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RESEARCH PAPER

Mitochondrial Phylogeny, Diversification, and Biogeographical History of the Carangids (Actinopterygii: Carangiformes) from the Odisha Coast, Bay of Bengal, India

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Abstract

Recent theoretical and practical advances in phylogenetic species delimitation have made biodiversity studies increasingly robust. In widespread taxa, delimiting species is a thought-provoking task; the carangid species are excellent example since they show high degree of phenotypic plasticity with a widespread distribution throughout the globe. The species of the family Carangidae are highly diverse, commercially important, and widely distributed across the Bay of Bengal. To date, information about genetic diversity, and phylogenetic relationships is not well established in the Bay of Bengal. In this study, we integrate two mitochondrial markers (COI and 16S rRNA) and 25 morphological characteristics to explore the phylogenetic relationships, and biogeographical history of the family Carangidae at the generic level. We collected 15 species among ten genera from the Odisha coast, Bay of Bengal, India. Phylogenetic analyses by both neighbor-joining (NJ) and maximum likelihood (ML) produce high congruence trees defining well-supported clades. Two species delimitation methods were analysed: Assemble Species by Automatic Partitioning (ASAP) and Poisson tree processes (PTP). In addition to phylogenetic analyses and species delimitation, the divergence time was estimated based on RelTime-ML method. Our results suggest that the studied carangids were diversified from the common ancestor in the early Cretaceous period (142 Mya).

Introduction

Tropical marine ecosystems are always a focus due to the existence of spectacular biodiversity. One such tropical marine ecosystem is the Bay of Bengal, which is often considered a mega-diverse region with overlapping features of many marine species. However, the biodiversity of most tropical marine ecosystems is under serious threat due to various anthropogenic stresses such as; climatic and habitat changes, oceanographic regimes, eutrophication, overfishing, increasing coastal development, and proliferation of invasive species. In the recent decade, these anthropogenic stresses individually or their synergistic combinations have driven diversity, mainly marine fish diversity, to a sharply declining condition on a global scale (Hilborn et al. 2003; Pauly 2008). This precarious trend has impacted a wide spectrum of fish species ranging from small pelagic foragers (Metian 2009) to large top-order predators (Myers and Worm 2003, 2004), thereby directly reducing the intrinsic and cultural values of the species as a result of the health and function of the ecosystem collapsing (Santos et al. 2011). Although the integral framework based on physical and organismal information about the species has been implemented for the conservation of marine fish diversity (Thrush and Dayton 2010), a lack of species information, such as biology and genetic structure, hinders such efforts (Reiss et al. 2009).

The carangiform family Carangidae is one of the most commercially important and exploited pelagic marine fish families. The family comprises 148 valid species in 30 genera (Fricke et al., 2019) distributed throughout the tropical and subtropical regions of the Atlantic, Indian, and Pacific Oceans (Nelson, 2006; Froese & Pauly, 2024). Frequently known variously by common names such as trevallies, kingfish, pilotfish, rainbow runner, pompanos, jack, and scads, the species of this family are important top-level predators and are highly prized food and sport fishes. All small or large carangid species are considered edible protein sources and are caught in large tons every year to meet the market demand. Despite their high commercial value and ecological importance, carangids are frequently subjected to overexploitation due to poor taxonomic classification (Laroche et al. 1984). Numerous synonyms in major taxonomic databases are a clear indication of taxonomic ambiguities in Carangids (Froese and Pauly, 2019) and are due to phenotypic plasticity and morphological similarities across species (Lakra et al. 2009). Additionally, significant alterations in morphology were observed in Carangids during different stages of development, which may lead to general taxonomic confusion.

Life's diversity can only be discovered by correctly delineating species boundaries because they determine whether different species belong to the same group of organisms (Dayrat, 2005). Species have traditionally been established based on morphology, known as "morphospecies". However, recent days, deep understanding of species diversity and their exploration is mostly achieved through phylogenetic approaches (Avise, 2000; Condamine et al. 2016). As an integrative discipline, phylogenetic approaches primarily focus on the processes to understand the genealogical relatedness that arise within and among closely related species across the landscape (Avise et al. 1987; Avise, 2000). The rapid irreversible changes in species composition in most marine ecosystems as a result of human activities have breathed a new urgency to carry out phylogenetic as well as phylogeographic analyses among the species (Elliott et al. 2015).

For ambiguous species delineation and better understanding of their diversity, molecular approaches using specific marker genes, such as mitochondrial (mt) or nuclear (n) DNA, have been extensively used in phylogenetic studies. Molecular phylogenetic analysis has provided imperative intuitions in species diversity by resolving complex taxonomic ambiguities (for instance, cryptic and invasive species) (Condamine et al. 2016). Earlier studies have provided evidence for the presence of genetic differentiation within species between the Bay of Bengal and Indian Ocean for instance *Tenualosa ilisha* (Verma et al. 2016), *Sardinella longiceps* (Sebastian et al. 2017).

The goal of the present study is mainly to focus on the molecular phylogeny of carangids on the Odisha coast, Bay of Bengal, with comprehensive genera-level sampling using mitochondrial cytochrome c oxidase 1 (COI) and large ribosomal subunit (16S rRNA) markers. We hypothesized that the diversified carangids are linked geographically with other marine environments. In addition, we compare the demographic patterns for the carangids, which provides novel insight into the evolutionary histories of these ecologically and economically important fish species in the Bay of Bengal.

Materials and Methods

Study Sites and Sample Collection

As this study is a part of a research expedition of Gopalpur-on-sea (Lat 19.26 Lon 84.86), Odisha coast, Bay of Bengal, our sampling sites are restricted to 16 fish landing centers (FLCs) located nearer to Gopalpur-on-sea. The specimen sampling was conducted without the necessity of any special permit. The specimens were rapidly identified in the field to the family level (Carangidae) by observing quick identification characters such as body shape and coloration and dorsal fin pattern and transported to the laboratory under freezing (-2° C) conditions. Furthermore, the fin clips and muscle samples were dissected from the onside and preserved in absolute ethanol under reduced temperature conditions. All the specimens were then vouchered and subjected to morphological identification.

Taxonomic Species Identification

All the collected specimens were identified as morpho-species, i.e., individuals harboring a similar morphology and were identified at the lowest possible taxonomical level by reviewing the keys and meristic characters (Fischer and Whitehead, 1974; Talwar and Jhingran, 1991; Nelson, 2006, Fricke et al., 2019). Further, the current valid scientific and vernacular names, and geographic distribution information were retrieved from the Fishbase (Froese and Pauly, 2024), Eschmeyer's Catalog of Fishes (Fricke et al., 2024). During taxonomic identification, undescribed species were morphologically sorted based on the comments of taxonomic experts and inconclusive taxonomic keys.

DNA Extraction, Polymerase Chain Reaction, and Sequencing

Genomic DNA was extracted from the muscle and fin clips by the rapid isolation method (Sambrook and Russell, 2000) with a final dilution volume of 50 µL. The purity and concentration of the extracted DNA were analyzed by a NanoDrop spectrophotometer. Furthermore, the extracted DNA was evaluated through 1.2% agarose gel electrophoresis. A partial fragment (~650 bp) of the mitochondrial cytochrome c oxidase subunit I (mtCOI) gene was amplified using specific primers (Ivanova et al. 2007). Furthermore, an ~500 bp

fragment of the large ribosomal subunit of the mitochondrial rRNA gene (16S rRNA) was amplified using reported primers (Yang et al. 2015). The detailed primer sequences and amplification conditions are given in Table 1. PCR amplifications were performed in 25-µl including 30ng DNA template, 5x PCR buffer, 10µM of each primer, and 1 U of *Taq* DNA polymerase enzyme. The final PCR products were purified using a QIAquick PCR purification kit according to the manufacturer's protocol and outsourced for bidirectional sequencing. The new sequences have been deposited in the GenBank database under the accession numbers shown in Table 2.

DNA Sequence Analysis and Validation

The generated chromatograms were subjected to a quality check through the measurement of their Phred scores (Richterich, 1998), and the continuous sequence reads having high-quality bases (Phred score, QV>20) were considered for analysis. Few nucleotides in the generated sequences showed the presence of heterozygous bases (i.e., double peaks). These nucleotides were treated as ambiguous sites and were excluded from further analysis. All the chromatograms (forward and reverse) were assembled, and the consensus barcode sequences were generated with the programs PIPEBAR and OverlapPER (Oliveira et al. 2018). All the sequences were aligned using *Auto* method in MAFFT v7.428 (Katoh & Standley, 2013), checked manually against non-conservative alignments in the program BioEdit. All the sequences then translated into protein sequences in MEGA X (Kumar et al. 2018) to check for the pseudogenes. Furthermore, to quantify genetic diversity indexes such as the number of polymorphic sites, number of haplotypes, and pairwise nucleotide substitution rate, DnaSP V 6.0 (Rozas et al. 2017) was used with the Kimura 2-parameter model. All analyses were performed separately for the mtCOI and 16S rRNA genes. All the sequences from both gene sets were compared with the NCBI (National Center for Biotechnology Information, www.ncbi.nlm.nih.gov) nucleotide database using the blastn search tool (http://blast.ncbi.nlm.nih.gov) to corroborate species identification and to discard possible sequence errors.

Partition Scheme and Model Selection

As an initial requirement for phylogenetic reconstruction, the best-fit nucleotide substitution model parameter was evaluated under the corrected Akaike information criterion (AICc) (Hurvich and Tsai, 1993) and was estimated with ModelTest-NG (Darriba et al., 2020). The protein-encoding sequence of COI was partitioned based on three codon positions, and the best codon substitution model was determined.

Phylogenetic Inference

Preceding the phylogenetic inference, tree reconstruction was performed by using both the distance-based neighbor-joining (NJ) and rapid bootstrapping maximum likelihood (ML) approaches. The nodes in the NJ tree were supported with 10,000 bootstrap replicates. Prior to analysis, the datasets comprised of both generated and acquired sequences were subjected to analysis of redundancy and possible occurrence of substitution saturation using MetaPIGA 2.0 (Helaers & Milinkovitch, 2010). Single-gene analyses for ML reconstruction were conducted using IQ-TREE v 1.6.12 (Nguyen et al., 2015) with implementation of best substitution models suggested by ModelTest-NG for each gene and 1000 ultrafast bootstrap replicates (Hoang et al. 2018). Furthermore, the two genes were concatenated into a single multigene alignment using FASconCAT-G (Kück and Meusemann, 2010), and a partitioned maximum likelihood (ML) analysis was carried out using IQ-TREE v 1.6.12 as described above. The resultant phylogenetic trees in both analyses were visualized and edited in TreeGraph 2 (Stöver & Müller, 2010).

Divergence Time Estimation

The timing of divergence was estimated using the RelTime approach based on the relative framework embedded in MEGA X (Kumar et al. 2018). The generated sequences of the mtCOI gene set along with some GenBank retrieved sequences were used for

Table 1. PCR primers and PCR reaction conditions used in this work

Fragment	Primer sequence (5'-3')	PCR conditions
COI*	**FishF2_t1:** 5'-TGT AAA ACG ACG GCC AGT CGA CTA ATC ATA AAG ATA TCG GCA C−3' **FishR2_t1:** 5'-CAG GAA ACA GCT ATG ACA CTT CAG GGT GAC CGA AGA ATC AGA A−3' **VF2_t1:** 5'-TGT AAA ACG ACG GCC AGT CAA CCA ACC ACA AAG ACA TTG GCA C−3' **FR1d_t1:** 5'-CAG GAA ACA GCT ATG ACA CCT CAG GGT GTC CGA ARA AYC ARA A−3'	The first step: Initial denaturation, 94°C for 5 min. The second step: involved 35 cycles of denaturation at 95°C for 30 s, annealing at 52°C for 40 s, extension at 72°C for 1 min, with a final extension for 10 min at 72°C.
16S rRNA	**16S-F:** TGTAAAACGACGGCCAGT ACCGTGCAAAGGTAGCATAAT **16S-R:** CAGGAAACAGCTATGACCTCCGGTCTGAACTCAGATCAC	The first step: Initial denaturation, 94°C for 4 min. The second step: involved 35 cycles of denaturation at 95°C for 30 s, annealing at 61°C for 10 s, extension at 72°C for 45 s, with a final extension for 5 min at 72°C.

Table 2. GenBank accession numbers acquired for Carangid species

Species	GenBank Accession No.	
	COI	**16S rRNA**
Megalaspis cordyla	MN562559	MT090176
*Atropus mentalis**	KX433139	MT093467
Trachinotus baillonii	MN562557	MT093492
Seriolina nigrofasciata	KY634861	MT093504
Ulua aurochs	MN872802	MT101854
Selar crumenophthalmus	MN623881, MN872805	MT102347
Trachinotus blochii	MN892527	MT102364
Atropus hedlandensis	MN562556	MT102408
Caranx heberi	MN892525	MT112179
Uraspis uraspis	MN623874	MT102407
Alepes djedaba	MN892529	MT122795
Alepes kleinii	MN623875	MT122796
Atropus Atropos	MN623880	MT122813
Alepes vari	MN872801	MT123333
Caranx ignobilis	MN640784	MT128988

divergence time analysis. The fossil calibration record of Eastmanalepes primaevus Eastman 1904 is commonly referred to as the oldest known carangid fossil from the Lutetian epoch deposits of Bolca (Italy, Harrington et al. 2016). The minimum age of 49 Mya and maximum age of 79.89 Mya were set for fossil calibration root age for the entire group of Carangidae. Furthermore, the fossil records were crosschecked by accessing fossil databases (https://fossilcalibrations.org/Browse.php and https://paleobiodb.org/).

Molecular Species Delimitation

DNA species delimitation analysis was carried out to correlate the morphological species identification data with our generated molecular data. Putative DNA species and their boundaries were delineated using the distance-based "Assemble Species by Automatic Partitioning" (ASAP; Puillandre et al., 2021) and the tree-based Poisson Tree Processes (PTP) model implemented in bPTP analysis on its online server (http://species.h-its.org/ptp/; Zhang et al., 2013). In the ASAP tool, molecular sequences are clustered into distinct groups by applying different thresholds on multiple partitions. Both the gene sets are analyzed separately with default parameter and the best-suited model were selected based on their ASAP score to delineate species. On the other hand, PTP model relies on intra- and interspecific substitution events (Zhang et al., 2013). Tree-based species delimitation methods require either ultrametric (sGMYC and bGMYC) or non-ultrametric trees (bPTP and mPTP) (Pons et al., 2006; Zhang et al., 2013; Tang et al., 2014). The ML tree from the COI matrix was used as an input guide tree with default parameter settings to explore the species boundary hypotheses. The analysis was run for 500,000 generations with a thinning of 500 and burn-in of 0.1, both with and without the out-group.

Results

During the study of marine fish diversity of Gopalpur-on-sea, Odisha coast, Bay of Bengal (Figure 1), we are able to record the number of carangids. Some of the recorded carangids were added to the ichthyo-faunal diversity of the Odisha coast, Bay of Bengal, as "first records". After thorough examination of various morphometric and meristic characteristics, the specimens were successfully assigned to their respective species. The recorded measurements are given in Table 3.

Genetic Diversity and DNA Phylogeny

To establish a phylogenetic relationship among the Carangids from the Odisha coast, Bay of Bengal, we analyzed two molecular markers. The mtCOI sequences were 652 bp in length, with 305 polymorphic sites, 267 parsimony-informative sites, 38 singletons and a nucleotide diversity (Pi) rate of 0.25522. Likewise, the 16S rRNA sequences were 528 bp in length, with 139 polymorphic sites, 116 parsimony-informative sites, 23 singletons and a nucleotide diversity rate of 0.10623. No gaps were detected for either the mtCOI or 16S rRNA gene sequences. In total, our analysis identified 16 (Hd=1.0000) and 11 (Hd=0.9848) haplotypes for the mtCOI and 16S rRNA gene sequences, respectively, where Hd indicates haplotype diversity. The substitution saturation test demonstrated that the generated sequences have little saturation ($P<0.0001$), thus validating their use of phylogenetic inference. Furthermore, the presence of any possible compositional bias was analyzed for the clustering of high confidence positional homology, which has been shown to alleviate strong branching within the closely related species group and increase tree accuracy. The decorated NJ and ML trees calculated from the trimmed alignments were in strong taxonomic agreement with

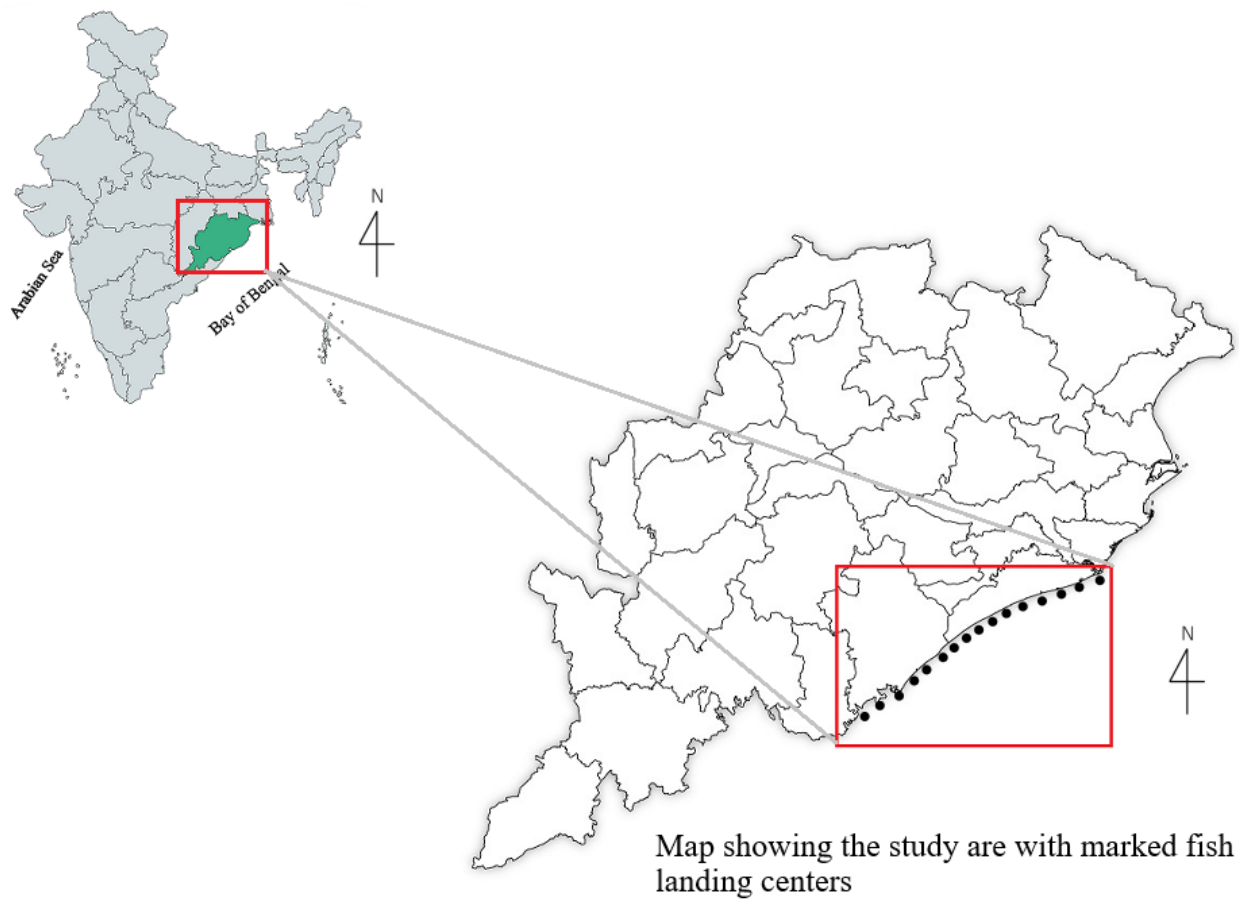

Figure 1. The location map of sampling sites of Carangidae species. The area within the rectangular box represents Odisha coast, North-East Bay of Bengal.

higher bootstrap values and recuperated more than 98% taxa as operationally monophyletic.

Phylogenetic Inference and Divergence Time Estimation

To compare the newly generated sequences of both mtCOI and 16S rRNA genes with those available sequences of carangid species, we retrieved some sequences for both gene sets from the NCBI nucleotide database to be used as both in-group and out-groups for phylogenetic reconstruction. The inferred NJ phylogenetic tree showed a highly congruent clustering of sequences according to their morphological identification with higher bootstrap branch support values, implying that all carangid species are operationally monophyletic. ModelTest-NG suggested TIM2e+G4, the best fit DNA substitution model for the mtCOI gene dataset, and GTR+F+G4 for the 16S rRNA gene dataset with the lowest AIC score. The dataset of 78 specimens comprised 40 COI and 38 16S rRNA sequences. The NJ (Figure 2 and Figure 2A) and partitioned ML (Figure 3) of both gene sets recovered similar topologies with moderate to strong branch support. The monophyly of all species is supported by higher bootstrap support values close to 100. The RelTime-based divergence time estimation recovered the stem-group age (i.e., the earliest possible origin) for the carangids as 147.24 million years ago (Mya) (95% confidence intervals) (Figure 4). The genus Caranx was estimated to be the most recent common ancestor (MRCA) by inferred ancestral sequence analysis. Divergence time estimates of *Atropos* and *Carangoides* indicate that they diverge almost simultaneously at 19.43 Mya (95% confidence intervals). However, the genera *Ulua* and *Uraspis* diverged from the stem group at 26.58 Mya and 49.48 Mya (95% confidence intervals), respectively. The divergence time estimates for the entire group of Carangids are in agreement with earlier studies on the diversification of ray-finned fishes (Near et al. 2012).

ASAP and bPTP Analyses

In the species delimitation analyses, the best score was identified using the ASAP method, with lower scores indicating better partitioning (Puillandre et al., 2021). The ASAP analyses for both the gene set (COI and 16S) supported all carangidae species partition (Figure 5 and 6). The generated results from ASAP and bPTP were concordant, which clearly showed carangid species delimitation. The bPTP method based on the ML option

Table 3. Morphometric and Meristic Counts of species of family Carangidae.

		Megalaspis cordyla	*Atropus mentalis*	*Trachinotus baillonii*	*Seriolina nigrofasciata*	*Ulua aurochs*	*Selar crumenophthalmus*	*Trachinotus blochii*	*Atropus hedlandensis*	*Caranx heberi*
Counts	**Dorsal fin rays**	VIII+18	VIII+22	VII+23	VIII+30	VIII+22	IX+27	VI+18	VIII+20	VIII-I + 20
	Anal fin rays	II+16	II+17	III+24	II+15	II+18	III+22	II+17	II+16	III + 15
	Pectoral fin rays	17	19	15	12	18	17	17	15	21
	Pelvic fin rays	4	5	4	5	4	4	4	5	I + 5
	Caudal fin rays	13	16	20	16	9	13	19	17	16
Body and Head	**Total Length (TL)**	255±0.81	156±0.66	311±0.88	161±0.88	141±0.87	153±0.39	235±0.49	140±0.46	198±0.16
	Standard length (SL)	228±0.95	130±0.88	220±0.76	126±0.31	119±0.76	137±0.44	172±0.43	113±0.31	158±0.26
	Measurements in % of SL									
	Body depth	23.68±0.19	51.53±0.17	42.27±0.43	31.74±0.62	52.1±0.60	27.13±0.39	58.72±0.25	52.21±0.31	38.6±0.15
	Head length (HL)	24.12±0.12	30.76±0.24	21.81±0.47	30.95±0.31	31.09±0.87	30.65±0.19	28.48±0.32	29.2±0.24	30.37±0.10
	Pre-pectoral length	24.56±0.22	30.76±0.24	23.18±0.65	30.95±0.33	35.29±0.69	32.11±0.43	29.06±0.36	30.97±0.15	31.64±0.10
	Pre-dorsal length	31.57±0.47	43.84±0.29	55.45±0.47	39.68±0.25	42.85±0.88	38.68±0.49	65.69±0.23	44.24±0.45	42.4±0.07
	Pre-anal length	47.36±0.29	59.23±0.32	52.72±0.59	73.8 ±0.37	59.66±0.71	40.87±0.23	54.06±0.28	61.94±0.17	59.49±0.20
	Caudal height	21.92±0.46	3.84±0.05	21.36±0.48	15.87±0.32	32.77±0.15	12.4±0.42	41.86±0.17	36.28±0.21	31.64±0.14
	Caudal-peduncle length	7.89±0.16	10.76±0.17	8.18±0.54	7.14±0.44	6.72±0.13	5.83±0.11	11.04±0.27	6.19±0.04	11.6±0.04
	Caudal-peduncle depth	3.07±0.05	6.15±0.07	9.09±0.60	8.73±0.69	9.24±0.24	6.56±0.17	8.72±0.05	6.19±0.04	3.4±0.02
	Measurements in % of HL									
	Eye diameter	25.45±0.18	32.5±0.24	29.16±0.54	30.76±0.61	29.72±0.36	30.95±0.30	28.57±0.12	42.42±0.14	27.08±0.04
	Snout length	7.27±0.08	12.5±0.29	14.58±0.42	12.82±0.48	16.21±0.29	11.9±0.20	10.2±0.12	12.12±0.25	29.16±0.07
	Inter-orbital width	25.45±0.13	32.5±0.26	37.5±0.44	35.89±0.89	32.43±0.35	26.19±0.44	34.69±0.18	33.33±0.19	30.23±0.07
	Pre-nasal length	21.81±0.31	35±0.33	25±0.41	28.2±0.67	35.13±0.25	26.19±0.33	22.44±0.13	30.3±0.11	25.58±0.02
Dorsal fin (mm)	**Dorsal fin base**	124±0.91	77±0.57	84±0.39	77±0.88	68±0.29	76±0.52	92±0.19	69 ±17	51.9±0.20
	Dorsal fin height	29±0.47	30±0.88	65±0.61	24±0.67	38±0.33	23±0.28	86±0.14	49±0.18	19±0.05
Anal fin (mm)	**Anal fin base**	80±0.62	49±0.86	79±0.59	34±0.63	46±31	54±0.37	72±0.25	52±0.25	37.3±0.07
	Anal fin height	26±0.62	27±0.76	89±0.40	17±0.91	19±0.12	16±0.36	72±0.28	25±0.17	19±0.04
Caudal fin (mm)	**Caudal fin length**	42±0.40	41±0.44	93±0.72	34±0.58	33±0.34	27±0.39	61±0.29	36±0.20	45±0.08
Pectoral fin (mm)	**Pectoral fin length**	72±0.71	49±0.60	36±0.29	20±0.63	40±0.37	19±0.26	34±0.18	42±0.22	42±0.06

Table 3. ***Continued.***

		Uraspis uraspis	*Alepes djedaba*	*Alepes kleinii*	*Atropus atropos*	*Alepes vari*	*Caranx ignobilis*
Counts	**Dorsal fin rays**	VIII+25	VIII+25	VIII+25	VIII+19	VIII+25	VIII+18
	Anal fin rays	II+17	II+22	II+19	II+17	II+21	II+15
	Pectoral fin rays	15	17	20	15	19	15
	Pelvic fin rays	5	4	5	5	4	5
	Caudal fin rays	16	14	13	16	15	16
Body and Head	**Total Length (TL)**	135±0.12	200±0.39	173±0.16	145±0.21	213±0.16	220±0.17
	Standard length (SL)	106±0.32	168±0.26	135±0.18	122±0.22	171±0.26	175±0.16
	Measurements in % of SL						
	Body depth	46.22±0.22	34.52±0.14	40.74±0.09	46.89±0.05	35.08±0.15	40±0.14
	Head length (HL)	44.33±0.19	25±0.12	25.92±0.08	24.13±0.10	25.14±0.10	30.28±0.04
	Pre-pectoral length	33.96±0.17	26.19±0.18	25.81±0.12	24.82±0.10	25.73±0.05	29.71±0.04
	Pre-dorsal length	42.45±0.25	33.92±0.15	34.81±0.14	35.86±0.07	35.08±0.20	33.71±0.07
	Pre-anal length	55.66±0.20	47.61±0.12	48.88±0.13	49.65±0.10	45.61±0.10	49.14±0.17
	Caudal height	35.84±0.16	18.45±0.07	20.74±0.19	36.88±0.07	19.29±0.04	40±0.16
	Caudal-peduncle length	5.66±0.04	8.92±0.04	10.37±0.12	6.55±0.02	8.77±0.02	9.71±0.06
	Caudal-peduncle depth	7.54±0.10	5.35±0.03	5.18±0.03	6.55±0.02	5.26±0.02	5.14±0.02
	Measurements in % of HL						
	Eye diameter	25.53±0.05	26.19±0.04	31.42±0.15	34.28±0.15	25.58±0.02	26.41±0.04
	Snout length	12.76±0.07	11.9±0.04	14.28±0.04	28.57±0.07	13.95±0.06	7.54±0.03
	Inter-orbital width	25.53±0.11	30.95±0.13	34.28±0.06	28.57±0.08	30.23±0.07	26.41±0.04
	Pre-nasal length	19.14±0.05	28.57±0.11	25.71±0.10	31.42 ±0.12	25.58±0.02	39.62±0.08
Dorsal fin (mm)	**Dorsal fin base**	60±0.28	95±0.15	91±0.17	70±0.19	95±0.23	109±0.13
	Dorsal fin height	26±0.14	25±0.13	22±0.08	25±0.17	19±0.05	37±0.11
Anal fin (mm)	**Anal fin base**	37±0.16	72±0.10	65±0.13	50±0.10	67±0.17	67±0.11
	Anal fin height	25±0.11	22±0.12	21±0.07	22±0.13	19±0.04	34±0.13
Caudal fin (mm)	**Caudal fin length**	38±0.19	45±0.12	40±0.10	39±0.13	45±0.08	53±0.10
Pectoral fin (mm)	**Pectoral fin length**	36±0.23	52±0.12	44±0.16	38±0.08	42±0.06	55±0.15

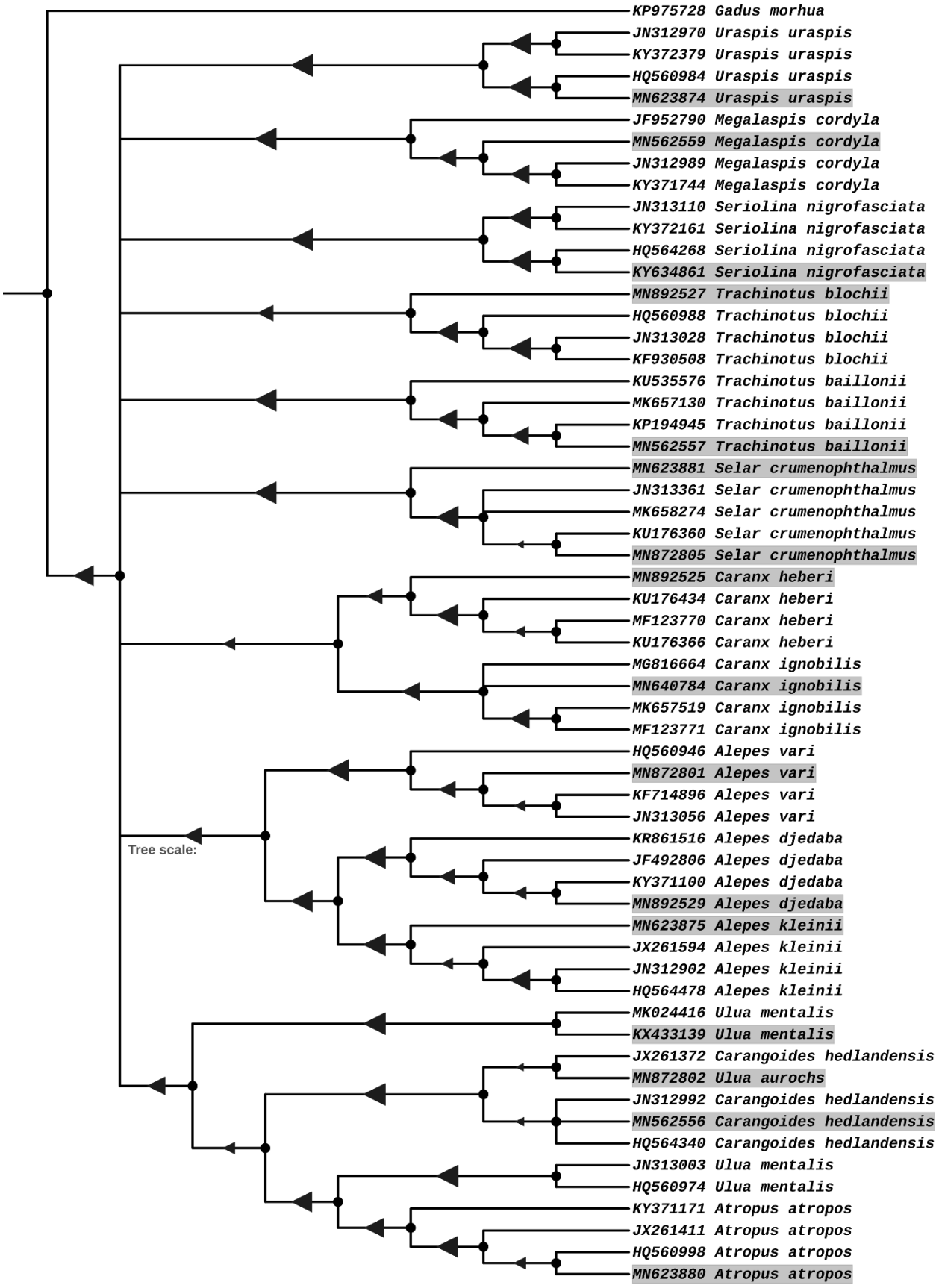

Figure 2. Evolutionary relationships among Carangidae taxa inferred through Neighbor-Joining (NJ) phylogeny based on mtCOI gene-set. The triangle (Blue color) shown next to the branches indicates percentage of replicate trees in which the associated taxa clustered together in the bootstrap test. The highlighted taxa along with their NCBI accession no. were generated in this study

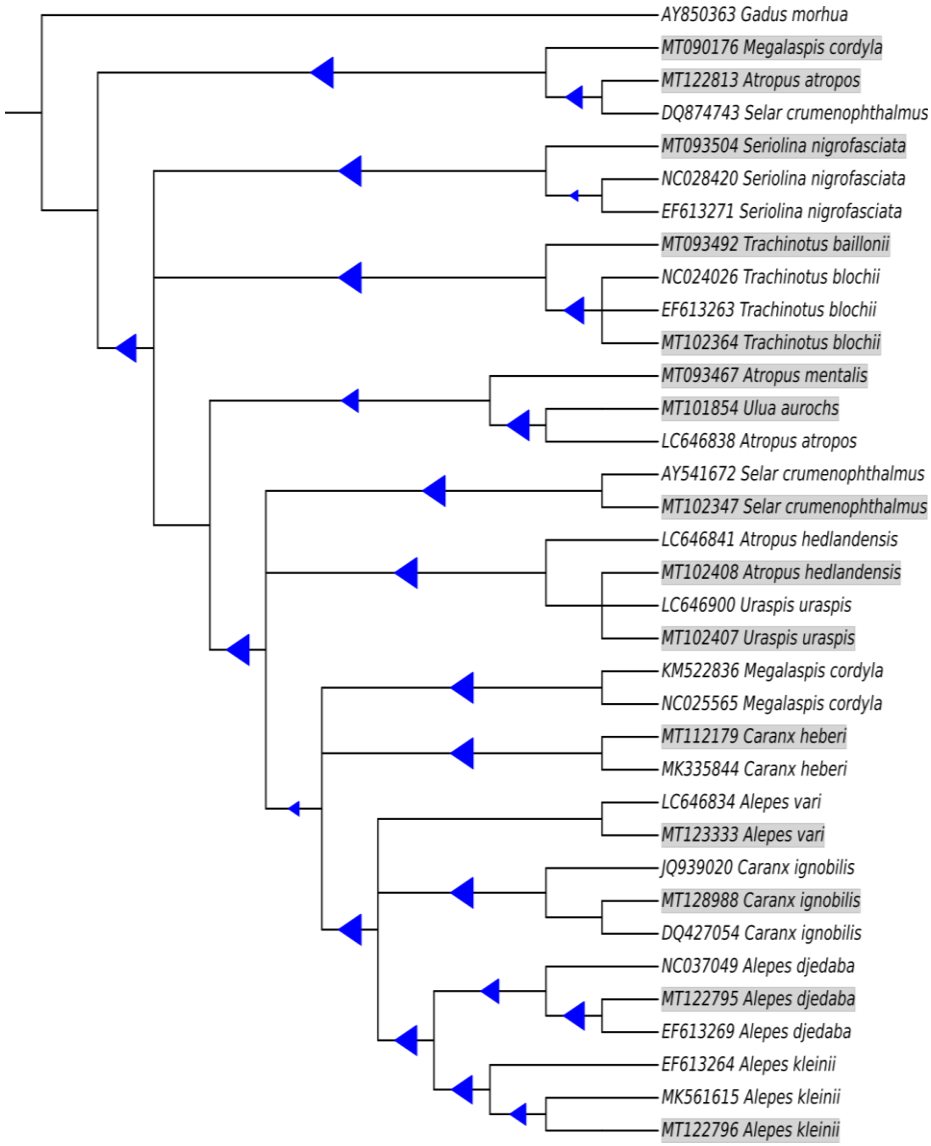

Figure 2A. Evolutionary relationships among Carangidae taxa inferred through Neighbor-Joining (NJ) phylogeny based on 16S rRNA gene-set. The triangle (Blue color) shown next to the branches indicates percentage of replicate trees in which the associated taxa clustered together in the bootstrap test. The highlighted taxa along with their NCBI accession no. were generated in this study

delimited a much more conservative number of all 15 species, with higher significance. All 15 COI haplotypes could be divided into 14 tentative species. The haplotypes for *Carangoides hedlandensis* and *Ulua aurochs* were grouped into the same tentative species. All other haplotypes of morphologically defined carangid species were divided according to their respective species (Table 4). These results are in agreement with our morphologically identified carangid species.

Discussion

The primary objectives of DNA-based taxonomy (DNA barcoding) are to classify unidentified specimens into specific species categories and to improve the identification of new and cryptic species. DNA barcoding also facilitates identification, particularly in microscopic, diverse life history stages, and other organisms with complex or inaccessible morphology (Hebert et al., 2003).

Morphology-based identification within the family Carangidae has been problematic. Our study aimed to explore the relationships among Carangid fishes using an integrative and exemplary approach. Specifically, we integrated discrete morphological traits with genome-scale data from representative taxa to develop a hypothesis about the relationships within Carangid fishes, providing credence to the effectiveness of integrative taxonomy in the identification and diversity studies of Carangids in the Bay of Bengal. In part, our study provides reference DNA barcode data that can be used in subsequent ecological, fisheries, food, forensic and other types of studies.

The monophyly of Carangids is corroborated by 25 morphological characters and DNA sequence data. The

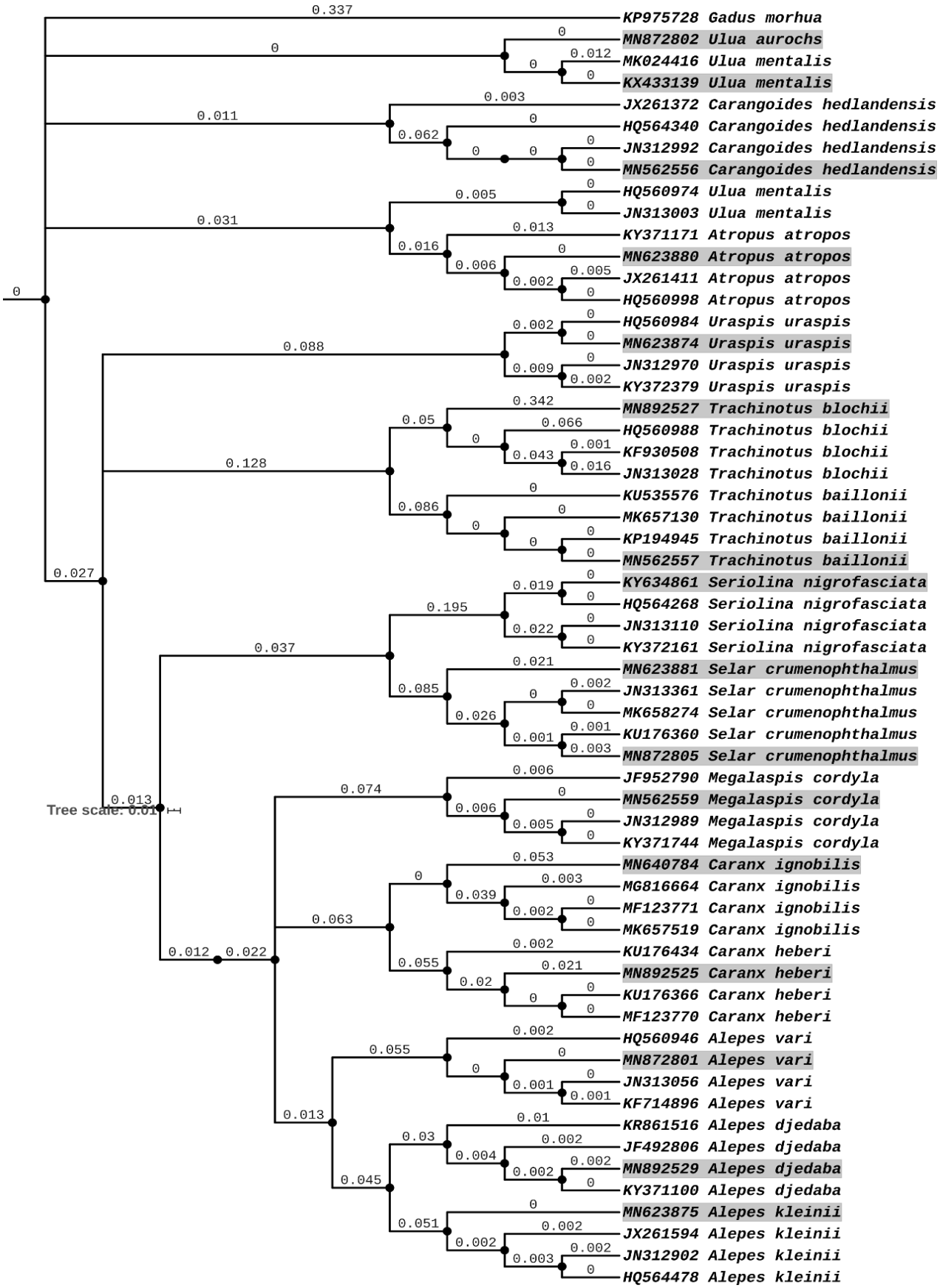

Figure 3. Evolutionary relationships among Carangidae taxa inferred through Maximum Likelihood phylogeny based on mtCOI gene-set. The best nucleotide substitution model predicted by the Model-Test-NG is 'TIM2e+G4'. The figures shown next to the branches indicates posterior probability values in which the associated taxa clustered together in the bootstrap test. The highlighted taxa along with their NCBI accession no. were generated in this study

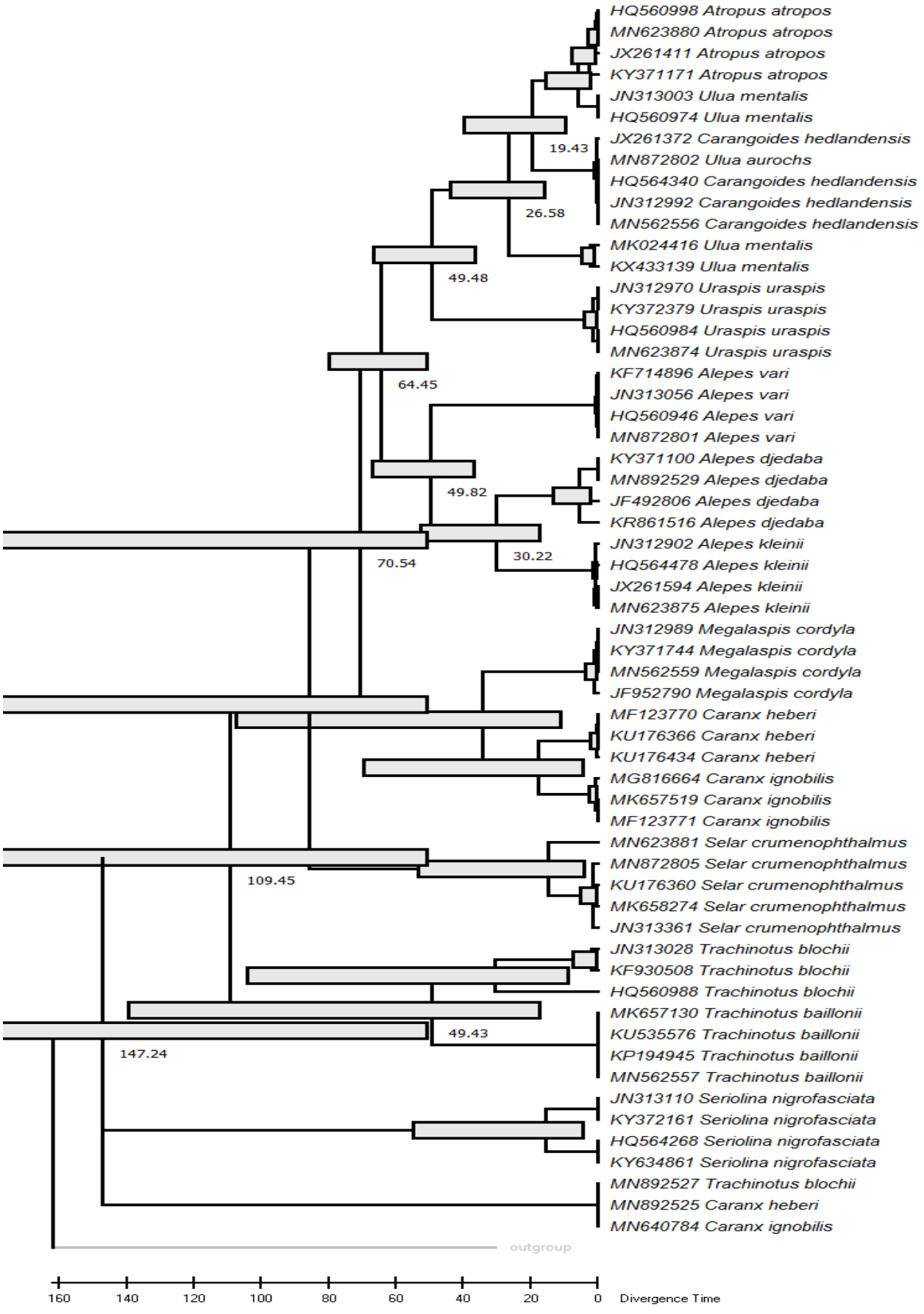

Figure 4. Divergence Time analysis using the RelTime method. The divergence time estimation recovered the stem-group age (i.e., the earliest possible origin) for the carangids as 147.24 million years ago (Mya) (95% confidence intervals)

generated NJ phylogenetic tree of the Carangids highlights that morphological characteristics provide essential prescience and resolve the relationships among the formed DNA-based clades between species. Phylogenetic relationships among species with COI-based NJ analysis were clearly established, and individuals from the same species were grouped in the same taxonomic cluster with 98–100% bootstrap support. Carangidae can be categorized into four tribes based on morphological evidence; the *Carangini*, *Trachinotini*, *Naucratini* and *Scomberoidini* (Smith-Vaniz, 1984) and the resultant phylogenetic tree in accordance with. However, the reconstructed phylogenetic tree based on COI gene (Figure 2) shows a different topology of mixed clade between *Carangoides* and *Ulua*. Generally, classification of both *Carangoides* and *Ulua* were very challenging due to morphological and meristic similarities across species, as well as plasticity in body shape, size and color patterns. Furthermore, both the genus typically undergoes significant changes in morphology and pigmentation during growth, which have likely resulted in specimen misidentification and contributed to overall taxonomic confusion (Nur et al., 2022). Also, taxonomic confusions among these two genus further supported by presence of numerous species synonyms in FishBase citations (Froese & Pauly, 2024). In another aspect, the phylogenetic analysis based on 16S rRNA shows a different topology forming a clade between *Atropus mentalis*, *Ulua aurochs*, and *Atropos atropos* (Figure 2A). Further, *Atropus hedlandensis* and *Uraspis uraspis* forms another clade. The observed different topology could be due to small sample size. Comparison of COI sequences of Carangid species from this study with conspecific sequences available from other geographical regions revealed the existence of several more complexes of potentially cryptic species from outside the Bay of Bengal.

The use of integrative taxonomic approaches has shown to be the best strategy to produce well-supported species delimitations (Carstens et al., 2013; Sheth & Thaker, 2017). In the species delimitation, both ASAP and bPTP identified 15 and 14 OTUs respectively. The remarkable consistency in OTU numbers across ASAP and bPTP analyses implies the robustness of species delimitation through DNA-based taxonomy. Only two species cannot be delimitated from each other by COI sequences, viz., *Carangoides hedlandensis* & *Ulua aurochs*. Both the species possess similar body and coloration pattern that are difficult to differentiate from each other. Incomplete lineage sorting of recent speciation or introgressive hybridization could result in two closely related species sharing haplotypes (Victor, 2015), a common phenomenon found in marine fishes (Allen et al., 2015; Huang et al., 2022). Combining various delimitation methods with the morphology of voucher specimens can effectively enhance the accuracy of identification. After careful comparison, the topologies of COI-based monophyletic genera are found out to be in accordance with those in phylogenetic tree reconstruction, suggesting COI markers may have resolutions at both generic and specie level.

Due to the limitation arising from the use of a single locus in biogeographic studies, our analysis cannot test the hypothesis that environmental changes and geographic isolation are contributors to the diversification of Carangids in the Bay of Bengal. However, our phylogeographic study reveals the presence of two geographically restricted matrilines of Carangids. In concordance with previous studies, this could indicate genetic adaptation of these populations in response to local selective challenges and environmental pressures. Although the paucity of genetic studies on African freshwater fishes hindered comparison, we note that previous studies have exemplified the roles of local adaptation to environmental changes on the population genetic structure of Bay of Bengal fauna. Further studies are therefore needed to investigate the evolutionary mechanisms or processes governing the diversification of Carangids in these regions, as well as to estimate the divergence events that could have accounted for the prolonged period of isolation of the matrilines. Understanding biogeography using molecular data is important for the interpretation of the distribution patterns of geographically distant populations. High haplotype diversity coupled with low nucleotide diversity, as was observed in carangid populations, is a pattern consistent with other catfish species, such as Chinese *Leiocassis longirostris* (Yang et al., 2012) and East African *B. docmak* (Basiita et al., 2017). Our study, therefore, provides evidence that historical biogeographic factors and contemporary environmental variations across sub-Saharan Africa accounted for the population divergence and geographic structuring within Carangids.

Conclusion

In conclusion, our study shows that multilocus mitochondrial DNA is an effective complementary tool to morphology in the identification and diversity study of carangids. Incorporation of newly acquired COI sequences with existing molecular data in global databases allowed investigation of the genetic diversity, population structure and historical demographics of carangids across the Bay of Bengal. The results obtained proved that the DNA barcode approach is effective in identifying genetic clusters as well as revealing hidden mitochondrial diversity. Our study thus gives credence to the effectiveness of integrative taxonomy in the identification and diversity studies of carangids.

Ethical Statement

This study did not require ethical approval as it did not involve human subjects, animal testing, or any procedures that would necessitate such approval

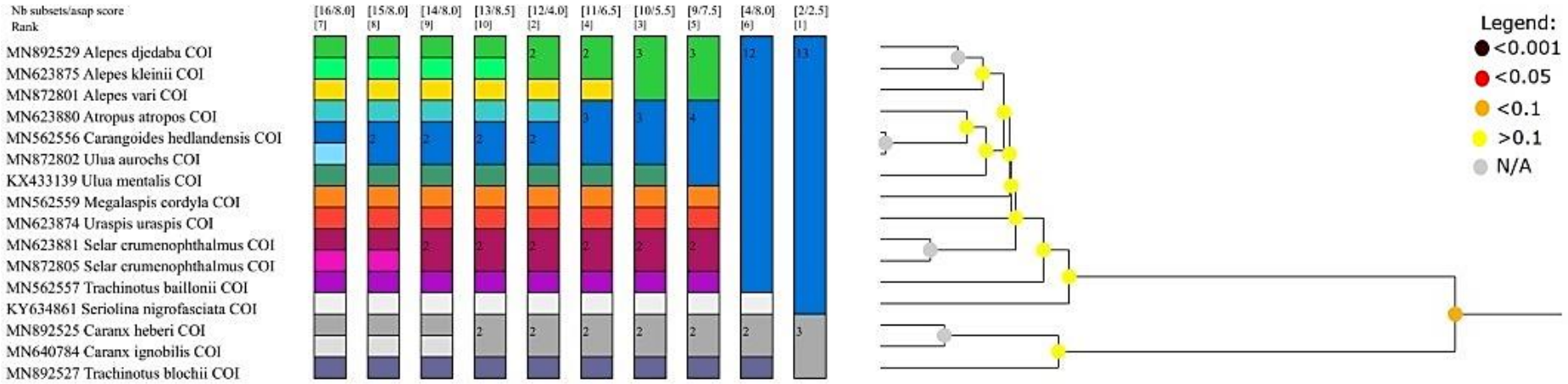

Figure 5. Assemble Species by Automatic Partitioning (ASAP) analyses of Carangidae species based on COI mitochondrial genetic markers. COI: Cytochrome c oxidase subunit 1. Different OTUs are represented by colors on the bar, and the number inside each color bar corresponds to the assigned specimen number for that OTU. The number of subsets, assigned total OTU number, ASAP score (where lower scores indicate better partitions), and the best rank-wise column (1-10) are included. Legend; Darker colors in the figure indicate lower probabilities, while a grey dot signifies that the probability was not computed. When a probability is very low (dark color), it suggests that the groups within the node likely correspond to different species.

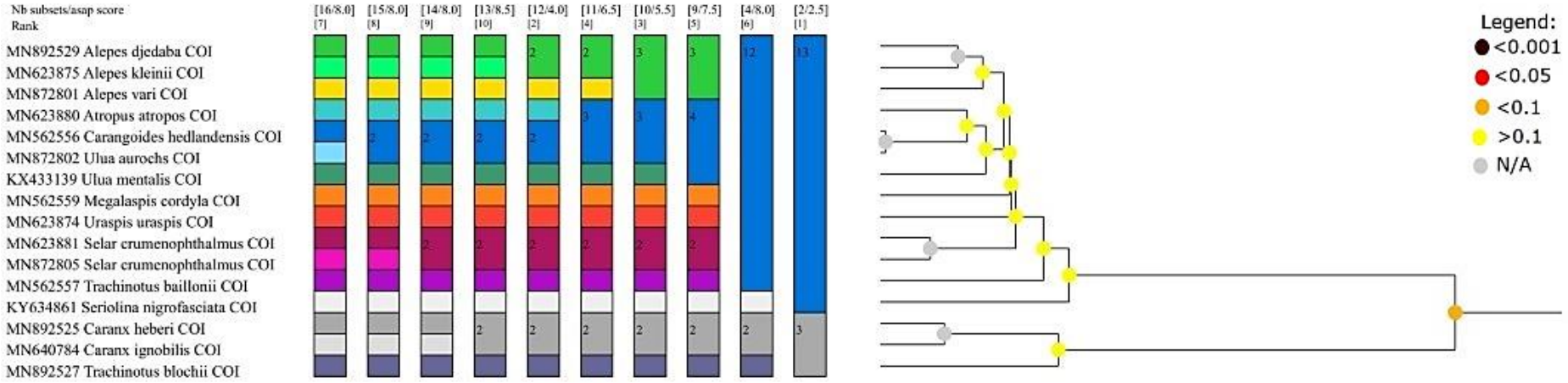

Figure 6. Assemble Species by Automatic Partitioning (ASAP) analyses of Carangidae species based on 16S rDNA mitochondrial genetic markers. Different OTUs are represented by colors on the bar, and the number inside each color bar corresponds to the assigned specimen number for that OTU. The number of subsets, assigned total OTU number, ASAP score (where lower scores indicate better partitions), and the best rank-wise column (1-10) are included. Legend; Darker colors in the figure indicate lower probabilities, while a grey dot signifies that the probability was not computed. When a probability is very low (dark color), it suggests that the groups within the node likely correspond to different species.

Table 4: Detailed result of the bPTP analysis

Marker	bPTP_Speices	Support	Number of haplotypes	Morphospecies
COI	Species 1	0.973	1	*Caranx ignobilis*
	Species 2	0.941	1	*Caranx heberi*
	Species 3	0.910	1	*Trachinotus blochii*
	Species 4	0.877	1	*Megalaspis cordyla*
	Species 5	0.840	1	*Seriolina nigrofasciata*
	Species 6	0.808	1	*Trachinotus baillonii*
	Species 7	0.779	1	*Uraspis uraspis*
	Species 8	0.340	2	*Selar crumenophthalmus*
	Species 9	0.427	1	*Alepes vari*
	Species 10	0.565	1	*Ulua mentalis*
	Species 11	0.225	1	*Alepes djedaba*
	Species 12	0.225	1	*Alepes kleinii*
	Species 13	0.353	1	*Atropus atropos*
	Species 14	0.179	2	*Carangoides hedlandensis* & *Ulua aurochs*

according to institutional and national guidelines. Furthermore, the study was conducted in accordance with environmental regulations and did not disturb or alter the natural populations or ecosystems under investigation.

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Author Contribution

BT: Conceptualization, Identification, Methodology; **SNS:** Data Curation, Methodology, Formal Analysis, and Writing –original draft; **URA:** Supervision, Writing –discussion and final editing.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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