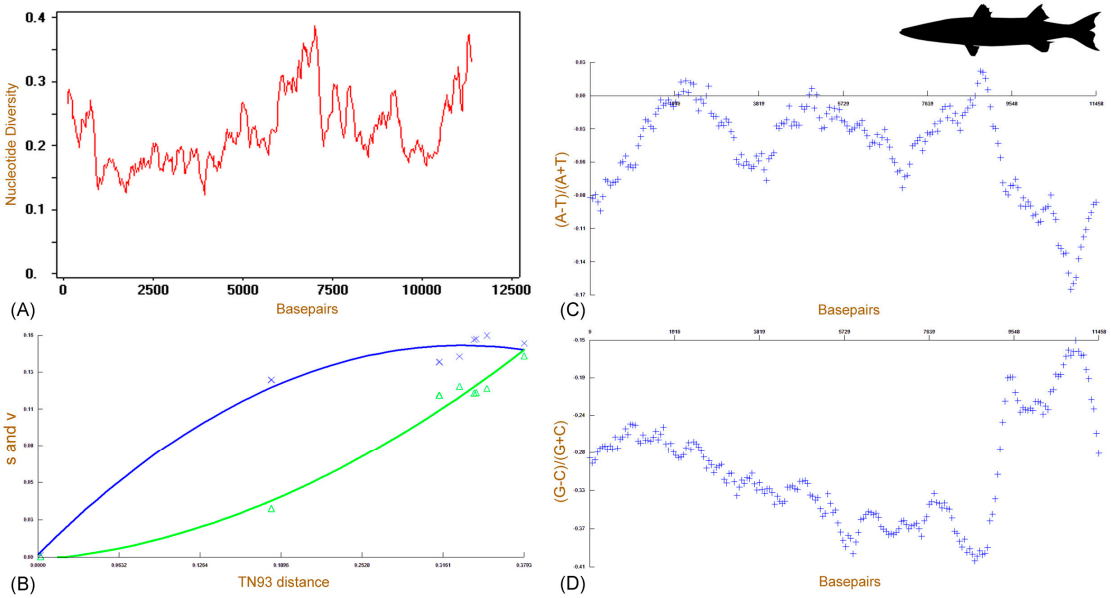

Figure 2. (**A**) Mitochondrial protein-coding gene genetic diversity (Pi) is displayed, providing insights into the genetic variation among six barracuda species; (**B**) the graph exhibits the relationship between transitions (s) and transversions (v) concerning divergence, utilizing the Tamura and Nei (1993) distance method. It effectively illustrates the saturation of transition codons in mitochondrial protein-coding genes, with crosses indicating transition events and triangles representing transversion events. The curves delineate the trends in the variance of transitions and transversions as genetic distance increases; (**C**) AT skew and (**D**) GC skew plots showcase the nucleotide composition patterns of the protein-coding genes of six barracuda fish mitogenomes.

3.3. Ribosomal RNA and Transfer RNA Genes

In *S. sphyraena*, the length of the *12S rRNA* was determined to be 965 bp, while the *16S rRNA* spanned 1781 bp, collectively accounting for 16.31% of the complete mitogenome. When compared to other *Sphyraena* species, the total length of rRNAs ranged from 2696 bp (*S. jello* and *S. barracuda*) to 2772 bp (*S. japonica* and *S. borealis*) (Table 2). The rRNA genes displayed an AT bias, with values ranging from 52.78% (*S. borealis*) to 53.93% (*S. sphyraena*). AT skews and GC skews exhibited variation, ranging from 0.134 (*S. japonica*) to 0.177 (*S. barracuda*) and from −0.108 (*S. barracuda*) to −0.056 (*S. borealis*), respectively (Table 2). The structural organization of these rRNA genes, particularly the conserved loops, provides valuable insights into the catalytic chemical processes underlying protein synthesis [64,65]. The studied taxon, *S. sphyraena*, harbored 22 tRNA genes within its mitogenome. The collective length of these tRNA genes was 1559 bp, contributing to 9.25% of the complete

mitogenome. In comparison with other *Sphyraena* species, the total length of tRNA ranged from 1551 bp (*S. borealis*) to 1562 bp (*S. jello*). The tRNA genes in *Sphyraena* species exhibited an AT bias, with values varying from 53.35% (*S. pinguis*) to 56.11% (*S. japonica*), and the AT skew ranged from 0.002 (*S. japonica*) to 0.024 (*S. sphyraena*) (Table 2). Identical anticodons were identified within all 22 transfer RNA (tRNA) genes across all *Sphyraena* species (Table S4). In *S. sphyraena*, wobble base pairings were identified in 13 tRNAs, with the highest number present in *trnA* and *trnE*. These wobble base pairings were observed in the DHU stem of seven tRNAs (*trnA*, *trnQ*, *trnW*, *trnC*, *trnG*, *trnR*, *trnP*), in the acceptor stem of nine tRNAs (*trnA*, *trnQ*, *trnC*, *trnY*, *trnD*, *trnK*, *trnR*, *trnE*, *trnP*), in the TψC stem of two tRNAs (*trnA*, *trnS2*), and in the anticodon stem of six tRNAs (*trnA*, *trnL2*, *trnY*, *trnS2*, *trnR*, *trnE*) (Figure 3).

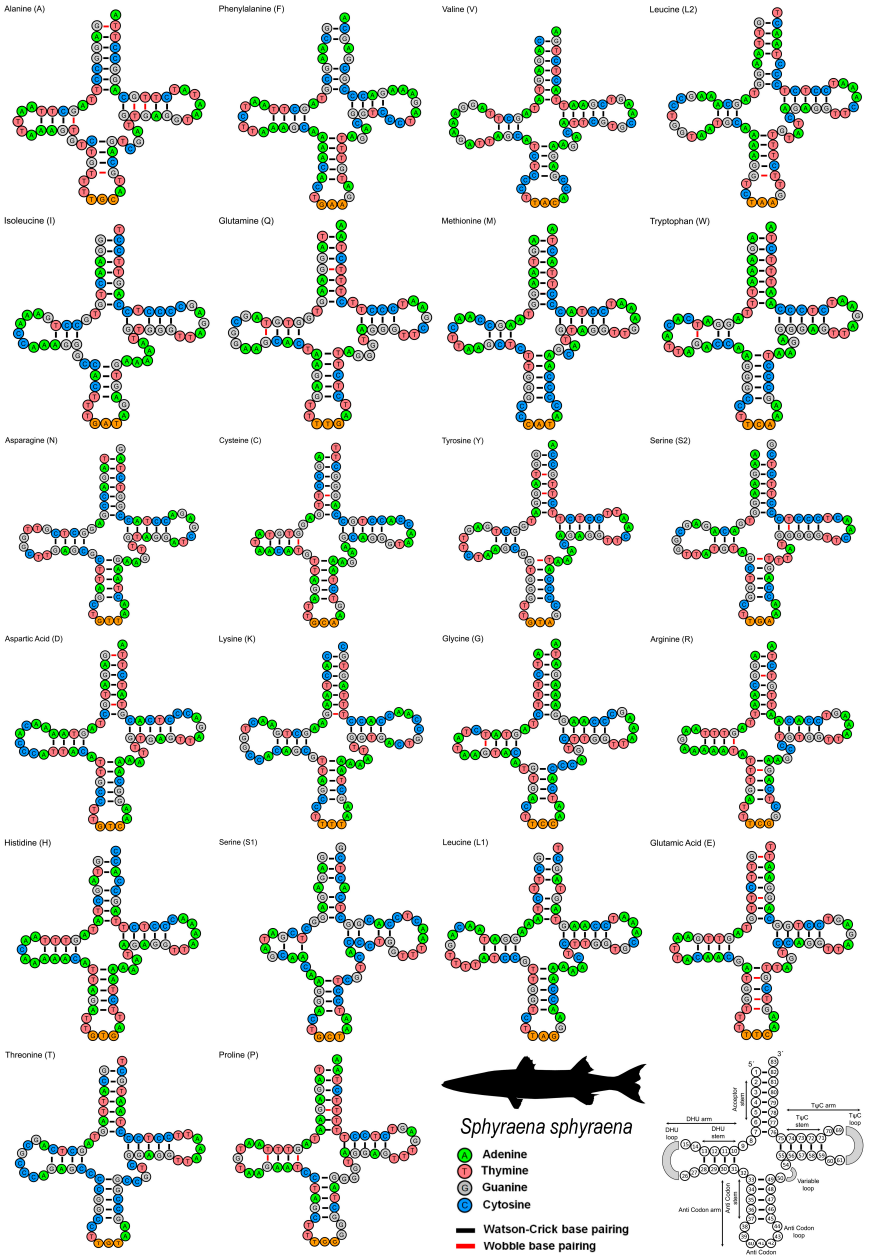

Figure 3. The secondary structures of 22 transfer RNAs (tRNAs) in the European Barracuda exhibit structural variations. These tRNAs are labeled with their complete names and single-letter amino acid codes following the IUPAC-IUB convention. The final structure provides information on the nucleotide positions and specifics of the tRNAs' stem-loop configuration.

3.4. Features of Control Region

The control region (CR) of *S. sphyraena* spans 850 base pairs, making up 5.05% of the total mitogenome. When compared to other *Sphyraena* species, the CR's length varied, ranging from 799 base pairs in *S. jello* to 858 base pairs in *S. japonica* (Table 2). The CRs exhibited an AT bias, with values ranging from 58.63% (*S. japonica*) to 63.77% (*S. sphyraena*), and the AT skews displayed variability, spanning from −0.038 (*S. japonica*) to 0.151 (*S. sphyraena*). In the scrutiny of the extant mitogenomes of five barracudas, a thorough examination of diverse domains was specifically undertaken for *S. barracuda* (AP006828) [66]. Comparative analysis with *S. barracuda* revealed the identification of four analogous blocks (CSB-D, CSB-I, CSB-II, and CSB-III) within the CR of *S. sphyraena* and other barracudas. This observation is consistent with patterns discerned in other teleost mitogenomes [66,67]. Among these blocks, CSB-I stood out as the longest, measuring 22 base pairs, while CSB-D, CSB-II, and CSB-III had lengths of 18 base pairs, 17 base pairs, and 20 base pairs, respectively (Figure 4). Comparative analyses unveiled significant nucleotide variability and parsimony-informative nucleotides within CSB-II and CSB-III when compared to the other two domains in *Sphyraena* CR's. In this investigation, the reliance on observed nucleotide variation within conserved domains in CRs is constrained, mirroring the imperative limitation in the blind dependence on CRs from other barracudas accessible in public databases. The lack of prior scrutiny on these datasets necessitates caution in their application. It is conjectured that the CRs in barracudas exhibit significant variability, potentially attributable to elevated substitution rates, a phenomenon notoriously challenging for alignment. Consequently, we advocate for targeted sequencing efforts directed at barracudas to substantiate assertions regarding the conserved CR domains. Nevertheless, this AT-rich regulatory region has the potential to assess population structures and distinguish differences among *Sphyraena* species, both between and within species, by scrutinizing these variable nucleotides. Similar to observations in other species, these conserved domains play a crucial role in the replication and transcription of mitochondrial genomes [68]. Analyzing the extensively variable control region of *S. sphyraena* across its geographical distribution in the East Atlantic Ocean, Mediterranean Sea, Tyrrhenian Sea, and Black Sea will offer valuable insights into estimating population structure and providing a detailed phylogeographic perspective, as previously inferred for the Great Barracuda [26].

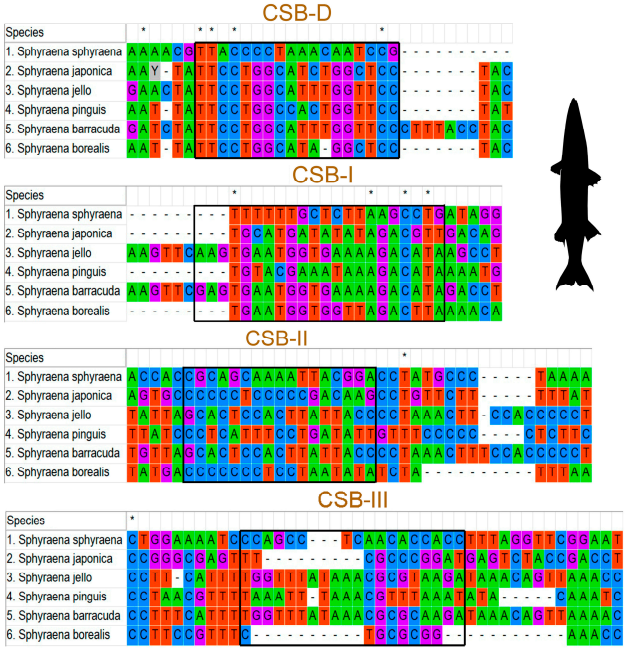

Figure 4. Comparison of length and nucleotide composition of four domains of five *Sphyraena* species control regions marked by black rectangles. The conserved nucleotides are marked in stars.

3.5. Genetic Distances and Matrilineal Phylogeny

The concatenated protein-coding genes (PCGs) of *S. sphyraena* exhibited 16.13–27.88% uncorrected p-distances and 18.8–35.6% K2P distances when compared to the other five *Sphyraena* species. Both the ML and BA phylogenies provided similar topologies and clear separation among all six *Sphyraena* species (Figure 5). It revealed a sister relationship between the European Barracuda, or Mediterranean Barracuda (*S. sphyraena*), with the Great Barracuda (*S. barracuda*) and the Pickhandle Barracuda (*S. jello*), which are widely distributed across the Indian, Pacific, and Atlantic oceans. On the other hand, the Japanese Barracuda (*S. japonica*) and the Northern Sennet (*S. borealis*), distributed in the eastern and western Pacific and western Atlantic Ocean, respectively, showed a close clustering with each other. Notably, the mitogenome-based topology revealed an ancestral cladding pattern of the Red Barracuda (*S. pinguis*) in comparison to other barracuda species (Figure 5). The current topology and the depicted evolutionary relationships are in agreement with previous research, demonstrating a monophyletic clustering of *Sphyraena* and congruent with the previous hypothesis on Sphyraenidae phylogeny [25,27,38]. However, an imperative need exists for the mitogenome analysis of the Yellowmouth Barracuda (*S. viridensis*), the nearest congener of *S. sphyraena*. This analysis is crucial for investigating the mitochondrial genome evolution of these two sympatric species in the northern Atlantic and Mediterranean Seas. Hence, for a more comprehensive understanding of the phylogenetic relationships, this study recommends generating additional mitogenomes from other *Sphyraena* congeners using live and/or museum specimens. Furthermore, the relationship among *Sphyraena* species does not exhibit a correlation with their geographical distribution and habitat types (reef and nonreef ecosystems). Conclusively, the mitogenomic data displayed adequate genetic variability suitable for both inter- and intraspecific examinations. The nucleotide information acquired effectively discriminates *S. sphyraena* from other *Sphyraena* species, offering novel insights into their evolutionary implications. In the recent past, a phylogenetic analysis of 20 species within the Sphyraenidae family was conducted, utilizing three mitochondrial loci (*COI*, *Cytb*, and *16S rRNA*). The resulting Bayesian analysis delineated three major clusters: (i) the '*Sphyraena barracuda*' group, (ii) the '*Sphyraena obtusata*' group, and (iii) the '*Sphyraena sphyraena*' group [69]. The TimeTree analysis provided additional insights, indicating the origin of these barracudas during the late Paleocene, with the radiation of extant phylogenetic lineages occurring from the middle Eocene to the Miocene period. The current phylogenetic trees revealed a close clustering of *S. barracuda* and *S. jello* within the '*S. barracuda*' group. Notably, the mitogenome sequence of *S. pinguis* cladded apart from other species, suggesting its placement under the '*S. obtusata*' group. However, the phylogenetic placement of both *S. sphyraena* and *S. japonica* under the '*S. sphyraena*' group displayed a paraphyletic clustering in the present analyses. Given this incongruency in cladistic patterns, the present study advocates for the inclusion of more mitogenomes from Sphyraenid species to elucidate a more comprehensive understanding of the evolutionary patterns and divergence times within the Percomorpha. Such data will contribute to clarifying the lineage diversification and colonization of barracudas in both reef-associated and non-reef ecosystems.

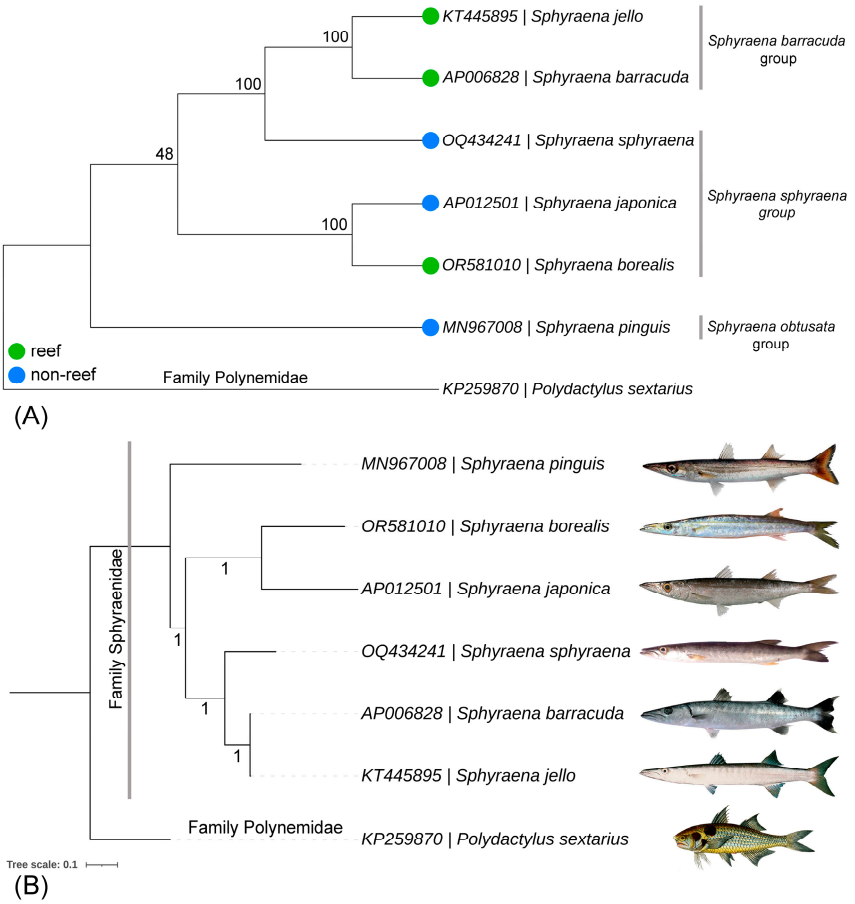

Figure 5. The maximum-likelihood (**A**) and Bayesian (**B**) matrilineal phylogenetic tree, constructed from the concatenated sequences of the 13 protein-coding genes (PCGs), provides insights into the evolutionary relationships among *Sphyraena* species. Bootstrap supports and Bayesian posterior probability values are indicated at each node, reflecting the statistical support for each branching point in the tree. Major groups and habitat types (reef and non-reef) of the barracudas are marked by a green and blue color circle, as shown in a previous study [69]. Representative photographs of the barracuda species and the blackspot threadfin were obtained from online sources and serve as visual references for the respective species.

4. Conclusions

In conclusion, this study delved into a comprehensive analysis of the mitogenome of *S. sphyraena*, providing a detailed understanding of its genetic composition and structural organization. Additionally, the research explored genetic distances and phylogenetic relationships, revealing the intricate evolutionary connections among Sphyraenidae species. Significantly, the study identified substantial genetic divergence between *S. sphyraena* and its congeners. The cladistic patterns, in conjunction with their distribution across different oceanic regions, offer valuable insights into the emergence and diversification of Sphyraenidae species. The genetic data, both partial loci and complete mitochondrial information, will be instrumental in assessing the population structure of barracudas in the near future, with potential implications for conservation efforts. This genetic knowledge will play a crucial role in formulating effective conservation strategies to protect species' diversity and ensure the sustainability of marine life, particularly within the Sphyraenidae family. In summary, the analysis of the mitogenome and evolutionary history of *S. sphyraena* provides invaluable insights into the genetic characteristics and evolutionary dynamics within the Atlantic Ocean.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes8120573/s1, Table S1. Mitogenomic dataset of barracuda fish species for comparative and phylogenetic analyses. The mitogenome of blackspot threadfin within the family Polynemidae is used as an outgroup taxon for phylogenetic construction. Table S2. Comparison of intergenic nucleotides and overlapping regions of six *Sphyraena* mitogenomes genes. Table S3. Comparison of start and stop codons of six *Sphyraena* mitogenomes PCGs. Table S4. Comparison of anticodons of six *Sphyraena* mitogenomes tRNA genes.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The genome sequence data supporting the findings of this study are openly accessible on the GenBank of NCBI website at https://www.ncbi.nlm.nih.gov, under the accession number OQ434241.

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