Zeng X, Cui M, Yu H, Pan X, Zheng P, Wei F. Phylogenetic relationships analysis of the family Scombridae (Actinopterygii, Scombriformes). *Israeli Journal of Aquaculture - Bamidgeh*. 2024;76(2):121-136. doi:10.46989/001c.94824

Original Research Articles

Phylogenetic relationships analysis of the family Scombridae (Actinopterygii, Scombriformes)

Xinru Zeng[1], Mengyao Cui[1], Haoyu Yu[1], Xiaoyuan Pan[1,2], Pingzhong Zheng[1], Fen Wei[1a]

[1] Coral Reef Research Center of China; Guangxi Laboratory on the Study of Coral Reefs in the South China Sea; School of Marine Sciences, Guangxi University, Nanning, Guangxi, China, [2] School of Resources, Environment and Materials, Guangxi University, Nanning, Guangxi, China

Keywords: Scombridae, MtDNA, NuDNA, phylogenetics

https://doi.org/10.46989/001c.94824

Israeli Journal of Aquaculture - Bamidgeh

Vol. 76, Issue 2, 2024

Scombridae is a family of pelagic marine fishes that comprises 16 genera and 51 species. This family has been of significant commercial importance throughout history; however, the phylogenetic relationships within the Scombridae have been disputed due to the unclear taxonomic boundaries of the suborder Scombroidei, which includes six families, including Scombridae. Despite this, only a limited number of studies have been conducted on the Scombridae. In our study, eight species covering five genera of the Scombridae were selected, and one nuclear (ITS) and three mitochondrial DNA markers (CO1, Cytb, and D-loop) were used to amplify gene fragments. Additionally, we included homologous sequences from other Scombridae fishes obtained from GenBank. Our analysis constructed phylogenetic relationships of 48 Scombridae species in 14 genera. The results demonstrated that the three phylogenetic trees (NJ, ML, and BI) exhibited similar topologies, containing three major clades. One major clade indicated that *Grammatorcynus bilineatus* and *Grammatorcynus bicarinatus* did not cluster with other species in the Scombridae; another clade comprised the genera *Scomber* and *Rastrelliger*; the third clade consists of the remaining Scombridae species. Notably, the study showed that Gasterochismatinae and Scombrinae were not sister groups; *Allothunnus* (tribe Thunnini) and *Cybiosarda* (tribe Sardini) clustered into a clade, suggesting that Sardini and Thunnini were non-monophyletic. Overall, this research enhances the understanding of phylogenetic relationships within the Scombridae and provides basic information to aid further research.

INTRODUCTION

The scombrid species of fish inhabiting the epipelagic realm have streamlined bodies with forked or lunate tails, allowing them to move efficiently over long distances for feeding and migration.[1] The Scombridae forms the largest and state-of-art family in Scombroidei.[2-4] It includes commercially valuable, edible, and recreational fish, and there are currently 51 valid species.[1,5] There are 22 species belonging to 11 genera of the family Scombridae recorded in China.[6] Previous studies on the taxonomy of scombroid fishes have mainly relied on traditional morphology.[2,7,8] The currently accepted classification is primarily based on the work of Collette and Monsch.[2,9] Collette[2] summarized available data on the scombroid fishes and divided the Scombridae into two subfamilies, Gasterochismatinae and Scombrinae, with only one genus (*Gasterochisma*) under Gasterochismatinae. The subfamily Scombrinae consists of four tribes: mackerels (Scombrini), Spanish mackerels (Scomberomorini), bonitos (Sardini), and tunas (Thunnini) (**Figure 1a**). Monsch[9] proposed that the family Scombridae consisted of subfamilies Scombrinae, Scomberomorinae, Sardinae (tribes Sardini and Thunnini), Acanthocybiinae and Xiphiinae (billfishes, tribes Xiphiini and Istiophorini) (**Figure 1b**).

Despite numerous morphological studies, unresolved taxonomic issues remain within the family. For instance, there has been disagreement regarding the hypothesis that *Gasterochisma* is a sister group to the Scombrinae.[10,11] The earliest differentiated clade within the Scombridae is also subject to debate. Collette and Nauen[8] considered that the Scombrini was the earliest differentiated group within Scombridae, whereas Johnson[12] alternatively proposed the hypothesis that *Grammatorcynus* was the earliest differentiated group. Additionally, the monophyletic status of Sardini and Thunnini has been disputed.[13] The instability of these classifications confounds the management of scom-

a Corresponding author: Fen Wei e-mail: weifensky@163.com

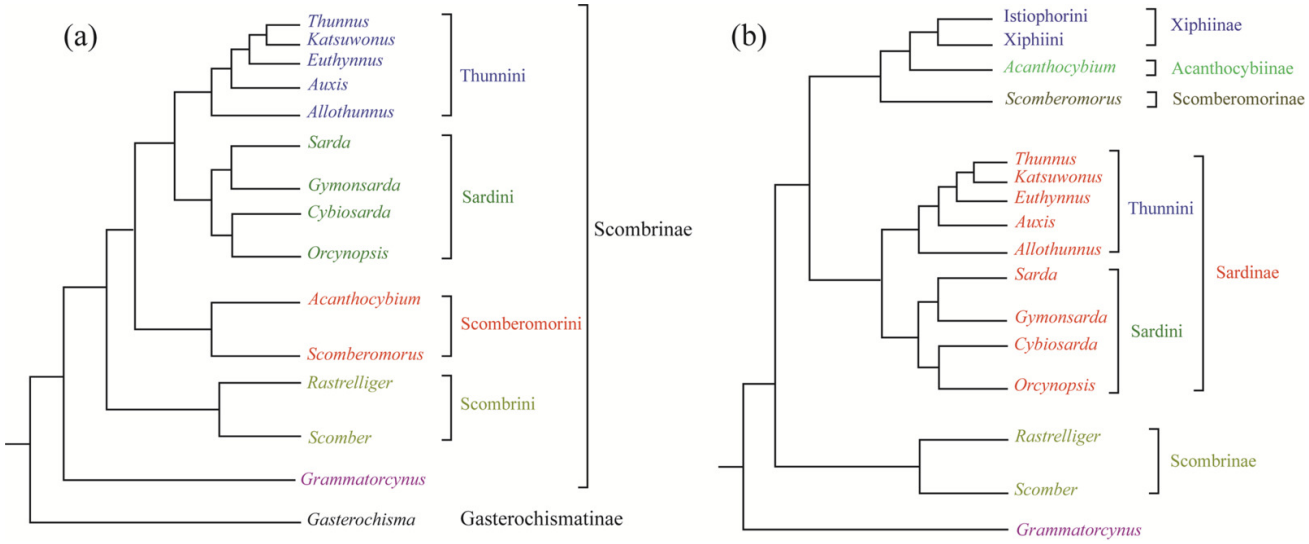

Figure 1. The cladistic hypothesis of the Scombroidei. (a) from Collette (1984)[2]; (b) from Monsch (2000).[9]

broid fishes as a fisheries resource and the understanding of the diversity and complexity of marine communities.

Many studies have recently been published on the phylogenetic relationships of the Scombridae. For instance, Block et al.[14] amplified the cytochrome b gene (Cytb) from 75 scombrid individuals, supporting the monophyly of endothermic tunas (*Auxis*, *Euthynnus*, *Katsuwonus*, and *Thunnus*) and suggesting *Gasterochisma melampus* falls within the radiation that included primitive scombrids (*Scomber*) and gempylids (*Ruvettus*). Dalziel et al.[15] discovered that the bonitos (tribe Sardini) were embedded within Thunnini based on the phylogenetic tree constructed by mitochondrial cytochrome c oxidase (COX II). Orrell et al.[16] used a single-copy nuclear Tmo-4C4 gene. They combined Tmo-4C4 with mitochondrial DNA cytochrome b (Cytb) to analyze phylogenetic relationships in the Scombridae, revealing that Gasterochismatinae and Scombrinae were sister groups. Jondeung and Karinthanyakit[17] concluded that the genera *Sarda* and *Thunnus* were sister taxa to each other, according to the analysis of mitochondrial genes Cytb and nicotinamide adenine dinucleotide dehydrogenase subunit 2 (nd2). Despite numerous attempts to resolve the evolutionary relationships within the Scombridae using both morphological and molecular data, the phylogenetic relationships of the scombrids remain unresolved.[18-21]

Furthermore, previous studies of scombroid relationships have mainly focused on composite taxa corresponding to either genera or tribes. For example, Chow and Kishino[22] investigated the phylogenetic relationships among species exclusively within the genus *Tunas* using Cytb and adenosine triphosphate (ATP) (two mitochondrial genes) and internal transcribed spacer (ITS) region (one nuclear gene). It was reported that gene trees derived from mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) data yielded concordant phylogenies supporting the genus Sarda's monophyly.[23] Jeena et al.[5] comprehensively reported phylogenetic relationships within the genus *Scomberomorus* using eight mitochondrial genes and three nuclear genes. However, to accurately determine the earliest diverging clade within the Scombridae, it is essential to consider the full range of taxa within the family. Focusing solely on a subset of species or genera may lead to incomplete or biased conclusions about evolutionary relationships. At present, only a few studies have exclusively evaluated the whole family. Hence, there is a critical need to investigate the phylogenetic relationships of Scombridae as a whole.

Most polymorphisms in mitochondrial genes are primarily concentrated in the D-loop region, exhibiting 5 to 10 times more significant variability than other mtDNA segments, rendering it an ideal locus for genetic differentiation studies.[24] Lee et al.[25] observed significant length variations in the D-loop structure when examining closely related teleost fish species, suggesting its suitability for rapid evolutionary analyses of species. Mitochondrial cytochrome b (Cytb) gene and CO1 were widely utilized sequences in research, extensively employed in systematic studies to resolve taxonomic discrepancies across various hierarchical levels.[26] Due to their robust applicability, these two genes have been extensively employed in phylogenetic investigations across diverse fish taxa. Due to its rapid variation and abundance of informative sites, the ITS sequence has emerged as a significant molecular marker in the phylogeny and taxonomy of lower taxonomic levels.[27] It can provide valuable information for distinguishing between closely related species and resolving evolutionary relationships.

This study aimed to investigate the phylogenetic relationships of Scombridae. For this purpose, we used one nuclear (ITS) and three mitochondrial DNA loci (CO1, Cytb, and D-loop) to reconstruct phylogenetic relationships of 48 Scombridae species from 14 genera combined with many sequences obtained from GenBank. The results provide valuable basic information and a greater understanding of Scombridae's classification system and phylogenetic relationship.

Table 1. The primers for PCR amplification and sequencing.

Gene fragment	Primer pair and sequence(5′–3′)	Amplification length (bp)	Annealing temperature (°C)	Reference
CO1	FishF1: TCAACCAACCACAAAGACATTGGCAC FishR1: TAGACTTCTGGGTGGCCAAAGAATCA	655	56	Ward et.al (2005)[28]
Cytb	L14724: GACTTGAAAAACCACCGTTG H15915: CTCCGATCTCCGGATTACAAGA	1140	55	Xiao et.al (2001)[29]
D-loop	D-Loop-F:TAACTCCCACCCCTAACTCC D-Loop-R: CCATTAACTTATGTAAGCGTCG	500	56	Grant and Bowen (1998)[30]
ITS	ITS-1F:TCCGTAGGTGAACCTGCGG ITS-1R: CGCTGCGTTCTTCATCG	1000	58	Chow et.al (2006)[31]

MATERIALS AND METHODS

SAMPLES COLLECTION AND ETHICS

Seventeen individuals of Scombridae were sampled. All the fish samples were bought from local fish dealers in Fude Market in Nanning, Guangxi province, China. All samples were preserved in 95% ethanol and transported to the laboratory to study coral reefs in the South China Sea, Guangxi University. They were identified at the family, genus, or species levels. The samples were morphologically identified concerning the "Key to Marine and Estuarial Fish of China" and the FishBase database (https://www.fishbase.se/search.php). As food fishes, no permits were required for sampling. All procedures followed corresponding regulations, by-laws, and Ethics.

DNA EXTRACTION, PCR AMPLIFICATION AND SEQUENCING

Genomic DNA was extracted from approximately 20 mg of muscle tissue in 95% ethanol using a marine animal tissue genomic DNA kit (Tiangen Biotech, China). Primers were adapted from the literature (**Table 1**). PCR amplifications were performed in 50 µL volumes of 1 µL of DNA template, 2.5 µL of each primer (10 mmol/L), 25 µL 2× PCR master mix, and 19 µL ddH_2O. We amplified and directly sequenced four genetic markers for all samples: CO1, Cytb, D-loop (mitochondrial markers), and ITS (nuclear marker).[28-31] The details of primers are shown in **Table 1**. All PCR products were subjected to 1% agarose gel electrophoresis to confirm amplification. Sangon Biotech (Shanghai, China) sequenced PCR products with single intense bands based on Sanger sequencing technology.

SEQUENCE ANALYSIS

Additional sequences from 40 species of 14 genera in the family were downloaded from GenBank (**Table 2**). Amplified sequences were aligned using the Clustal W algorithm,[32] manually edited using Seqman v. 7.1.0 to remove gaps and severe base pair mismatches. All generated sequences were compared to the sequences of nucleotide databases using the search tool BLAST.[33] After confirming the accuracy of sequences, all amplified sequences were deposited in GenBank. Base composition, transition/transversion ratios, and the analysis of molecular diversity indicators, such as polymorphic sites, conserved sites, parsimony-informative sites, and singleton sites, were determined using MEGA X.[34] Distance matrices from aligned nucleotide sequences were determined by applying the Kimura 2-parameter and the Tamura-Nei model using the pairwise distance between genera calculation of MEGA v. 7.0.[35,36] DAMBE v. 5.3.19 was used to analyze substitution saturation. A scatter plot was constructed with F84 model-corrected distances as the horizontal axis and the number of transitions and transversions as the vertical axis.[37]

MOLECULAR PHYLOGENETIC ANALYSIS

We selected Carangidae (*Trachurus trachurus)* and Percichthyidae (*Lateolabrax japonicus)*, closely related to Scombridae, as the outgroup taxa. A partition-homogeneity test was run in PAUP 4.0b10[38] to examine whether the sequences from the four loci should be combined in a single dataset analysis. No conflicting phylogenetic signals between the datasets were detected. The multigene analysis focused on increasing the phylogenetic signals, thereby verifying the validity of the primary investigation results. For phylogenetic analyses, CO1, Cytb, D-loop, and ITS region sequences were concatenated into one partitioned dataset. Three methods, neighbor-joining (NJ), maximum likelihood (ML), and Bayesian inference (BI), were employed to reconstruct the phylogenetic relationships within Scombridae. Phylogenies were constructed using the NJ tree through MEGA 7.0.26, the ML tree via RAxML, and the BI tree executed with MrBayes 3.2.[35,39,40]

Three phylogenetic tree optimality criteria were employed. The NJ analysis was done in two-parameter mode.[41] We selected the best-fitting models for ML and BI using the Akaike Information Criterion (AIC) as implemented in MrModeltest 1.0b.[42-44] The model GTR+I+G was suggested as the best fit for CO1 and Cytb; the model GTR+G was the op-

Table 2. The species used in this study and GenBank accession numbers.

NO.	Specie	CO1	Cytb	D-loop	ITS
1	*Scomber scombrus*	MT456185	EU492105	AB120717	LC464971
2	*Scomber japonicus* (this study)	OQ781868	OR357873	/	OR343904
3	*Scomber colias*	KM538536	EU224080	NC013724	LC464972
4	*Scomber australasicus* (this study)	OQ781874	OR357879	OR357886	OR343906
5	*Scomberomorus regalis*	GU225663	EU349370	/	/
6	*Scomberomorus commerson* (this study)	OQ781871	OR357876	OR357883	/
7	*Scomberomorus sinensis*	/	DQ497891	NC033887	/
8	*Scomberomorus guttatus* (this study)	OQ781872	OR357877	OR357884	/
9	*Scomberomorus maculatus*	MN869898	EU349362	/	/
10	*Scomberomorus cavalla*	DQ835914	L11543	/	/
11	*Scomberomorus niphonius* (this study)	OQ781869	OR357874	OR357881	/
12	*Scomberomorus tritor*	/	AF231666	/	/
13	*Scomberomorus concolor*	MN869897	KX462516	NC033531	/
14	*Scomberomorus semifasciatus*	DQ107657	OM799593	NC021391	/
15	*Scomberomorus sierra*	HQ974552	KX462527	/	/
16	*Scomberomorus koreanus*	OM416538	DQ497884	OL362240	/
17	*Scomberomorus queenslandicus*	DQ107667	AY390590	/	/
18	*Scomberomorus plurilineatus*	JF494460	/	/	/
19	*Scomberomorus brasiliensis*	JX124893	DQ080322	ON885865	/
20	*Scomberomorus munroi*	DQ107675	NC021390	NC021390	/
21	*Auxis rochei*	HM389992	EU708971	AB105165	EU708978
22	*Auxis thazard*	HM390182	EF141173	AB105447	AB193567
23	*Sarda sarda*	KJ709601	EF392614	/	/
24	*Sarda chiliensis*	GU440511	EU349339	/	/
25	*Sarda australis*	DQ107715	/	/	/
26	*Sarda orientalis* (this study)	OQ781873	OR357878	OR357885	/
27	*Thunnus thynnus*	KT352985	EU036523	KF906720	/
28	*Thunnus alalunga*	MZ050652	DQ198012	AB101291	AB211999
29	*Thunnus albacares*	MN549777	EU708975	JN572794	EU708982
30	*Thunnus atlanticus*	HM389683	AB098104	NC025519	AB212040
31	*Thunnus obesus*	MT455858	DQ198013	LC498079	AB212016
32	*Thunnus orientalis*	DQ107631	EU708976	KU058180	EU708983
33	*Thunnus tonggol*	JN312309	EU708977	MW658109	EU708984
34	*Thunnus maccoyii*	KF528372	AB098105	AB536519	AB212013
35	*Acanthocybium solandri*	MN549715	EF141172	AP012945	/
36	*Grammatorcynus bilineatus*	HQ564453	DQ497833	NC051931	/
37	*Grammatorcynus bicarinatus*	KP194447	AY390594	/	/
38	*Euthynnus alletteratus*	HM390234	EF439531	KJ573349	/
39	*Euthynnus affinis* (this study)	OQ781867	OR357872	OR357880	/
40	*Euthynnus lineatus*	GU440322	EU349380	/	/
41	*Rastrelliger kanagurta* (this study)	OQ781877	OR357875	OR357882	/
42	*Rastrelliger faughni*	JN312963	DQ497844	/	/
43	*Katsuwonus pelamis*	MZ050591	EU708973	KY353410	EU708980
44	*Cybiosarda elegans*	DQ107697	AY390597	/	/
45	*Allothunnus fallai*	GU440212	EU935745	/	/
46	*Gasterochisma melampus*	HQ956188	HQ425781	NC020671	/
47	*Gymnosarda unicolor*	KJ534628	DQ497834	AP012510	/
48	*Rastrelliger brachysoma*	DQ107680	AB507240	AB507210	/
The total of sequence		46	48	28	14

Table 3. Summary statistics for the genes used in this study (involved all sequences by PCR amplification and download from GenBank).

	CO1	Cytb	D-loop	ITS
Aligned sites (bp)	575	311	216	517
A%	23.6	22.6	35.2	13.9
G%	17.4	16.1	18.3	31.0
C%	29.6	31.0	18.9	38.9
T%	29.3	30.2	27.5	16.2
A+T	52.9	52.8	62.7	30.1
Variable sites	230 (40%)	140 (45%)	205 (95%)	379 (73%)
Parsimony-informative sites	203 (35%)	124 (40%)	171 (79%)	298 (58%)

timal model for D-loop and ITS. BI analysis used four independent MCMC chains run simultaneously for 10 million generations while sampling one tree per 1000 replicates. Bayesian posterior probabilities (BPP), the frequencies of nodal resolution, were mapped on the BI tree. We visually inspected the trace files in Tracer v. 1.7[45] to verify that the chains had reached convergence. For ML, nodal support was assessed using a nonparametric bootstrap sampling of 1000 replicates. The output trees were further edited by Figtree v. 1.4.3 software (http://influenza.bio.ed.ac.uk/software/Figtree).

RESULTS

SAMPLE IDENTIFICATION

According to the morphological characteristics, 17 fish samples representing 5 genera 8 species were identified as *Sarda orientalis* (Linnaeus, 1758), *Euthynnus affinis* (Cantor, 1850), *Scomber japonicas* (Honttuyn, 1782), *Scomber australasicus* (Cuvier, 1832), *Scomberomorus niphonius* (Cuvier, 1832), *Rastrelliger kanagurta* (Cuvier, 1816), *Scomberomorus commerson* (Lacepède, 1800), and *Scomberomorus guttatus* (Bloch & Schneider, 1801). The morphology of eight species was presented in the Supporting Information (**Supplementary Figure S1**)

SEQUENCE FEATURES

The concatenated dataset comprised 1619 aligned sites: 575 bp from CO1, 311 bp from Cytb, 216 bp from D-loop, and 517 bp from ITS. The sequences downloaded by amplification and database in this study included 46 CO1 sequences, 48 Cytb sequences, 28 D-loop sequences and 14 ITS sequences (**Table 2**). Through the analysis of individual gene datasets (the sequence by PCR amplification and download from GenBank), the mtDNA (CO1, Cytb , and D-loop) exhibited a bias toward A+T in base composition, with percentages of 52.9%, 52.8%, and 62.7%, respectively. The ITS regions displayed A+T base content of 30.1% (**Table 3**). The results of the saturation analysis revealed that the base mutations in the single gene were suitable for phylogenetic analyses and did not reach saturation (**Figure 2**).

SPECIES DIVERGENCE

Using Tamura-Nei mean values, a genetic pairwise distance matrix was generated between the 14 genera (**Table 4**). The inter-genera genetic distances ranged from 0.086 to 0.253, with an average genetic distance of 0.154. The smallest genetic distance was observed between *Sarda* and *Allothunnus* (0.086), and the largest genetic distance was observed between *Rastrelliger* and *Gymnosarda* (0.253).

PHYLOGENETIC RELATIONSHIPS

The trees constructed by analyses of the concatenated data using the methods of NJ, ML, and BI were consistent for well-supported nodes (**Figures 3-5**). The low nodal support values in Figures 3-5 were mainly reflected in the branch nodes above the genus level. The source file of three phylogenetic trees was in supplementary files. The Scombridae formed three monophyletic groups, one consisting of *Grammatorcynus* (BI BPP = 1.0, ML BS = 100% and NJ BS =100%), another consisting of *Rastrelliger* and *Scomber* (BI BPP = 1.0, ML BS = 80% and NJ BS =68%) and the other comprising *Scomberomorus*, *Acanthocybium*, *Sarda*, *Auxis*, *Katsuwonus*, *Euthynnus*, and *Thunnus* (BI BPP = 0.563, ML BS = 42%, and NJ BS = 46%) (**Figures 3-5**).

All results revealed that *Grammatorcynus bilineatus* and *Grammatorcynus bicarinatus* did not cluster with others in the family Scombridae, forming a distinct genus clade (**Figures 3-5**). The NJ tree believed that Scombrini was the earliest differentiated group within Scombididae (**Figure 3**).

Rastrelliger and *Scomber* were recovered as sister taxa, forming the Scombrini. The monophyly of the Scombrini was highly supported in both sets of analyses (BI BPP = 1.0, ML BS = 80%) (**Figure 4 and 5**). *Thunnus* was a highly monophyletic group, and *Auxis*+*Katsuwonus*+*Euthynnus* (BI BPP = 1.0, ML BS = 96%, and NJ BS = 96%) were sister groups to each other, forming the Thunnini (**Figures 3-5**). Within *Thunnus*, we recovered a deep split between a clade with *T. orientalis* and *T. alalunga*, and a subclade containing all remaining *Thunnus* species (*Thunnini thynnus*, *Thunnini maccoyii*, *Thunnini obesus*, *Thunnini tonggol*, *Thunnini albacares*, and *Thunnini atlanticus*). *Katsuwonus* + *Auxis* + *Euthynnus* was the next lineage to branch off, followed by a

Table 4. Pairwise Tamura–Nei mean genetic distances of Scombridae genera sampled in this study.

Genus	1.*Euthynnus*	2.*Scomber*	3.*Scomberomorus*	4.*Rastrelliger*	5.*Sarda*	6.*Thunnus*	7.*Cybiosarda*	8.*Allothunnus*	9.*Auxis*	10.*Grammatorcynus*	11.*Gasterochisma*	12.*Acanthocybium*	13.*Gymnosarda*	14.*Katsuwonus*
1.*Euthynnus*														
2.*Scomber*	0.171													
3.*Scomberomorus*	0.163	0.188												
4.*Rastrelliger*	0.192	0.177	0.190											
5.*Sarda*	0.123	0.171	0.149	0.173										
6.*Thunnus*	0.131	0.169	0.163	0.183	0.120									
7.*Cybiosarda*	0.116	0.166	0.147	0.156	0.102	0.109								
8.*Allothunnus*	0.095	0.157	0.142	0.154	0.086	0.094	0.088							
9.*Auxis*	0.115	0.160	0.168	0.190	0.126	0.125	0.109	0.088						
10.*Grammatorcynus*	0.177	0.199	0.180	0.185	0.167	0.171	0.160	0.150	0.170					
11.*Gasterochisma*	0.174	0.192	0.183	0.209	0.157	0.161	0.146	0.135	0.156	0.194				
12.*Acanthocybium*	0.158	0.187	0.176	0.223	0.140	0.145	0.128	0.108	0.157	0.184	0.167			
13.*Gymnosarda*	0.165	0.184	0.185	0.253	0.138	0.158	0.125	0.106	0.153	0.184	0.177	0.172		
14.*Katsuwonus*	0.111	0.165	0.171	0.193	0.134	0.134	0.113	0.102	0.094	0.177	0.154	0.155	0.161	

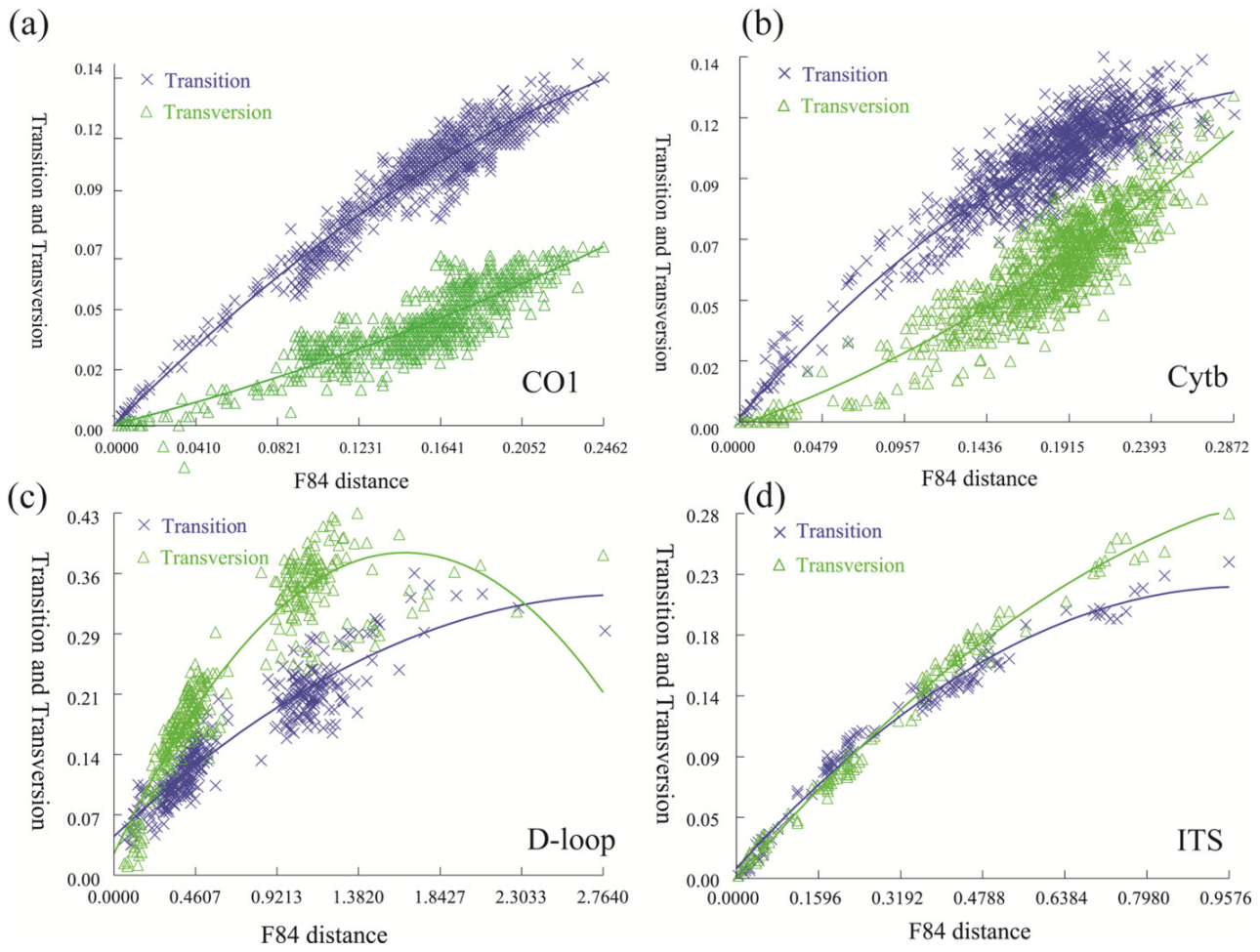

Figure 2. Saturation plot for transition and transversion of gene sequences. (a) CO1; (b) Cytb; (c) D-loop; (d) ITS

The crosses (×) show the number of transition events, and the triangles (△) show the number of transversion events. The x-axis shows the genetic distance based on the F84 model, and the y-axis shows the proportion of transitions or transversions, which is calculated by multiplying the number of transitions or transversions by the sequence length. The curves show the trends of the variance of transitions and transversions with the genetic distance increasing.

clade formed by all extant *Thunnus*. The Sardini consists of *Sarda*, *Cybiosarda*, and *Allothunnus*. However, the evolutionary relationship between *Acanthocybium solandri* and *Gymnosarda unicolor* was doubtful. The NJ Tree showed that *G .unicolor* belonged to the Sardini, and *A. solandri* differentiated separately (**Figure 3**); the ML tree revealed that *A. solandri* converged into one clade with *G. unicolor* (ML BS = 56%) (**Figure 4**); the BI tree indicated that *G. unicolor* was clustered with *Scomberomorus cavalla*, and *A. solandri* was a sister group to the rest of *Scomberomorus*. Two major clades were within the *Scomberomorus* subclade (1.0 BPP) (**Figure 5**). The topology of the three evolutionary trees was not precisely consistent. Still, they all indicated a common class group, the regalis group: *S. tritor*, *S. maculatus*, *S. concolor*, *S. sierra*, *S. brasiliensis*, and *S. regalis* (**Figures 3-5**).

DISCUSSION

GASTEROCHISMATINAE IS NOT A SISTER GROUP TO SCOMBRINAE

Our results all supported that *Gasterochisma* and Scombrinae are not sister taxa. The phylogenetic placement of *Gasterochisma* has been debated due to the presence of distinctive primitive features, as well as uniquely derived characteristics, making it difficult to classify using traditional morphological criteria.[11] The currently accepted phylogeny in **Figure 1a** suggested that the subfamily Gasterochismatinae was closely related to Scombrinae, potentially indicating a sister group relationship.[2,46] Block et al.[14] proposed that *Gasterochisma* was not closely related to Scombrinae, but the strength of the branch nodes in the phylogenetic tree constructed using the Cytb gene was weak. Finnerty[47] speculated that the limited resolution of the single-gene informative locus and the high degree of saturation and homogeneity in the Cytb gene might obscure relationships among nodes in the tree. To address the issue of Cytb gene saturation, researchers have suggested two approaches: sequencing slower-evolving genes, such as the 16s ribosomal gene, or using multiple molecular markers for analysis.[48,49] Reeb[50] utilized 16S rDNA (475 bp) and maximum likelihood distance and neighbor-joining algorithms to construct phylogenetic trees, concluding that *Gasterochisma* belonged to Scombrinae but were not sister groups. Our research provides further evidence supporting

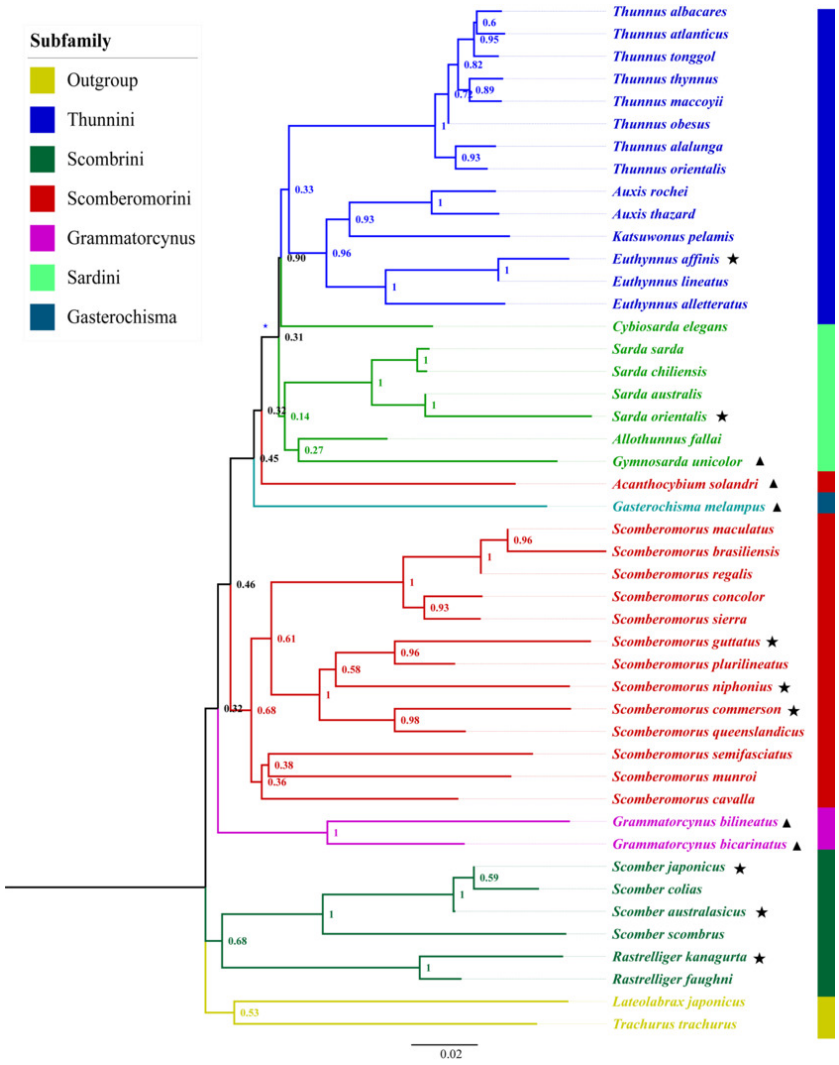

Figure 3. Neighbor-joining (NJ) tree derived from concatenated sequence dataset (CO1+Cytb+D-loop+ITS). Nodal support values are indicated on the branches. The specific symbol (★) denotes the samples in this study and the symbol (▲) indicates some special species in result. The scale represents the length of the genetic distance.

this conclusion, indicating that Gasterochismatinae and Scombrinae are not sister taxa.

GRAMMATORCYNUS IS THE EARLIEST DIVERGENT LINEAGE OF THE SCOMBRIDAE

This study's phylogenetic analysis of ML and BI methods indicated that *Grammatorcynus* was the earliest diverging clade in Scombridae. The NJ tree showed that the phylogenetic position of *Grammatorcynus* was located between Scombrini and Scomberomorini. There has been controversy about which group of Scombridae was the earliest to differentiate. According to traditional morphology, Scombrini was considered the earliest differentiated group in the Scombridae, while *Grammatorcynus* occupied an intermediate evolutionary position between Scombrini and Scomberomorini.[12,51] However, Johnson[12] observed that the skeletal morphology of *Grammatorcynus* differed from other Scombridae species in lacking a fourth pharyngeal dental plate. Consequently, Johnson[12] proposed that *Grammatorcynus* was the earliest diverging clade within the Scombridae. With the rapid development of molecular biology, the study of phylogenetic relationships on Scombridae is improving. Orrell et al.[16] utilized the maximum likelihood method with the Tmo-4C4 gene sequencing to construct a phylogenetic tree and their findings were consistent with the traditional taxonomic classification which suggested Scombrini as the earliest-differentiated taxon within Scombridae. However, using the same gene, the maximum parsimony method showed *Grammatorcynus* as the earliest divergent branch.[16] Santini et al.[52] utilized phylogenetic analysis of seven genetic loci, including both nuclear and mitochondrial genes, to identify Scombrini as the earliest divergent clade within the Scombridae. However, Miya et al.[53] reconstructed phylogenetic trees for the mackerel family based on mitochondrial CO1 and Cytb genes, all indicating that *Grammatorcynus* was the most basal clade. The different branching hypotheses might be caused by the different algorithms and datasets using construct phylogenetic trees. The topological structure of the three algorithms in this study was not completely consistent. Unlike the analysis of ML and BI, the NJ tree be-

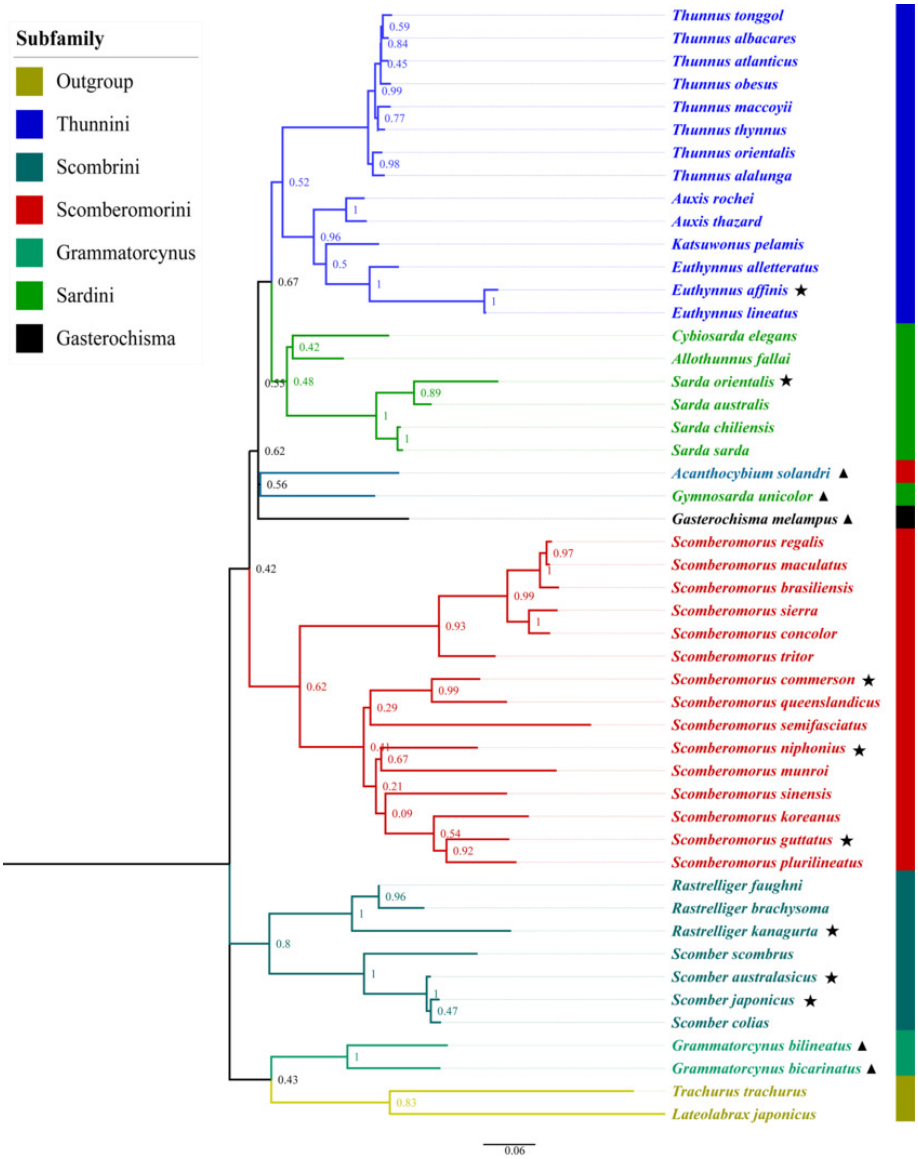

Figure 4. Maximum likelihood (ML) tree derived from concatenated sequence dataset (CO1+Cytb+D-loop+ITS). Nodal support values are indicated on the branches. The specific symbol (★) denotes the samples in this study and the symbol (▲) indicates some special species in result. The scale represents the length of the genetic distance.

lieved that Scombrini was the earliest differentiated group within Scombididae. Many factors affected the topologies of phylogenetic trees, including the choice of ingroup representation, the evolution of genes, long-branch attraction (LBA), and the method of tree construction.[54] Due to different evolutionary patterns between genes, some mitochondrial genes might not have enough information loci to construct interspecific phylogenetic relationships.[55,56] Accordingly, different genetic markers are suitable for phylogenetic analysis at different taxonomic levels. Thus, our results suggested that Scombrini was a group separately differentiated from the rest of Scombridae and *Grammatorcynus* was the earliest divergent lineage in the Scombridae.

SARDINI AND THUNNINI ARE NON-MONOPHYLETIC

Our analyses showed that *Allothunnus* (tribe Thunnini) and *Cybiosarda* (tribe Sardini) clustered into a clade (BI BPP = 0.688, ML BS = 56%, and NJ BS = 27%). The low nodal support values are shown in Figures 3-5. Overall, the low support rate of nodes in phylogenetic trees was the result of inherent complexities in the evolutionary process, limitations of these genes which their information loci were insufficient to support evolutionary relationships above the genus level and the stochastic nature of molecular evolution.[57]

The ML tree revealed that *Acanthocybium* (tribe Scomberomorini) and *Gymnosarda* (tribe Sardini) clustered into a single clade. In addition, the BI tree analysis indicated *Auxis*+*Euthynnus*+*Katsuwonus* did not cluster into a clade with *Thunnus*. All results consistently supported Sar-

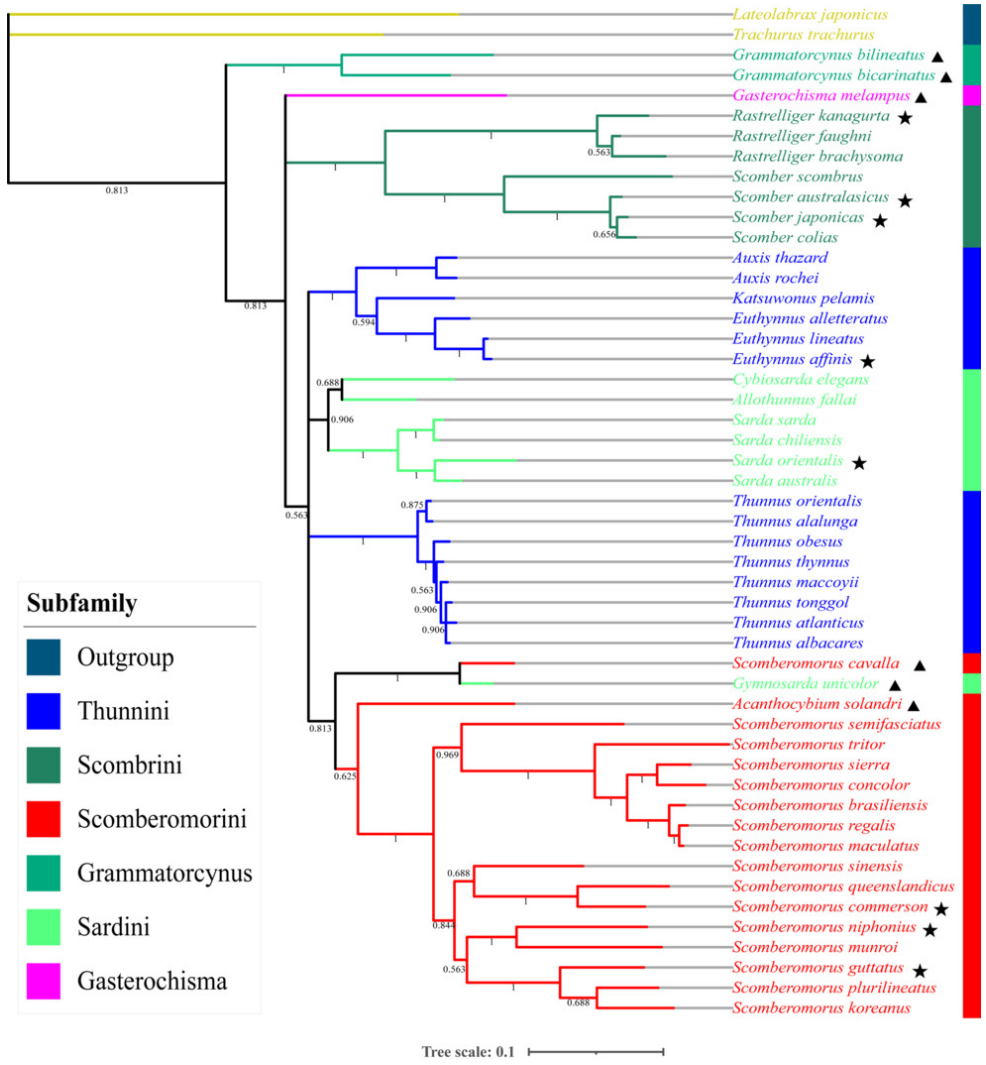

Figure 5. Bayesian inference (BI) phylogenetic hypothesis of Scombridae relationships based on analysis of the concatenated dataset (CO1+Cytb+D-loop+ITS) using Mrbyes 3.2. Nodal support values are indicated on the branches. The specific symbol (★) denotes the samples in this study and the symbol (▲) indicates some unique species in result. The scale represents the length of the genetic distance.

dini and Thunnini as non-monophyletic groups and showed they were clustered. These findings contradicted the conventional analysis of morphological data.[23,58] Only a few studies have performed phylogenetic relationships, specifically on these two taxa, Sardini and Thunnini. The available molecular studies can only support the monophyly of genera *Sarda*, *Auxis*, *Euthynnus*, *Katsuwonus*, and *Thunnus* and cannot support the monophyly of Sardini and Thunnini.[23,58] Santini et al.[52] revealed the clade of *Acanthocybium*+*Gymnosarda* and believed that the clade of *Katsuwonus*+*Auxis*+*Euthynnus* was not clustered with *Thunnus*. Monsch[59] suggested that *Gymnosarda* was a link between Sardinae and Scomberomorinae. Monsch[21] reported that the genus *Auxides* might be the immediate sister group of *Scomber* and *Rastrelliger*. All of these contradicted the monophyletic character of Sardini and Thunnini. Thus, we confirmed the non-monophyly of the Sardini and Thunnini as recognized by Santini et al.[52] and Monsch.[9,21] Nevertheless, additional morphological data and more comprehensive molecular data are needed to study the two groups in the future deeply.

CONCLUSION

This study investigated the molecular evolutionary relationships of 48 species from 14 genera found within the Scombridae. In summary, we propose that (1) Gasterochismatinae and Scombrinae are not sister groups; (2) *Grammatorcynus* likely represent the earliest divergent lineage in the Scombridae; and (3) Sardini and Thunnini are non-monophyletic. However, the phylogenetic relationship within the *Scomberomorus* genus and the classification of tribes require further clarification. Hence, additional morphological and molecular data (e.g., ITS), including an expansion of the molecular dataset through incorporating more genes and taxa, such as *Orcynopsis*, is necessary to enhance our understanding and refinement of the systematic phylogenetic relationships of this family.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTION PER CREDIT

Methodology: Xinru Zeng (Equal), Haoyu Yu (Equal). Formal Analysis: Xinru Zeng (Equal), Mengyao Cui (Equal). Investigation: Xinru Zeng (Equal), Haoyu Yu (Equal), Pingzhong Zheng (Equal). Data curation: Xinru Zeng (Equal), Mengyao Cui (Equal), Haoyu Yu (Equal). Writing – original draft: Xinru Zeng (Equal). Writing – review & editing: Xinru Zeng (Equal), Pingzhong Zheng (Equal), Fen Wei (Equal). Conceptualization: Fen Wei (Lead). Supervision: Fen Wei (Lead). Project administration: Fen Wei (Lead). Funding acquisition: Fen Wei (Lead).

ACKNOWLEDGMENTS

We thank the Coral Reef Research Center of China for the infrastructure provided. This work was supported by funding from the National Natural Science Foundation of China (Nos. 42090041 and 42030502) and the Science and Technology Project of Guangxi (Nos.AA17204074, AD17129063, 2018GXNSFBA050023). We thank the reviewers for their meaningful comments.

DATA AVAILABILITY STATEMENT

The data presented in this study are openly available in GenBank with Reference accession numbers OQ781867-OQ781879, OR343904-OR343906, and OR357872-OR357886.

Submitted: January 16, 2024 CST. Accepted: March 04, 2024 CST. Published: May 09, 2024 CST.

REFERENCES

1. Nelson JS, Grande TC, Wilson MVH. *Fishes of the World*. 5th ed. John Wiley & Sons; 2016. doi:10.1002/9781119174844

2. Collette BB. Scombroidei: development and relationships. Ontogeny and Systematics of Fishes. *American Society of Ichthyologists and Herpetologists, Special Publication*. 1984;476:591-620. doi:10.5962/p.226794

3. Fricke R, Eschmeyer W, Van der Laan R. *Catalog of Fishes: Genera, Species, References*. California Academy of Sciences; 2018.

4. Zeng XS, Sun CH, Huang XY, et al. DNA barcoding of Scomberomorus (Scombridae, Actinopterygii) reveals cryptic diversity and misidentifications. *ZooKeys*. 2022;1135:157-170. doi:10.3897/zookeys.1135.93631. PMID:36761794

5. Jeena NS, Rahuman S, Roul SK, et al. Resolved and Redeemed: A New Fleck to the Evolutionary Divergence in the Genus Scomberomorus Lacepède, 1801 (Scombridae) With Cryptic Speciation. *Frontiers in Marine Science*. 2022;9(888463). doi:10.3389/fmars.2022.888463

6. Wu HL, Zhong JS, Zhao SL. Retrieval of marine and estuarine fish systems in China. *China Agriculture Press Beijing*. 2021;9787109279995.

7. Collette BB. Morphology, systematics, and biology of the Spanish Mackerels (*Scomberomorus, Scombridae*). *Fishery Bulletin*. 1984;82(4):545-692. https://api.semanticscholar.org/CorpusID:90868243

8. Collette BB, Nauen C. *FAO Species Catalogue. v. 2: Scombrids of the World. An Annotated and Illustrated Catalogue of Tunas, Mackerels, Bonitos, and Related Species Known to Date*. Food & Agriculture Organization of the United Nations; 1983.

9. Monsch K. *The Phylogeny of the Scombroid Fishes*. University of Bristol; 2000.

10. Carpenter K, Collette BB, Russo J. Unstable and stable classifications of scombroid fishes. *Bulletin of Marine Science* . 1995;56(2):379-405. doi:10.1515/botm.1995.38.1-6.175

11. Collette BB, Reeb C, Block BA. Systematics of the tunas and mackerels (Scombridae). *Fish Physiology*. 2001;19:1-33. doi:10.1016/s1546-5098(01)19002-3

12. Johnson G. Scombroid phylogeny: an alternative hypothesis. *Bulletin of Marine Science*. 1986;39(1):1-41. doi:10.1201/b10194-13

13. Banford HM, Bermingham E, Collette BB, McCafferty SS. Phylogenetic systematics of the Scomberomorus regalis (Teleostei: Scombridae) species group: molecules, morphology and biogeography of Spanish mackerels. *Copeia*. 1999;(3):596-613. doi:10.2307/1447593

14. Block BA, Finnerty JR, Stewart AFR, Kidd J. Evolution of endothermy in fish: mapping physiological traits on a molecular phylogeny. *Science*. 1993;260(5105):210-214. doi:10.1126/science.8469974

15. Dalziel AC, Moyes CD, Fredriksson E, Lougheed SC. Molecular evolution of cytochrome c oxidase in high-performance fish (*Teleostei: Scombroidei*). *Journal of Molecular Evolution*. 2006;62(3):319-331. doi:10.1007/s00239-005-0110-7

16. Orrell T, Collette BB, Johnson G. Molecular data support separate scombroid and xiphioid clades. *Bulletin of Marine Science*. 2006;79(3):505-519.

17. Jondeung A, Karinthanyakit W. The complete mitochondrial DNA sequence of the short mackerel (*Rastrelliger brachysoma*), and its phylogenetic position within Scombroidei, Perciformes. *Mitochondrial DNA*. 2010;21(2):36-47. doi:10.3109/19401731003622529

18. Abdussamad EM, Sukumaran S, K. O. Ratheesh A, et al. Scomber indicus, a new species of mackerel (Scombridae: Scombrini) from Eastern Arabian Sea. *Indian Journal Of Fisheries*. 2016;63(3):1-10. doi:10.21077/ijf.2016.63.3.59184-01

19. Bannikov AF. A New Genus and Species of Scombrid Fish (Perciformes, Scombroidei, Scombridae) from the Lower Oligocene of the Caucasus. *Paleontological Journal*. 2020;54(1):59-67. doi:10.1134/s0031030120010037

20. Monsch KA. Revision of the scombroid fishes from the Cenozoic of England. *Earth and Environmental Science Transactions of the Royal Society of Edinburgh*. 2004;95(3-4):445-489. doi:10.1017/s0263593300001164

21. Monsch KA. A revision of scombrid fishes (Scombroidei, Perciformes) from the Middle Eocene of Monte Bolca, Italy. *Palaeontology*. 2006;49(4):873-888. doi:10.1111/j.1475-4983.2006.00566.x

22. Chow S, Kishino H. Phylogenetic relationships between tuna species of the genus Thunnus (Scombridae: Teleostei): inconsistent implications from morphology, nuclear and mitochondrial genomes. *Journal of Molecular Evolution*. 1995;41(6):741-748. doi:10.1007/bf00173154

23. Viñas J, Alvarado Bremer JR, Pla C. Phylogeography and phylogeny of the epineritic cosmopolitan bonitos of the genus *Sarda* (Cuvier): inferred patterns of intra- and inter-oceanic connectivity derived from nuclear and mitochondrial DNA data. *Journal of Biogeography*. 2010;37(3):557-570. doi:10.1111/j.1365-2699.2009.02225.x

24. Rosel P, Dizon A, Haygood M. Variability of the mitochondrial control region in populations of the harbour porpoise, Phocoena, on interoceanic and regional scales. *Canadian Journal of Fisheries and Aquatic Sciences*. 1995;52(6):1210-1219. doi:10.1139/f95-11

25. Lee WJ, Conroy J, Howell WH, Kocher ThomasD. Structure and evolution of teleost mitochondrial control regions. *Journal of molecular evolution*. 1995;41(1):54-66. doi:10.1007/bf00174041

26. Tobe SS, Kitchener AC, Linacre AMT. Reconstructing mammalian phylogenies: a detailed comparison of the cytochrome b and cytochrome oxidase subunit I mitochondrial genes. *PloS one*. 2010;5(11):e14156. doi:10.1371/journal.pone.0014156. PMID:21152400

27. Yang RH, Su JH, Shang JJ, et al. Evaluation of the ribosomal DNA internal transcribed spacer (ITS), specifically ITS1 and ITS2, for the analysis of fungal diversity by deep sequencing. *PloS one*. 2018;13(10):e0206428. doi:10.1371/journal.pone.0206428. PMID:30359454

28. Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN. DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 2005;360(1462):1847-1857. doi:10.1098/rstb.2005.1716. PMID:16214743

29. Xiao W, Zhang Y, Liu H. Molecular systematics of Xenocyprinae (Teleostei: Cyprinidae): taxonomy, biogeography, and coevolution of a special group restricted in East Asia. *Molecular Phylogenetics and Evolution*. 2001;18(2):163-173. doi:10.1006/mpev.2000.0879

30. Grant W, Bowen B. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *Journal of Heredity*. 1998;89(5):415-426. doi:10.1093/jhered/89.5.415

31. Chow S, Nakagawa T, Suzuki N, Takeyama H, Matsunaga T. Phylogenetic relationships among *Thunnus* species inferred from rDNA ITS1 sequence. *Journal of Fish Biology*. 2006;68(A):24-35. doi:10.1111/j.0022-1112.2006.00945.x

32. Thompson PW, Philipp R, Boyd B. In: *Yearbook of the NCTM*. ; 1994.

33. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *Journal of Molecular Biology*. 1990;215(3):403-410. doi:10.32388/rhq6vj

34. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*. 2018;35(6):1547-1549. doi:10.1093/molbev/msy096. PMID:29722887

35. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*. 2016;33(7):1870-1874. doi:10.1093/molbev/msw054. PMID:27004904

36. Srivathsan A, Meier R. On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. *Cladistics*. 2012;28(2):190-194. doi:10.1111/j.1096-0031.2011.00370.x

37. Xia X, Xie Z, Salemi M, Chen L, Wang Y. An index of substitution saturation and its application. *Molecular Phylogenetics and Evolution*. 2003;26(1):1-7. doi:10.1016/S1055-7903(02)00326-338

38. Swofford DL, Sullivan J. Phylogeny inference based on parsimony and other methods using PAUP*. In: *The Phylogenetic Handbook: A Practical Approach to DNA and Protein Phylogeny*. Cáp 7; 2003:160-206. doi:10.1017/CBO9780511819049.010

39. Ronquist F, Teslenko M, van der Mark P, et al. MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology*. 2012;61(3):539-542. doi:10.1093/sysbio/sys029. PMID:22357727

40. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*. 2014;30(9):1312-1313. doi:10.1093/bioinformatics/btu033. PMID:24451623

41. Kimura MA. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*. 1980;16(2):111-120. doi:10.1007/bf01731581

42. Bozdogan H. Model selection and Akaike's information criterion (AIC): The general theory and its analytical extensions. *Psychometrika*. 1987;52(3):345-370. doi:10.1007/bf02294361

43. Huelsenbeck JP, Crandall KA. Phylogeny estimation and hypothesis testing using maximum likelihood. *Annual Review of Ecology and Systematics*. 1997;28(1):437-466. doi:10.1146/annurev.ecolsys.28.1.437

44. Nylander J. *MrModeltest 1.0 b. a Simplified Version of David Posada's Modeltest 3.06*. Department of Systematic Zoology; 2003.

45. Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*. 2018;67(5):901-904. doi:10.1093/sysbio/syy032. PMID:29718447

46. Collette BB, Russo JL. Interrelationships of the Spanish mackerels (Pisces: Scombridae: Scomberomorus) and their copepod parasites. *Cladistics*. 1985;1(2):141-158. doi:10.1111/j.1096-0031.1985.tb00418.x

47. Finnerty J. volution of cytochrome b in Scombroidei (Teleostei): molecular insight into billfish (Istiophoridae and Xiphiidae) relationships. *Fishery Bulletin*. 1995;93(1):78-96. doi:10.2307/1446950

48. Hrbek T, Seckinger J, Meyer A. A phylogenetic and biogeographic perspective on the evolution of poeciliid fishes. *Molecular Phylogenetics and Evolution*. 2007;43(3):986-998. doi:10.1016/j.ympev.2006.06.009

49. Meynard CN, Mouillot D, Mouquet N, Douzery EJP. A phylogenetic perspective on the evolution of Mediterranean teleost fishes. *PLoS One*. 2012;7(5):e36443. doi:10.1371/journal.pone.0036443. PMID:22590545

50. Reeb CA. Molecular insights into the evolution of a circumtropical fish (Coryphaena hippurus) and an Indo-Pacific group of mollusks (Cellana).

51. Collette BB. Adaptations and systematics of the mackerels and tunas. *The Physiological Ecology of Tunas*. Published online 1978:7-39. doi:10.1016/b978-0-12-639180-0.50009-4

52. Santini F, Carnevale G, Sorenson L. First molecular scombrid timetree (Percomorpha: Scombridae) shows recent radiation of tunas following invasion of pelagic habitat. *Italian Journal of Zoology*. 2013;80(2):210-221. doi:10.1080/11250003.2013.775366

53. Miya M, Friedman M, Satoh TP, et al. Evolutionary origin of the Scombridae (tunas and mackerels): members of a Paleogene adaptive radiation with 14 other pelagic fish families. *PloS one*. 2013;8(9):e73535. doi:10.1371/journal.pone.0073535. PMID:24023883

54. Wang J, Lu B, Zan R, et al. Phylogenetic Relationships of Five Asian Schilbid Genera Including Clupisoma (Siluriformes: Schilbeidae). *PloS one*. 2016;11(1):e0145675. doi:10.1371/journal.pone.0145675. PMID:26751688

55. Cummings MP, Otto SP, Wakeley J. Sampling properties of DNA sequence data in phylogenetic analysis. *Molecular Biology and Evolution*. 1995;12(5):814-822. doi:10.1093/oxfordjournals.molbev.a040258

56. Kocher TD, Carleton KL. Base substitution in fish mitochondrial DNA: patterns and rates. *Molecular systematics of fishes*. Published online 1997:13-24. doi:10.1016/b978-012417540-2/50003-8

57. Gatesy J, Springer MS. Phylogenetic analysis at deep timescales: unreliable gene trees, bypassed hidden support, and the coalescence/concatalescence conundrum. *Molecular Phylogenetics and Evolution*. 2014;80:231-266. doi:10.1016/j.ympev.2014.08.013

58. Puncher GN, Arrizabalaga H, Alemany F, et al. Molecular identification of Atlantic Bluefin Tuna (Thunnus thynnus, Scombridae) larvae and development of a DNA character-based identification key for Mediterranean Scombrids. *Plos One*. 2015;10(7):e0130407. doi:10.1371/journal.pone.0130407. PMID:26147931

59. Monsch KA. A new fossil bonito (Sardini, Teleostei) from the Eocene of England and the Caucasus, and evolution of tail region characters of its Recent relatives. *Paleontological Research*. 2000;4:75-80. doi:10.2517/PRPSJ.4.75

SUPPLEMENTARY MATERIALS

Supplementary 1

Download: https://ija.scholasticahq.com/article/94824-phylogenetic-relationships-analysis-of-the-family-scombridae-actinopterygii-scombriformes/attachment/199232.pdf