



Phylogenetic And Genetic Diversity Of Some Carangid Species From The Egyptian Red Sea Using Divergent Domain D11 Of 28S rRNA Gene

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ARTICLE INFO

Article History:

Received: Nov. 13, 2020

Accepted: Dec. 30, 2020

Online: Jan. 11, 2021

Keywords:

Carangidae
Phylogeny
Ribosomal genes
28S rRNA Gene
Divergent Domain D11

ABSTRACT

Study of molecular diversity and phylogenetic analysis of eight Carangid species, which are ecologically and economically important species, was carried out using Divergent Domain D11 of 28S rRNA Gene. The size of the consensus sequences of Divergent Domain D11 of 28S rRNA Gene ranged from 490 in *Carangoides bajad*, to 496 in *Carangoides malabaricus*. The average content of A+T was lower than C+G. The average content of C+G slightly changing among the eight fishes, it was 55.3% in *Carangoides bajad*; 55.7% in *Carangoides chrysophrys*; 55.5% in *Carangoides malabaricus*; 55.6% in *Caranx melampygus*; 55.3% in *Caranx sexfasciatus*; 55.6% in *Elagatis bipinnulata*; 55.5% in *Scomberoides lysan* and 55.9% in *Trachinotus ovatus*. Genetic distance among Carangid species ranged from 0.000% to 0.005%. Overall the distance value among all fishes was 0.005%. The phylogenetic relations among our species were inferred by three methods, the Maximum Likelihood, Neighbor Joining and Minimum Evolution. The three different phylogenetic approaches resulted mostly in similar tree topologies and the clades. Results of Divergent Domain D11 of 28S rRNA revealed the species belong to two genera *Carangoides* and *Caranx* were genetically related to each other more than the rest genera; *Elagatis*, *Scomberoides* and *Trachinotus*. This investigation could be utilized for genetic relationship and association analyses in the taxonomy studies of Carangid fishes.

INTRODUCTION

The Carangid fishes are considered among the very economically significant coastal pelagic fishes of the world, they are distributed to a large degree in all tropical and subtropical seas (Lin and Shao, 1999). Family Carangidae is named for the genus *Caranx* and comprises a varied group of fishes known by several common names like jacks, pilotfish, trevallies, pompanos, amberjacks, kingfish, scads, rainbow runners (Honebrink, 2000). These fishes have an elongated, slightly compressed and deep body with separate dorsal fins, two anterior spines on the anal fin, a narrow caudal peduncle, and a forked caudal fin (Chan et al., 1974; Honebrink, 2000). Significant changes in morphology and pigmentation occur during growth in carangids (Bohlke and chaplin,

1993) these changes have likely lead to misidentification of samples, and contributed to general taxonomic bewilderment (Jaafar *et al.*, 2012). Oftenly, morphological identification has some difficulties, because of inter- and intraspecific variation, while molecular markers can reliably, accurately and rapidly identify species as well as variants and cryptic taxa (Holland *et al.*, 2004; Le Roux and Wieczorek, 2009; Garcia-Morales and Elias-Gutierrez, 2013).

DNA barcoding analyses on aquatic biota have been executed and are actually receiving a lot of interest, especially efforts to increase the database on GenBank (Andriyono and Suciyono, 2020). Compared to the morphological identification method, DNA barcoding has a reliability that is close to 100% matched (Meyer and Paulay, 2005).

Ribosomal genes and its associated spacers are considered among the most versatile sequences for phylogenetic examinations (HersHKovitz and Lewis 1996; Coleman, 2000 and 2003; Coleman and Vacquier, 2002; Álvarez and Wendel, 2003; Müller *et al.*, 2007; Wickramasinghe *et al.*, 2009; Yan *et al.*, 2013). Verma and Serajuddin (2012) reported that, studies based on ribosomal RNA genes have been used in many animals and plants to examine, the evolutionary linkages as well as the description of genome structure. Uses of large (28S rRNA) and small (18S rRNA) subunit ribosomal DNA produced abundant gravid resolution among the Metazoa (Medina *et al.*, 2001). The 28S ribosomal RNA is considered one of the main components of all eukaryotic cells as well as it is the structural ribosomal RNA for the large subunit (LSU) of eukaryotic ribosome (Lodish and Darnell, 1995; Awasthi *et al.*, 2016). The large subunit ribosomal DNA (LSU) or 28S rRNA is a mosaic of many variable and conservative fragments, and are widely used as a phylogenetic marker (Shylla *et al.*, 2013). In eukaryotes, the 28S rRNA region contains 12 divergent domains or expansion segments, which vary extremely in nucleotide composition and length among species (Hassouna *et al.* 1984; De Rijk *et al.*, 1995). Divergent domains are used in a large degree to study the relationships of species by using a phylogenetic analysis method in diverse organisms (Vidigal *et al.*, 2000; Vidigal *et al.*, 2004; He *et al.*, 2005). DNA barcoding is a faster and more accessible method for species identification (Hebert *et al.*, 2003; Hebert *et al.*, 2010). Templonuevo *et al.* (2018) revealed the usefulness of DNA barcoding as an efficient and accurate tool for the identification of several species of Carangidae and Lutjanidae families.

In view of this, our study was carried out to estimate the degree of genetic divergence and puzzle out phylogenetic relationship among some species of Carangid fish using Divergent Domain D11 of 28S rRNA Gene.

MATERIALS AND METHODS

Samples collection:

Fish samples of family Carangidae (*Carangoides bajad*, *Carangoides chrysophrys*, *Carangoides malabaricus*, *Caranx melampygus*, *Caranx sexfasciatus*, *Elagatis bipinnulata*, *Scomberoides lysan* and *Trachinotus ovatus*) were collected from the Egyptian Red Sea, Hurghada. Then were identified morphologically according to (Randall, 1982; Lin and Shao, 1999; Joshi *et al.*, 2011). The muscles tissues were isolated and preserved at -80°C until used.

DNA Extraction and PCR amplification:

The Total genomic DNA was extracted from the preserved muscles tissues using the DNA extraction method of QIAamp DNA Mini kit (Qiagen, Hidden, Germany) by following the manufacturer's guidelines. The used primers to amplify the divergent domain D11 28S rDNA in Carangid were according to (Zardoya and Meyer, 1996; Verma *et al.*, 2011; Awasthi *et al.*, 2016). The PCR reactions comprised of 10 pmoles of each forward and reverse primers, 25 µL PCR master mix and 50-90 ng of genomic DNA and in a final reaction volume of 50 µL. The PCR conditions were performed as the following; an initial denaturation at 94 °C for 4 minutes, followed by 30 cycles of denaturation at 94 °C for 60s, annealing at 50°C for 60s and an extension at 72 °C for 60s with post cycling extension at 72°C for 7 min. The Amplification products were electrophoresed in 1.5% agarose gel stained with ethidium bromide.

The Sequencing of PCR Products and phylogenetic tree construction:

All DNA sequencing was carried out by Macrogen (Seoul, South Korea), using the same primer used in amplification. The sequences were submitted to the National Center for Biotechnology Information (GenBank/NCBI) for obtaining accession numbers MW139283 - MW139290 (Table 1). Sequence alignment was performed using MUSCLE (Edgar, 2004) with default settings. Some segments of the long extension of the out-group were discarded because they did not align with our samples. The divergent domains D11 of 28S tree was rooted with out-group sequences from *Cottus bairdii* (GenBank accession number AY539122.1), *Cottus carolinae* (GenBank accession number AY539123.1) and *Cottus poecilopus* (GenBank accession number AY539124.1) (Table 1).

Table 1: The understudying Carangid fishes with outgroup from the GenBank/ NCBI based on divergent domains D11 of 28S sequence.

No.	Species	Accession number
1	<i>Carangoides bajad</i>	MW139283
2	<i>Carangoides chrysophrys</i>	MW139284
3	<i>Carangoides malabaricus</i>	MW139285
4	<i>Caranx melampygus</i>	MW139286
5	<i>Caranx sexfasciatus</i>	MW139287
6	<i>Elagatis bipinnulata</i>	MW139288
7	<i>Scomberoides lysan</i>	MW139289
8	<i>Trachinotus ovatus</i>	MW139290
Out group	<i>Cottus bairdii</i>	AY539122.1
	<i>Cottus carolinae</i>	AY539123.1
	<i>Cottus poecilopus</i>	AY539124.1

Phylogenetic trees analyses were performed with MEGA version 7.0 18 (**Kumar et al., 2016**) using Maximum likelihood (ML), Neighbour Joining (NJ) and Minimum Evolution (ME) methods of trees construction and using 1000 bootstrap iterations (**Felsenstein, 1985**). Sequence divergences were calculated using Kimura 2-parameter distances (**Kimura, 1980**) to provide a graphical representation of divergence between species.

RESULTS AND DISCUSSION

According to **Torres and Santos (2019)** the identification of species is considered an integral step in surveillance biodiversity. Carangidae is among the very economically important coastal pelagic fishes of the world and this family is considered one of the bonefish families with about 148 species belonging to 32 genera (**Nelson, 2006**). **Damerou et al. (2018)** declared that, the phylogenetic relationships of family Carangidae still remained uncertain, in this respect, studies of molecular analysis are beneficial to illustrate the status and phylogenetic linkages of problematic taxa.

Zordoya and Meyer (1996) used 28S rRNA genes to analyze the genetic linkages of many animals likes; lungfishes, coelacanth, rainbow trout, eel, sturgeon and tetrapods and reported that, the divergent domain of 28S rRNA genes are beneficial in phylogenetic studies (**Awasthi et al., 2016**).

The sequencing of divergent domains D11 of 28S in eight fishes from family Carangidae (*Carangoides bajad*, *Carangoides chrysophrys*, *Carangoides malabaricus*, *Caranx melampygus*, *Caranx sexfasciatus*, *Elagatis bipinnulata*, *Scomberoides lysan* and *Trachinotus ovatus*) produced nucleotide length ranging from 490 bp to 496 bp. The nucleotide sequences were submitted to the GenBank under accession numbers (MW139283 - MW139290) (Table 1).

The results indicate *Carangoides malabaricus* has the longest (496 bp.) nucleotide sequences of divergent domain D11 of 28S, while *Carangoides bajad* has the shortest nucleotide sequences (490 bp.). The average nucleotide frequencies of adenine (A),

thymine (T), cytosine (C) and guanine (G) were 21.3, 23.2, 23.9 and 31.6% respectively. More details about nucleotide frequencies, A+T contents, C+G contents and their averages were given in (Table 2). The average content of C+G ranged from 55.3 to 55.9%, which was higher than the A+T in all species. This was concurred with (Zardoya and Meyer, 1996; Verma *et al.*, 2011; Awasthi *et al.*, 2016) who proclaim high G+C content of Divergent domains 11 of 28S rRNA in many fishes.

Table 2: Nucleotide frequencies, A+T contents, C+G contents and their averages of divergent domains D11 of 28S sequence in 8 Carangid fishes.

No.	Species	Base pair length	Nucleotide Number %				A+T Content (%)	C+G Content (%)
			A%	T%	C %	G%		
1	<i>Carangoides bajad</i>	490	21.4	23.3	23.7	31.6	44.7	55.3
2	<i>Carangoides chrysophrys</i>	492	21.1	23.2	24.0	31.7	44.3	55.7
3	<i>Carangoides malabaricus</i>	496	21.1	23.4	23.6	31.9	44.5	55.5
4	<i>Caranx melampygus</i>	491	21.2	23.2	23.8	31.8	44.4	55.6
5	<i>Caranx sexfasciatus</i>	495	21.3	23.4	23.8	31.5	44.7	55.3
6	<i>Elagatis bipinnulata</i>	491	21.4	23.0	24.0	31.6	44.4	55.6
7	<i>Scomberoides lysan</i>	494	21.2	23.3	23.7	31.8	44.5	55.5
8	<i>Trachinotus ovatus</i>	495	21.5	22.6	24.4	31.5	44.1	55.9
	Average %	493	21.3	23.2	23.9	31.6	44.45	55.55

The final alignments consisted of 499 bp. Out of them 486, 9 and 4 were conserved sites, variable sites and Parsimony informative sites respectively (Fig. 1). The high conserved sites of divergent domains D11 of 28S in the under studied fishes was in agreement with (Jansen *et al.*, 2006) who reported that, within a genome, the genes coding for 18S, 5.8S and 28S rRNA are highly conserved due to concerted evolution of intra- and inter-chromosomal loci.

Pairwise genetic distances among 8 Carangid fishes and the out group, were estimated by MEGA version 7 (Kumar *et al.*, 2016) using the K²P method with gamma correction. The P-distances among all fish species ranged from 0.000 to 0.005%. Overall the distance value among all fish species was 0.005% (Table 3).

Table 3: Pairwise distances divergent domains D11 of 28S sequence using Kimura 2- parameter among 8 Carangid fishes additional to the outgroup.

Species	1	2	3	4	5	6	7	8	9	10	11
1 <i>Carangoides bajad</i>		0.002	0.003	0.002	0.002	0.004	0.003	0.002	0.004	0.004	0.005
2 <i>Carangoides chrysophrys</i>	0.002		0.002	0.000	0.000	0.003	0.002	0.003	0.004	0.004	0.004
3 <i>Carangoides malabaricus</i>	0.005	0.002		0.002	0.002	0.004	0.003	0.004	0.005	0.005	0.005
4 <i>Caranx melampygus</i>	0.002	0.000	0.002		0.000	0.003	0.002	0.003	0.004	0.004	0.004
5 <i>Caranx sexfasciatus</i>	0.002	0.000	0.002	0.000		0.003	0.002	0.003	0.004	0.004	0.004
6 <i>Elagatis bipinnulata</i>	0.007	0.005	0.007	0.005	0.005		0.002	0.003	0.003	0.003	0.004
7 <i>Scomberoides lysan</i>	0.005	0.002	0.005	0.002	0.002	0.002		0.004	0.003	0.003	0.004
8 <i>Trachinotus ovatus</i>	0.002	0.005	0.007	0.005	0.005	0.005	0.007		0.004	0.004	0.005
9 <i>Cottus bairdii</i>	0.009	0.007	0.009	0.007	0.007	0.005	0.005	0.009		0.000	0.002
10 <i>Cottus carolinae</i>	0.009	0.007	0.009	0.007	0.007	0.005	0.005	0.009	0.000		0.002
11 <i>Cottus poecilopus</i>	0.012	0.009	0.012	0.009	0.009	0.007	0.007	0.012	0.002	0.002	

MW139283	1	Carangoides bajad	- - - - - C	CGTGA	- GC	AGGA	GGCG	AGCC	CCG	AGCC	GGCT	CTC	CGCT	TCT	GGCG	CTCAA	GC	GC	[80]
MW139284	1	Carangoides chrysophrys	- - - - - C																[80]
MW139285	1	Carangoides malabaricus	T T A	G C C G															[80]
MW139286	1	Caranx melampygus	- - - - - C																[80]
MW139287	1	Caranx sexfasciatus	- T T A C C																[80]
MW139288	1	Elagatis bipinnulata	- - - - A C															T	[80]
MW139289	1	Scomberoides lysan	- - - A C T															T	[80]
MW139290	1	Trachinotus ovatus	- - - A C C																[80]
MW139283	1	Carangoides bajad	C C G G C T C	- T	G C C G G G C G T G	A C C C G C T C C G G G G A C A G T G G C A G G T G G G G A G T T T S A C T G G	[120]												
MW139284	1	Carangoides chrysophrys	- -																[120]
MW139285	1	Carangoides malabaricus	- -																[120]
MW139286	1	Caranx melampygus	- -																[120]
MW139287	1	Caranx sexfasciatus	- -																[120]
MW139288	1	Elagatis bipinnulata	C - - C																[120]
MW139289	1	Scomberoides lysan	C - -																[120]
MW139290	1	Trachinotus ovatus	C C G C																[120]
MW139283	1	Carangoides bajad	G G C G G T A C A C C T G T C A A A C G	G T A A C G C A G G T G T C C T A A G G C G A G C T C A G G G A G G A C A G A A	[180]														
MW139284	1	Carangoides chrysophrys	- -																[180]
MW139285	1	Carangoides malabaricus	- -																[180]
MW139286	1	Caranx melampygus	- -																[180]
MW139287	1	Caranx sexfasciatus	- -																[180]
MW139288	1	Elagatis bipinnulata	- -																[180]
MW139289	1	Scomberoides lysan	- -																[180]
MW139290	1	Trachinotus ovatus	- -																[180]
MW139283	1	Carangoides bajad	A C C T C C C G T G G A G C A G A A G G	G C A A A G C T C G C T T G A T C T T G A T T T T C A G T A T G A A T A C A G	[240]														
MW139284	1	Carangoides chrysophrys	- -																[240]
MW139285	1	Carangoides malabaricus	- -																[240]
MW139286	1	Caranx melampygus	- -																[240]
MW139287	1	Caranx sexfasciatus	- -																[240]
MW139288	1	Elagatis bipinnulata	- -																[240]
MW139289	1	Scomberoides lysan	- -																[240]
MW139290	1	Trachinotus ovatus	- -																[240]
MW139283	1	Carangoides bajad	A C C G T G A A A G C G G G C C T C A	C G A T C C T T C T G A C T T T T T G G G T T T T A A G C A G G A G G T G T C A	[300]														
MW139284	1	Carangoides chrysophrys	- -																[300]
MW139285	1	Carangoides malabaricus	- -																[300]
MW139286	1	Caranx melampygus	- -																[300]
MW139287	1	Caranx sexfasciatus	- -																[300]
MW139288	1	Elagatis bipinnulata	- -																[300]
MW139289	1	Scomberoides lysan	- -																[300]
MW139290	1	Trachinotus ovatus	- -																[300]
MW139283	1	Carangoides bajad	G A A A A G T T A C C A C A G G G A T A	A C T G G C T T G T G G C G G C C A A G C G T T C A T A G C G A C G T C G C T T	[360]														
MW139284	1	Carangoides chrysophrys	- -																[360]
MW139285	1	Carangoides malabaricus	- -																[360]
MW139286	1	Caranx melampygus	- -																[360]
MW139287	1	Caranx sexfasciatus	- -																[360]
MW139288	1	Elagatis bipinnulata	- -																[360]
MW139289	1	Scomberoides lysan	- -																[360]
MW139290	1	Trachinotus ovatus	- -																[360]
MW139283	1	Carangoides bajad	T T T G A T C C T T C G A T G T C G G C	T C T T C C T A T C A T T G T G A A G C A G A A T T C A C C A A G C G T T G G A	[420]														
MW139284	1	Carangoides chrysophrys	- -																[420]
MW139285	1	Carangoides malabaricus	- -																[420]
MW139286	1	Caranx melampygus	- -																[420]
MW139287	1	Caranx sexfasciatus	- -																[420]
MW139288	1	Elagatis bipinnulata	- -																[420]
MW139289	1	Scomberoides lysan	- -																[420]
MW139290	1	Trachinotus ovatus	- -																[420]
MW139283	1	Carangoides bajad	T T G T T C A C C C A C T A A T A G G G	A A C G T G A G C T G G G T T T A G A C C G T C G T G A G A C A G G T T A G T T	[480]														
MW139284	1	Carangoides chrysophrys	- -																[480]
MW139285	1	Carangoides malabaricus	- -																[480]
MW139286	1	Caranx melampygus	- -																[480]
MW139287	1	Caranx sexfasciatus	- -																[480]
MW139288	1	Elagatis bipinnulata	- -																[480]
MW139289	1	Scomberoides lysan	- -																[480]
MW139290	1	Trachinotus ovatus	- -																[480]
MW139283	1	Carangoides bajad	T T A C C C T A C T G A T G A T G T G	[499]															
MW139284	1	Carangoides chrysophrys	- -																[499]
MW139285	1	Carangoides malabaricus	- -																[499]
MW139286	1	Caranx melampygus	- -																[499]
MW139287	1	Caranx sexfasciatus	- -																[499]
MW139288	1	Elagatis bipinnulata	- -																[499]
MW139289	1	Scomberoides lysan	- -																[499]
MW139290	1	Trachinotus ovatus	- -																[499]

Fig. 1. Alignment of partial sequences of distances divergent domains D11 of 28S gene among 8 Carangid fishes. Dots indicate identical nucleotides and A,T,C and G indicate the difference nucleotides

Among Carangid fishes the highest P-distance (0.004) was found between *Carangoides bajad* and *Elagatis bipinnulata*, also between *Carangoides malabaricus* and both *Elagatis bipinnulata* and *Trachinotus ovatus* and likewise between *Scomberoides lysan* and *Trachinotus ovatus*. While, the lowest value (0.000) was found between *Carangoides chrysophrys* and both *Caranx melapygus* and *Caranx sexfasciatus*. Also was found between, *Caranx melapygus* and *Caranx sexfasciatus*.

Jacobina et al. (2014) worked on three species of family Carangidae; *Carangoides bartholomaei*, *Caranx latus* and *Caranx lugubris* and reported that, the phylogenetic linkage between *Caranx* and *Carangoides* is corroborate by the common karyotypes among *Carangoides bartholomaei* and *Caranx latus*, as well as the presence of 18S rDNA sites, which appear at equilocal positions on the short arm of the first chromosome pair in three species. Our results of divergent domains D11 of 28S sequencing revealed low genetic distance values among, species of both genera *Carangoides* and *Caranx*. This indicated that, both genera *Carangoides* and *Caranx* were distantly related to each other more than the rest genera; *Elagatis*, *Scomberoides* and *Trachinotus*. This was according to **(Kaleshkumar et al., 2015)** who said that, closely related species have the lowest genetic distance, while the highest genetic distance refers to highly diverged case.

Taxonomic analysis of genus *Caranx* has shown that some species with a wide geographic distribution and cryptic taxonomic features constitute species complex **(Smith-Vaniz and Carpenter, 2007)**. Three phylogenetic methods implicated; Maximum likelihood (ML), Neighbor Joining (NJ) and Minimum Evolution (ME), to confirm the phylogenetic relations among eight carangid fishes by using the sequences of divergent domains D11 of 28S (Figs. 2-4). The three methods indicated nearly the same results and assured that, species under both genera *Carangoides* and *Caranx* were closely related to each others. This was in agreement with **(Smith-Vaniz, 1984; Reed et al., 2002)** who reported that, *Caranx* displays morphological similarities that make it difficult to differentiate from other *Carangoides* members. These similarities led many authors to classify these species into the same genus. Likewise, **Thu et al. (2019)** referred to complicated position of the phylogenetic linkage of the genera in the subfamily Caranginae.

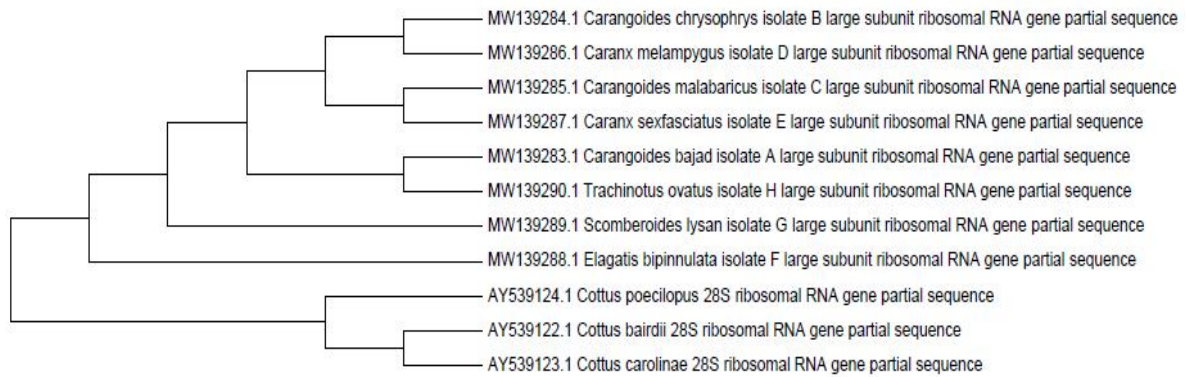


Fig.2. Phylogenetic tree using the Maximum likelihood method among understudying fishes based on divergent domains D11 of 28S sequences.

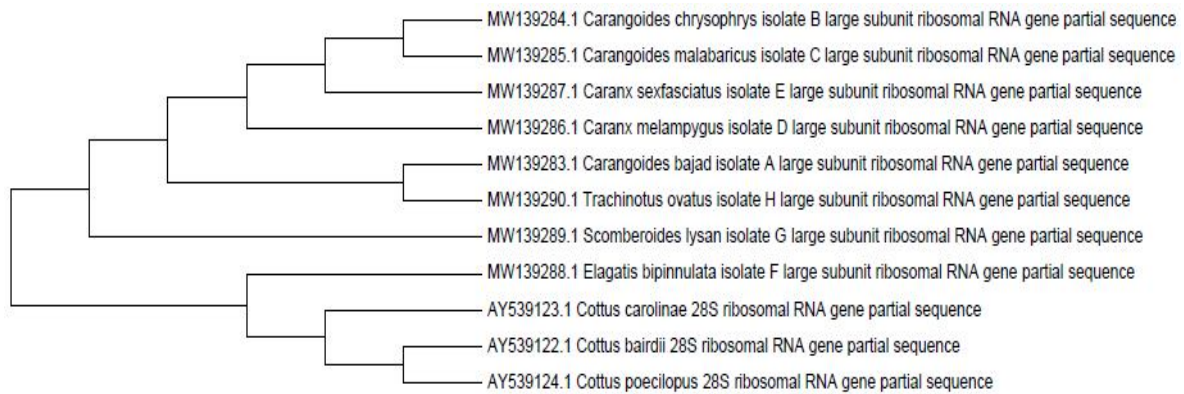


Fig.3. Phylogenetic tree using the Neighbor Joining method among understudying fishes based on divergent domains D11 of 28S sequences.

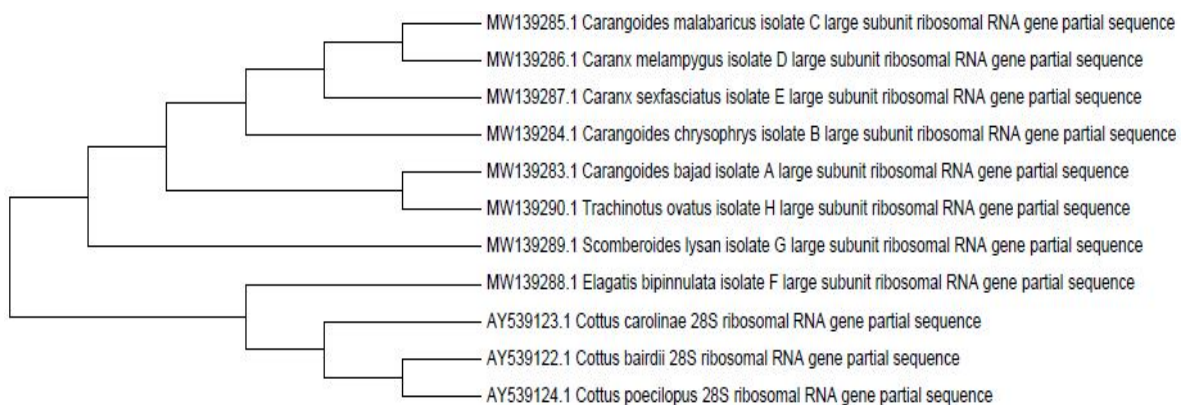


Fig.4. Phylogenetic tree using Minimum Evolution (ME) method among understudying fishes based on divergent domains D11 of 28S sequences.

The study of **Verma and Serajuddin (2012)** affirms that the ribosomal genes possess the general proclivity of variability between the species as well as the preservedness in the same family. They also provided that 28S rRNA gene was found to be shorter in fishes as compared to mammals. The 28S rRNA gene is formed by many highly conserved cores interrupted by divergent domains evolve rapidly with substitution rates which are at least two orders of magnitude higher than those of core regions that creating possibility for variations in these fast evolving divergent domains (**Olsen and Woese, 1993**). These evolving domains are considered to be better to analysis the phylogenetic linkages between closely related species (**Awasthi et al., 2016**). The variations in 28S rRNA gene are because of some unique sites embedded within the largely conserved secondary structure of the genes (**Verma and Serajuddin, 2012**).

Several studies using Divergent domains of 28S rRNA gene were occurred in fishes, mouse and humans and revealed that, 28S rRNA gene was found to be shorter in fishes as compared to mammals (**Hassouna et al., 1984; Awasthi et al., 2016**). Recently, many studies were carried out to investigate the phylogenetic relationships among species and genera of family Carangidae using different molecular markers like (**Damerou et al., 2018; Templonuevo et al., 2018; Thu et al., 2019; Torres and Santos, 2019; Li et al., 2020**).

CONCLUSION

The classification analysis of family Carangidae is controversial. Our study was performed to examine the phylogenetic relationships among eight Carangid species using Divergent Domain D11 of 28S rRNA Gene. The results of Divergent Domain D11 of 28S rRNA illustrated closely genetic of species under two genera *Carangoides* and *Caranx*, more than the rest genera; *Elagatis*, *Scomberoides* and *Trachinotus*. The data reported here may be employed in study and analysis the phylogenetic variety and relationships among species and genera of family Carangidae.

REFERENCES

- Álvarez, I. and Wendel, J. F.** (2003). Ribosomal ITS sequences and plant phylogenetic inference. *Mol. Phylogenet. Evol.*, 29(3): 417-434.
- Andriyono, S. and Suciyo S.** (2020). Molecular Identification and Phylogenetic Tree Reconstruction of Marine Fish from the Essential Wetland Area of Banyuwangi, Indonesia. *E.J.A.B.F.*, 24(2): 427-439.
- Awasthi, M.; Kashyap, A. and Serajuddin, M.** (2016). Molecular Phylogeny of Four Gouramis Based on Divergent Domain D11 of 28S rRNA Gene. *Advan. Biol. Res.*, 10(6): 351-353.

- Bohlke, J. E. and Chaplin, C. C. G.** (1993). Fishes of the Bahamas and adjacent tropical water. In: Honebrink RR (2002) A review of the biology of the family carangidae, with emphasis on species found Hawaiian waters. DAR Technical Report 20-01.
- Chan, W.; Talbot, F. and Sukhavaisidh, P.** (1974). The Carangidae. In W. Fischer & P. J. P. Whitehead (Eds.), F.A.O. Species Identification Sheets for Fishery Purposes in Eastern Indian Ocean (Fishing Area 57) and Western Central Pacific (Fishing Area 71), vol 1.
- Coleman, A. W.** (2000). The significance of a coincidence between evolutionary landmarks found in mating affinity and a DNA sequence. *Protist.*, 151(1): 1-9.
- Coleman, A. W.** (2003). ITS2 is a double-edged tool for eukaryote evolutionary comparisons. *Trends. Genet.*, 19(7): 370-375.
- Coleman, A. W. and Vacquier, V. D.** (2002). Exploring the phylogenetic utility of ITS sequences for animals: a test case for Abalone (*Haliotis*). *J. Mol. Evol.*, 54(2): 246-257.
- Damerau, M.; Freese, M. and Hanel, R.** (2018). Multi-gene phylogeny of jacks and pompanos (Carangidae), including placement of monotypic vadigo *Campogramma glaycos*. *J. Fish. Biol.*, 92(1): 190-202.
- De Rijk, P.; Van de Peer, Y.; Van den Broeck, I. and DeWachter, R.** (1995). Evolution according to large ribosomal subunit RNA. *J. Mol. Evol.*, 41(3): 366-375.
- Edgar, R. C.** (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.*, 32(5): 1792-1797.
- Felsenstein, J.** (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39(4): 783-791.
- Garcia-Morales, A. E. and Elias-Gutierrez, M.** (2013). DNA barcoding freshwater Rotifera of Mexico: evidence of cryptic speciation in common rotifers. *Mol. Ecol. Resour.*, 13(6): 1097-1107.
- Hassouna, N.; Michot, B. and Bachellerie, J. P.** (1984). The complete nucleotide sequence of mouse 28S rRNA gene: implications for the process of size increase of the large subunit rRNA in higher eukaryotes. *Nucleic Acids Res.*, 12(8): 3563-3583.
- He, M.; Huang, L.; Shi, J. and Jiang, Y.** (2005). Variability of ribosomal DNA ITS-2 and its utility in detecting genetic relatedness of Pearl Oyster. *Mar. Biotechnol.*, 7(1): 40-45.
- Hebert, P. D. N.; Cywinska, A.; Ball, S. L. and de Waard, J. R.** (2003). Biological identifications through DNA barcodes. *Proc. Biol. Sci.*, 270(1512): 313-321.
- Hebert, P. D. N.; deWaard, J. R. and Landry, J. F.** (2010). DNA barcodes for 1/1000 of the animal kingdom. *Biol. Lett.*, 6(3): 359-362.
- Hershkovitz, M. A. and Lewis, L. A.** (1996). Deep level diagnostic value of the rDNA-ITS region: the case of an algal interloper. *Mol. Biol. Evol.*, 13(9): 167-177.

- Holland, B. S.; Dawson, M. N.; Crow, G. L. and Hofman, D. K.** (2004). Global phylogeography of *Cassiopea* (Scyphozoa: Rhizostomeae): molecular evidence for cryptic species and multiple invasions of the Hawaiian Islands. *Mar. Biol.*, 145: 1119-1128.
- Honebrink, R. R.** (2000). A Review of the Family Carangidae, With Emphasis on Species Found in Hawaiian Waters. DAR Technical Report (Honolulu: Department of Land and Natural Resources). 20-01: 1-43.
- Jaafar, T. N. A. M.; Taylor, M. I.; Nor, S. A. M.; de Bruyn, M. and Carvalho, G. R.** (2012). DNA Barcoding Reveals Cryptic Diversity within commercially Exploited Indo-Malay Carangidae (Teleostei: Perciformes). *PLoS One*, 7(11): e49623.
- Jacobina, U.P.; Bertollo, L. A. C.; Cioffi, M. B. and Molina W. F.** (2014). Physical mapping of 18S and 5S genes in pelagic species of the genera *Caranx* and *Carangoides* (Carangidae). *Genet. Mol. Res.*, 13(4): 9628-9635.
- Jansen, G.; Devaere, S.; Weekers, P. H. H. and Adriaens, D.** (2006). Phylogenetic relationship and divergence time estimate of African anguilliform catfish (Siluriformes: Clariidae) inferred from ribosomal genes and spacer sequences. *Mol. Phylogenet. Evol.*, 38(1): 65-78.
- Joshi, K. K.; Nair, R. J.; Abdussamad, E. M.; Thomas, S.; Kakati, V. S.; Jasmine, S.; Varghese, M.; Sreeram, M. P.; Sukumaran, S.; George, R. M. and Manisseri, M. K.** (2011). The Carangids of India- A Monograph. Central Marine Fisheries Research Institute. *Fish and Fisheries*, 16(3): 543-546.
- Kaleshkumar, K.; Rajaram, R.; Vinothkumar, S.; Ramalingam, V. and Meetei, K. B.** (2015). DNA barcoding of selected species of pufferfishes (Order: Tetraodontiformes) of Puducherry coastal waters along south-east coast of India. *Indian J. Fish.*, 62(2): 98-103.
- Kimura, M.** (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.*, 16(2): 111-120.
- Kumar, S.; Stecher, G. and Tamura, K.** (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.*, 33(7): 1870-1874.
- Le Roux, J. and Wiczoek, A. M.** (2009). Molecular systematics and population genetics of biological invasions: towards a better understanding of invasive species management. *Ann. Appl. Biol.*, 154(1): 1-17.
- Li, Z.; Li, M.; Xu, S.; Liu, L.; Chen, Z. and Zou, K.** (2020). Complete Mitogenomes of Three Carangidae (Perciformes) Fishes: Genome Description and Phylogenetic Considerations. *Int. J. Mol. Sci.*, 21(13): 4685.
- Lin, P. L. and Shao, K. T.** (1999). A Review of the Carangid fishes (Family Carangidae) from Taiwan with Description of Four New Records. *Zool. Stud.*, 38(1): 33-68.

- Lodish, H. F. and Darnell, J. E.** (1995). Molecular cell biology. Scientific American Books 3rd ed.
- Meyer, C. P. and Paulay, G.** (2005). DNA barcoding: error rates based on comprehensive sampling. PLoS boil., 3(12): e422.
- Medina, M.; Collins, A. G.; Silberman, J. D.; Sogin, M. L.** (2001). Evaluating hypotheses of basal animal phylogeny using complete sequences of large and small subunit rRNA. Proc. Natl. Acad. Sci. U. S. A., 98(17): 9707-9712.
- Müller, T.; Philippi, N.; Dandekar, T.; Schultz, J. and Wolf, M.** (2007). Distinguishing species. RNA, 13(9): 1469-1472.
- Nelson, J. S.** (2006). Fishes of the world. John Wiley and Sons, Inc. New York. 4thed. 601pp.
- Olsen, G. J. and Woese, C. R.** (1993). Ribosomal RNA : a key to phylogeny. F. A. S. E. B., 7(1): 113-123.
- Randall, J. E.** (1982). The diver guide to Red Sea reef fishes. Publishing limited 20 Berkeley street, Berkeley square London Wix 5AE.
- Reed, D. L.; Carrpenter, K. E. and deGravelle, M. J.** (2002). Molecular systematic of the Jacks (Perciformes: Carangidae) based on mitochondrial cytochrome b sequences using parsimony, likelihood, and Bayesian approaches. Mol. Phylogenet. Evol. 23(3): 513-524.
- Shylla, J. A.; Ghatani, S. and Tandon, V.** (2013). Utility of divergent domains of 28S ribosomal RNA in species discrimination of paramphistomes (Trematoda: Digenea: Paramphistomoidea). Parasitol. Res., 112(12): 4239-4253.
- Smith-Vaniz, W. F.** (1984). Carangidae: Relationships. In: Ontogeny and Systematics of Fishes (Moser HG, Richards WJ, Cohen DM, Fahay MP, et al, eds.). Am. Soc. Ichthyol. Herpetol., 522-530.
- Smith-Vaniz, W. F. and Carpenter, K. E.** (2007). Review of the crevalle jacks, *Caranx hippo* complex (Teleostei: Carangidae), with a description of a new species from West Africa. Fisheries Bulletin, 105(2): 207-233.
- Templonuevo, R. M.; Alcantara, S.; Juanico, C. S. and Yambot, A.** (2018). DNA barcoding of two commercially important fish families (Carangidae and Lutjanidae) collected from Cuyo, Palawan, Philippines. International Journal of Agricultural Technology 14(7): 2051-2066.
- Thu, P. T.; Linh, N. M.; Quan, N. V.; Chien, P. V.; Ly, D. H. and Hiep, L. B. H.** (2019). DNA barcoding for identification of some fish species (Carangidae) in Vietnam coastal area. Vietnam Journal of Marine Science and Technology, 19(4): 527-536.
- Torres, S. K. M. and Santos, B. S.** (2019). Species Identification Among Morphologically-Similar *Caranx* species. Turk. J. Fish.& Aquat. Sci., 20(2): 159-169.
- Verma, J.; Lakra, W. S.; Kushwaha, B.; Serajuddin, M.; Kumar R. and Nagpure, N. S.** (2011). Phylogenetic Relationship Between Four Species Using Divergent

- Domain D9 And D11 In Family: Siluridae (Pisces). International Journal of Innovations in Biological and Chemical Sciences, 1: 12-15.
- Verma, Y. and Serajuddin, M.** (2012). Phylogenetic Relationship Between Four Species Using Divergent Domain D8 In Family: Siluridae. International Journal of Life science and Pharma Research, 2(3): 240-244.
- Vidigal, T. H. D.A.; Kissinger, J. C.; Caldeira, R. L.; Pires, E. C. R.; Monteiro, E.; Simpson, A. J. G. and Carvalho, O. S.** (2000). Phylogenetic relationships among Brazilian Biomphalaria species (Mollusca Planorbidae) based upon analysis of ribosomal ITS-2 sequence; Parasitology, 121: 611-620.
- Vidigal, T. H. D.A.; Spatz, L.; Kissinger, J. C.; Redondo, R. A. F.; Pires, E. C. R.; Simpson, A. J. G. and Carvalho, O. S.** (2004). Analysis of the first and second internal transcribed spacer sequences of the ribosomal DNA in Biomphalaria tenagophila complex (Mollusca: Planorbidae). Mem. Inst. Oswaldo. Cruz., 9(2): 153-158.
- Wickramasinghe, S.; Yatawara, L.; Rajapakse, R. P. V. J. and Agatsuma, T.** (2009). *Toxocara canis* and *Toxocara vitulorum*: molecular characterization, discrimination, and phylogenetic analysis based on mitochondrial (ATP synthase subunit 6 and 12S) and nuclear ribosomal (ITS-2 and 28S) genes. Parasitol. Res., 104(6): 1425-1430.
- Yan, H.; Lou, Z.; Li, L.; Ni, X.; Guo, A.; Li, H.; Zheng, Y.; Dyachenko, V. and Jia, W.** (2013). The nuclear 18S ribosomal RNA gene as a source of phylogenetic information in the genus Taenia. Parasitol. Res., 112(3): 1343–1347.
- Zardoya, R. and Meyer, A.** (1996). Evolutionary relationships of the coelacanth, lungfishes and tetrapods based on 28S ribosomal RNA gene. Proc. Natl. Acad. Sci. U. S. A., 93(11): 5449-5454.