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DNA fingerprinting of the carangid fish species from the Iraqi marine waters by using RAPD technique

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Abstract
Twenty species of the family Carangidae from Iraqi marine waters, northwest, Arabian Gulf were identified depending on RAPD-PCR technique. Six primers were used: P1 (212), P2 (239), P3 (244), P4 (250), P5 (265) and P6 (347), which showed evident banding patterns and distinguishable differences among species. The number of bands generated varied from 104 in primer 4 to 230 in primer 6. The genetic distance (D) among carangid fish species ranged from 57% between *A. ciliaris* and *A. indica* to 97% between *C. bajad* and *S. nigrofasciata*. The conclusions of cluster analysis about the phylogenetic relationships of carangid fishes indicate two main branches, the first branch with six groups included 14 species (*A. ciliaris, A. indica, A. atropos, A. mate, C. armatus, C. bajad, C. chrysophrys, C. malabaricus, G. speciosus, M. cordyla, P. niger, S. commersonnianus, S. tol, S. crumenophthalmus*), the second branch with two groups included six species (*A. djedaba, A. kleinii, A. vari, S. nigrofasciata, T. mookalee, U. helvola*). The more relative species among carangid fishes were *A. ciliaris* and *A. indica*.

Keywords: Fingerprinting, RAPD, PCR, carangidae, Iraq

1. Introduction
The family Carangidae forms one of the largest families of bony fish, there are 149 species belong to 30 genera distributed widely in the world [1]. Their habits range from pelagic to demersal, majority of them are semi-pelagic, whose body shapes vary from elongate and fusiform to deeply ovate and strongly compressed [2]. Carangidae is named for the genus *Caranx*, first described by Lacépède in 1801, previous descriptions of some carangid species but assigned to other genera [3].
DNA-based techniques offer an alternative approach to for species identification and have recently started to be applied towards a wide variety of fish, including closely related species belonging to the same family and genus [4]. The analysis of PCR-RAPD results have found a wide range of applications in gene mapping, population genetics and molecular evolutionary genetics. This could be attributed to their efficiency in generating large numbers of markers in a short period [5].
Several studies have been conducted on fish using RAPD technique [6-10]. In this study, RAPD-PCR was used to examine the DNA-fingerprint and identification of carangid fish species from the Iraqi marine waters, northwestern Arabian Gulf.

2. Materials and Methods
2.1 Fish sampling
A total of 418 specimens of carangid fish (Table 1) were collected from Iraqi marine waters, northwestern Arabian Gulf (29° 46' 50"N 48° 39' 46" E to 29° 78' 83"N 48° 75' 78"E) using trawl net, and from the landing site in Al-Faw port, 100 km south of Basrah city during the period from January 2014 to June 2015.

2.2 DNA extraction
Genomic DNA was extracted from 20 mg of muscle tissues of fish, according to Invitrogen kit instructions (Pure linkgenomic DNA kit, USA).

Table 1: Species of Carangidae from the Iraqi marine waters.

Genus	Species
Alectis	*A. ciliaris* (Bloch, 1787)
	A. indica (Rüppell, 1830)
Alepes	*A. djedaba* (Forsskål, 1775)
	A. kleinii (Bloch, 1793)
	A. vari (Cuvier, 1833)
Atropus	*A. atropos* (Bloch & Schneider, 1801)
Atule	*A. mate* (Cuvier, 1833)
Carangoides	*C. armatus* (Rüppell, 1830)
	C. bajad (Forsskål, 1775)
	C. chrysophrys (Cuvier, 1833)
	C. malabaricus (Bloch & Schneider, 1801)
Gnathanodon	*G. speciosus* (Forsskål, 1775)
Megalaspis	*M. cordyla* (Linnaeus, 1758)
Parastromateus	*P. niger* (Bloch, 1795)
Scomberoides	*S. commersonnianus* Lacepède, 1801
	S. tol (Cuvier, 1832)
Selar	*S. crumenophthalmus* (Bloch, 1793)
Seriolina	*S. nigrofasciata* (Rüppell, 1829)
Trachinotus	*T. mookalee* Cuvier, 1832
Uraspis	*U. helvola* (Forster, 1801)

2.3 RAPD protocol

Six primers were used in PCR-RAPD technique which were as follow: P1 (212): GCT GCG TGA C, P2 (239): CTG AAG CGG A, P3 (244): CAG CCA ACC G, P4 (250): CGA CAG TCC C, P5 (265): CAG CTG TTC A and P6 (347): TTG CTT GGC G [8]. PCR was performed in a total volume of 25 µL, containing 12.5 µL red master mix, 2 µL primer, 3 µL genomic DNA, 7.5 µL distilled water. PCR cycling conditions were 94 °C, 1.5 min for initial denaturation, then 40 cycles of 38 °C, 2 min, 72 °C, 2 min, 91 °C, 1 min. An additional step of 72 °C (5 min) was performed for final extension. Amplification products were analyzed by 1.5% agarose gel electrophoresis (80 V and 50 min) and staining with ethidium bromide. The samples migrated with the 100 bp ladder. Gel profile was checked by UV transilluminator and photographs were taken by Photonyx S 140 direct copy system (Nyx Technik Company, USA).

2.4 Data analysis

The genetic similarity (GS) between the species was calculated based on pair comparison between them for primers using the formula Nei and Li [11]:

GSxy= 2 Nxy / (Nx + Ny)

Where, Nx and Ny were the number of bands in individuals X and Y. Nxy was the number of shared bands.

The similarity values were converted into genetic distance using the formula:

D = 1 - GS [11]

3. Results

The DNA fingerprints were identified of twenty species of the family Carangidae from the Iraqi marine waters, using Polymerase Chain Reaction-Random Amplified Polymorphic DNA (PCR-RAPD). In this study, six primers were selected to identify species showed evident banding patterns (Figures 1-6) and distinguishable differences among them. The number of bands generated varied from 104 of primer 1 to 230 of primer 6.

The primer 1 produced 163 bands for 20 species of family Carangidae, the number of bands produced varied from five in *C. bajad* (350-1320 bp) and *T. mookalee* (170-620 bp) to 17 (170-1770 bp) in *A. vari*. The number of bands produced of primer 2 was 149, no bands were shown in *C. chrysophrys* and *A. djedaba*, the highest number of bands was 12 in *A. mate* (160-1520 bp), *C. malabaricus* (160-1700 bp) and *S. nigrofasciata* (100-1340 bp). The primer 3 produced 143 bands, the number of bands produced varied from one (270 bp) in *A. djedaba* to 12 (120-1400 bp) in *S. nigrofasciata*. The primer 4 produced fewer bands, 104, no bands was shown in *A. vari*, the highest number of bands was nine in *A. Atropos* (140-1000 bp) and *S. Crumenophthalmus* (130-1000 bp). The primer 5 produced 190 bands, the number of bands produced varied from five (220-830 bp) in *C. bajad* to 17 (140-1330 bp) in *A. mate*. The primer 6 was superior to the rest of the primers used, producing 230 bands for the carangid fish species, the number of bands produced varied from six in *S. tol* (390-1400 bp) and *S. Nigrofasciata* (180-1300 bp) to 15 in *A. atropos* (170-1400 bp), *C. armatus* (170-1400 bp), *C. bajad* (160-1450 bp) and *C. Chrysophrys* (160-1400 bp).

The genetic distance (D) among carangid fish species according to PCR-RAPD technique ranged from 57% between *A. ciliaris* and *A. indica* to 97% between *C. bajad* and *S. nigrofasciata*. The genetic distance ranged from 68% to 84% among species of genus *Carangoides* and from 62% to 76% among species of genus *Alepes* and reached 80% between species of genus *Scomberoides* (Table 2).

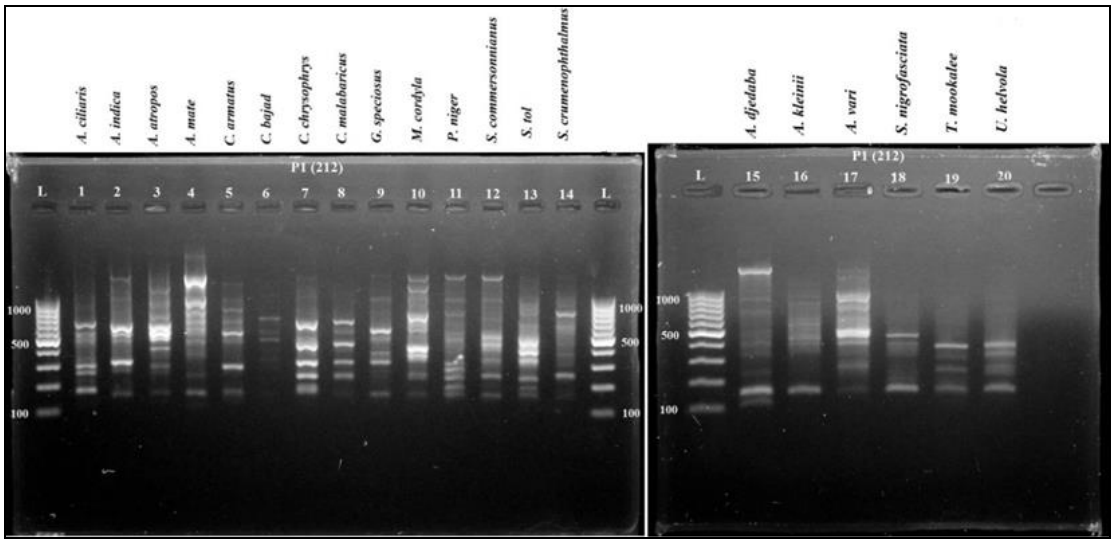

Fig 1: RAPD profile obtained using primers 1(212) of carangid fish species

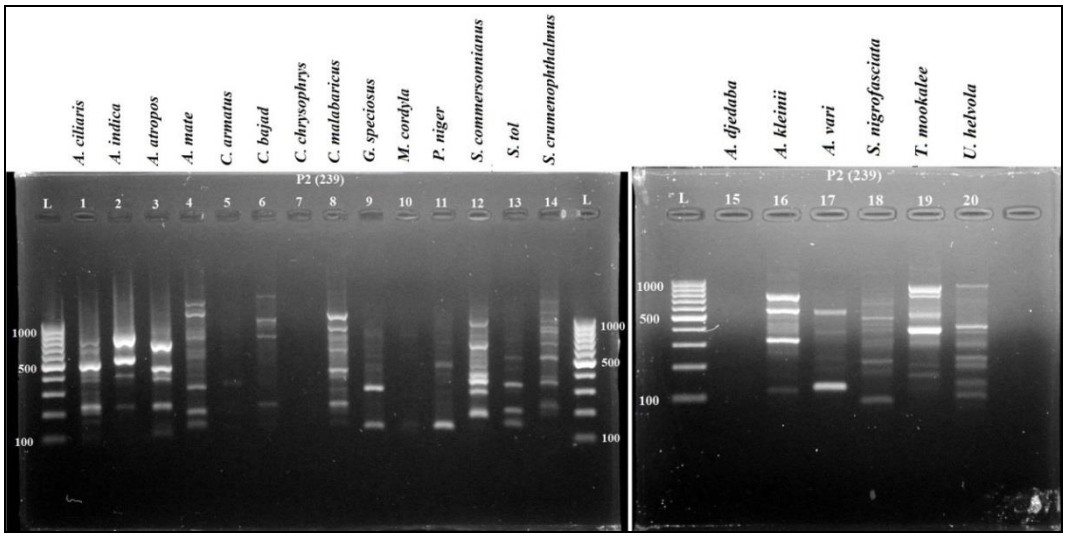

Fig 2: RAPD profile obtained using primers 2(239) of carangid fish species

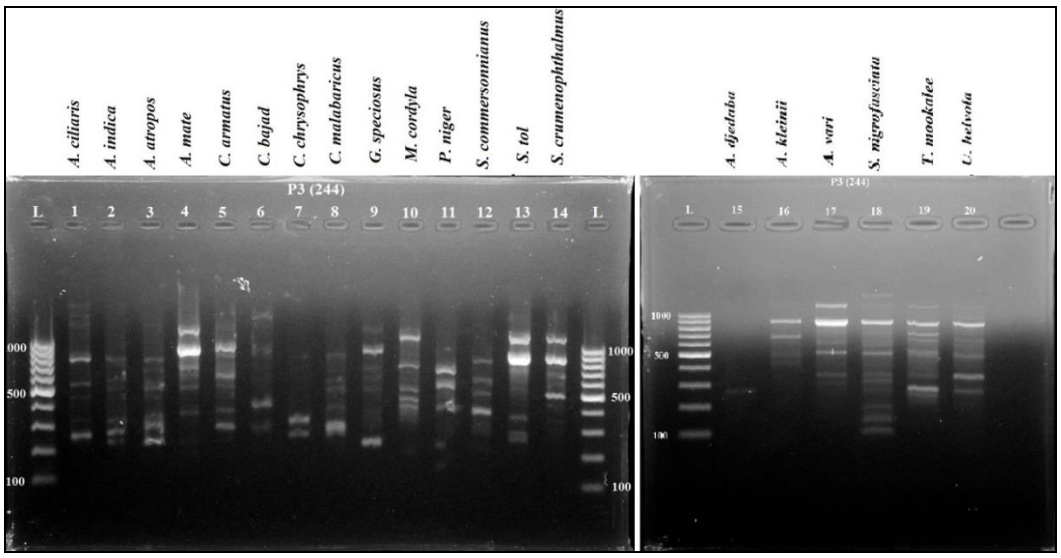

Fig 3: RAPD profile obtained using primers 3(244) of carangid fish species

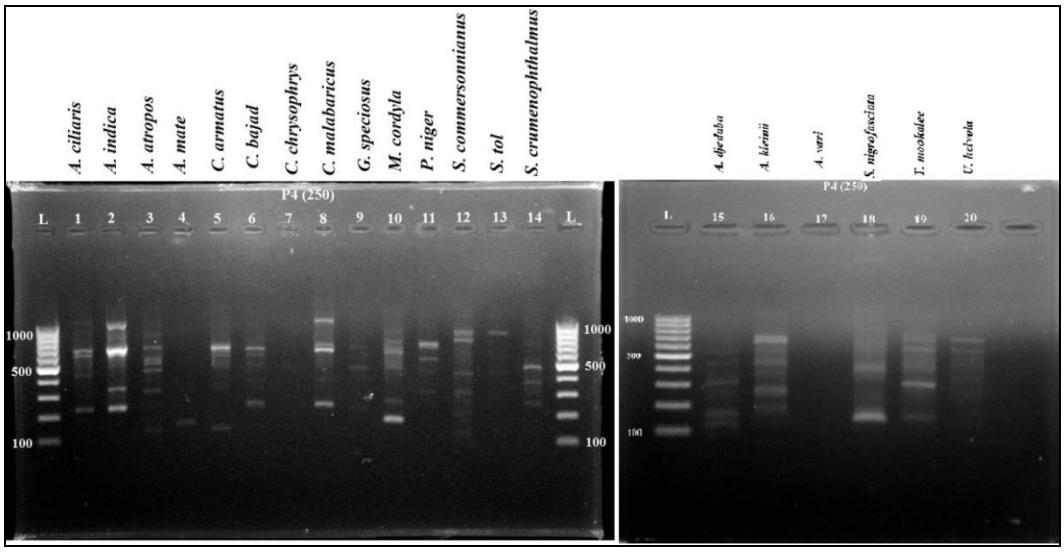

Fig 4: RAPD profile obtained using primers 4(250) of carangid fish species

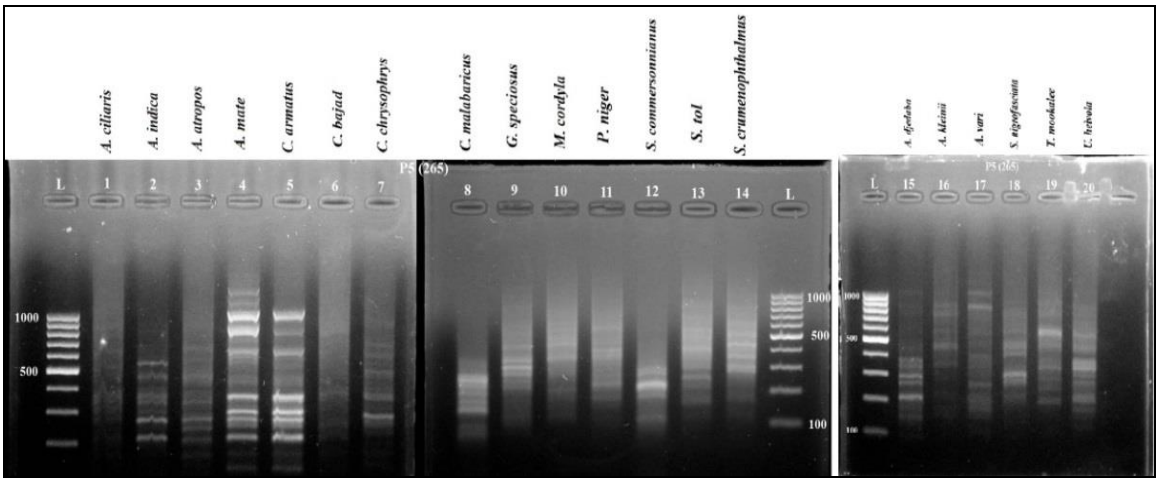

Fig 5: RAPD profile obtained using primers 5(265) of carangid fish species

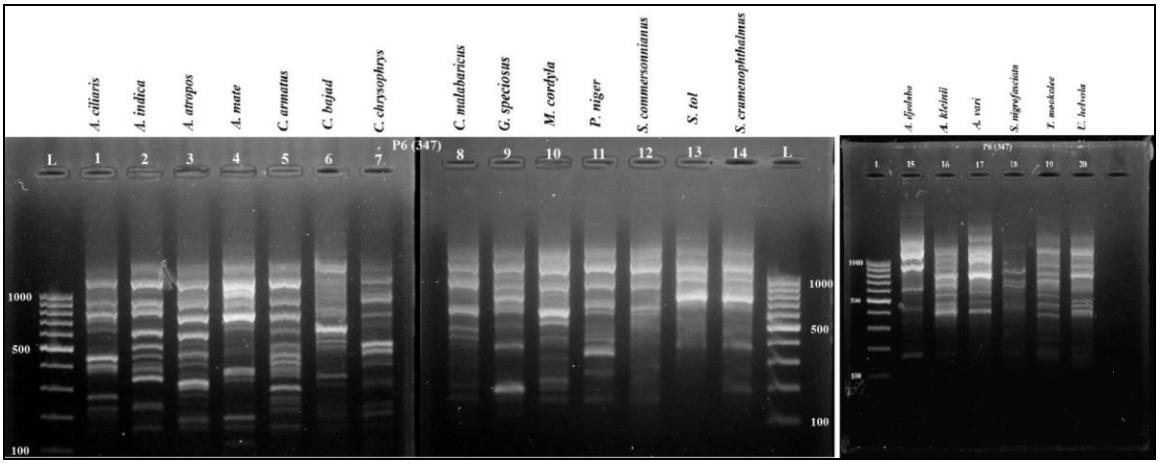

Fig 6: RAPD profile obtained using primers 6(347) of carangid fish species

The genetic similarity (GS) among carangid fish species ranged from 3% between *C. bajad* and *S. nigrofasciata* to 42% between *A. ciliaris* and *A. indica*. The genetic similarity ranged from 16% to 32% among species of genus *Carangoides* and from 24% to 38% among species of genus *Alepes* and reached 19% between species of genus *Scomberoides* (Table 3).

The conclusions of cluster analysis about the phylogenetic relationships of carangid fish indicate two main branches, the first branch with six groups included 14 species (*A. ciliaris*, *A. indica*, *A. atropos*, *A. mate*, *C. armatus*, *C. bajad*, *C. chrysophrys*, *C. malabaricus*, *G. speciosus*, *M. cordyla*, *P. niger*, *S. commersonnianus*, *S. tol*, *S. crumenophthalmus*), the second branch with two groups included six species (*A. djedaba*, *A. kleinii*, *A. vari*, *S. nigrofasciata*, *T. mookalee*, *U. helvola*). The more relative species among carangid fish were *A. ciliaris* and *A. indica* (Figure 7).

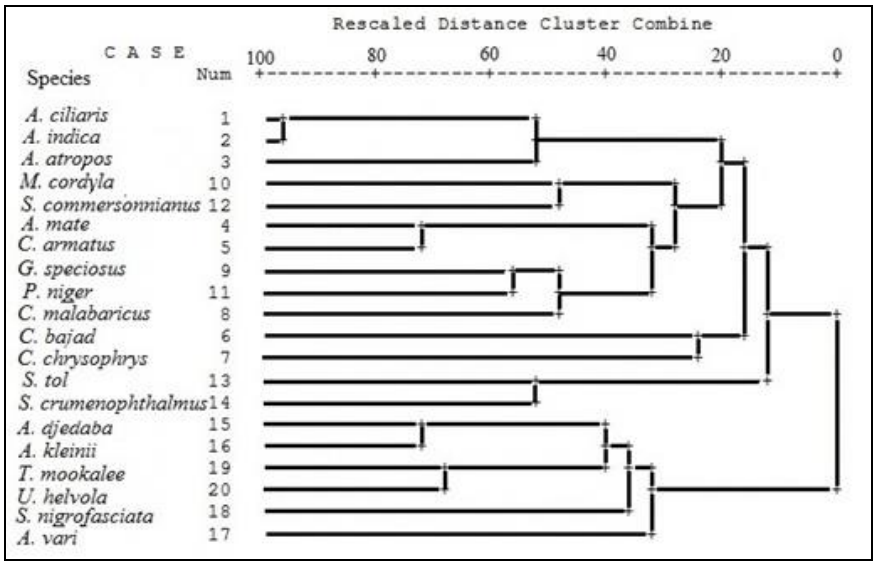

Fig 7: Cluster analysis for phylogenetic relationships of carangid fish species based on RAPD bands

Table 2: Genetic distance (D)% for carangid fish species.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1																			
2	57																		
3	72	68																	
4	87	80	81																
5	70	74	78	60															
6	84	77	88	85	68														
7	86	78	84	78	76	77													
8	80	80	77	74	70	83	84												
9	81	64	74	76	71	77	78	71											
10	86	82	79	81	77	89	82	73	73										
11	85	70	83	84	70	82	81	71	67	76									
12	83	79	80	86	76	85	81	76	76	74	68								
13	86	80	77	83	81	90	80	87	72	91	77	80							
14	81	88	84	83	86	89	82	86	77	82	83	77	67						
15	90	83	88	92	80	88	86	86	92	89	83	83	79	90					
16	86	92	87	90	83	91	89	94	91	90	94	88	89	86	62				
17	85	79	84	86	90	93	90	88	85	88	87	94	87	96	70	76			
18	90	87	93	86	90	97	93	90	89	92	94	94	85	94	75	78	74		
19	84	86	90	92	88	95	91	82	83	88	90	96	81	94	73	69	76	67	
20	80	86	87	85	92	85	91	86	87	92	92	86	87	88	70	74	76	76	63

Species **:**1 **-***A. ciliaris*, 2 **-***A. indica*, 3 **-***A. atropos*, 4 **-***A. mate*, 5 **-***C. armatus*, 6 **-***C. bajad*, 7 **-***C. chrysophrys*, 8 **-***C. malabaricus*, 9 **-***G. speciosus*, 10 **-***M. cordyla*, 11 **-***P. niger*, 12 **-***S. commersonnianus*, 13 **-***S. tol*, 14 **-***S. crumenophthalmus*, 15 **-***A. djedaba*, 16 **-***A. kleinii*, 17 **-***A. vari*, 18 **-***S. nigrofasciata*, 19 **-***T. mookalee*, 20 **-***U. helvola***.**

Table 3: Genetic similarity (GS) % of carangid fish species.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1																			
2	42																		
3	27	32																	
4	12	20	19																
5	29	26	28	39															
6	15	22	11	15	32														
7	14	21	16	22	24	23													
8	20	20	23	26	30	17	16												
9	19	35	26	24	28	22	21	29											
10	13	18	21	19	22	10	17	26	26										
11	14	30	16	16	30	17	19	28	32	24									
12	16	21	20	14	23	15	18	24	23	25	31								
13	13	20	23	17	18	10	20	12	27	8	22	19							
14	18	12	16	17	13	10	18	13	23	17	16	22	32						
15	10	16	12	8	20	12	13	13	7	11	16	16	20	9					
16	14	8	12	10	17	9	11	6	8	9	6	12	10	13	38				
17	14	21	16	14	10	7	9	12	15	11	12	6	13	4	30	24			
18	10	12	7	14	10	3	7	10	10	7	6	6	15	6	25	21	26		
19	15	14	10	8	11	4	9	17	16	11	10	4	19	5	26	30	23	32	
20	20	14	12	14	8	15	9	13	12	7	8	14	13	11	29	25	24	23	36

Species **:**1 **-***A. ciliaris*, 2 **-***A. indica*, 3 **-***A. atropos*, 4 **-***A. mate*, 5 **-***C. armatus*, 6 **-***C. bajad*, 7 **-***C. chrysophrys*, 8 **-***C. malabaricus*, 9 **-***G. speciosus*, 10 **-***M. cordyla*, 11 **-***P. niger*, 12 **-***S. commersonnianus*, 13 **-***S. tol*, 14 **-***S. crumenophthalmus*, 15 **-***A. djedaba*, 16 **-***A. kleinii*, 17 **-***A. vari*, 18 **-***S. nigrofasciata*, 19 **-***T. mookalee*, 20 **-***U. helvola***.**

4. Discussion

RAPD technique has been used in a wide range of applications, including gene mapping, population genetics, and molecular evolutionary genetics. This is mainly due to its efficiency in producing large numbers of markers in a short period [5]. The importance of DNA fingerprinting using RAPD-PCR is relatively simple to obtain reliable, simple data for preparation, it also requires small amounts of DNA without the requirement for sequencing of the genome of the species [12]. Six primers were used in the present study. These primers have greatly succeeded in amplifying the genome of the carangid fish, by the number of bands produced, subsequently dan identified the species with high accuracy. This was agreed with [8], they used the same six primers to identify of hybrids belonging to Carangidae. The success of the RAPD technique in the identification of other populations of fish has been observed in several studies [13, 14, 10, 15, 16]. The primer 6 was superior to the rest of the primers used, producing 230 bands. These number of produced bands are relatively high compared to some studies using the same technique [9, 17, 18].

The percentages of genetic variation according to RAPD-PCR showed the extent of variation among species of Carangidae, both at species and genus level. The genetic variance between species of the same genus was lower than that of different genera, thus increasing the affinity of traits at the species level and increasing their frequency at the genus level. As a result, the availability of RAPD-PCR data can provide an insight into the taxonomic status of fish. Despite the controversy over the different fish groups [19]. The low genetic similarities

between many species of the carangid fish and increased genetic variation among them can be attributed to the fact that a large proportion of DNA in the cell is noncoding, where genetic changes can accumulate which not subject to normal selection. RAPD analysis reveals a lot of genetic variations accumulated in the genome, which are not transcribed and translated into proteins [20].

Phylogenetic analysis allows a comprehensive understanding of the origin and evolution of species [21]. Though some carangid fish species were from the same genus, the analysis of phylogenetic tree showed that some species genetically distant from each other. This agree with [22]. The reason may be competition between species if environmental conditions are excluded [23].

It can be concluded that the use of RAPD technique gives a broad understanding of the distinction of carangid fish species and their Phylogenetic relationships.

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