Morphological and Molecular Systematic of Carangids (Genus: *Alepes*), with New Record of *Alepes vari* from the Iraqi Marine Waters,Northwest Arabian Gulf

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ABSTRACT-- ***Morphometric and meristic characteristics and DNA fingerprint analysis were applied to identify three species of Carangidss belonging to the genus Alepes [A. djedaba (Forsskål, 1775), A. kleinii (Bloch, 1793) and A. vari (Cuvier, 1833)] from Iraqi marine waters, during the period from January 2014 to June 2015. A. vari was recorded for the first time in Iraqi waters. Second dorsal fin rays ranged from 22 to 24 in A. djedaba, 25 to 26 in A. kleinii and 26 to 28 in A. vari. Anal fin rays were 19 - 20 in A. djedaba, 21 - 22 in A. kleinii and 20 - 22 in A.vari. Gill rakers on lower limb were 27 - 31 in A. djedaba, 27 – 29 in A. kleinii and 22 – 23 in A. vari. The DNA fingerprints of species of the genus Alepes were identified using Polymerase Chain Reaction-Random Amplified Polymorphic DNA (PCR-RAPD) with six primers: P1 (212), P2 (239), P3 (244), P4 (250), P5 (265), and P6 (347). The numbers of bands generated by primers were 37 in A. djedaba, 51 in A. kleinii and 49 in A. vari. The genetic similarity was 0.38 between A. djedaba and A. kleinii, 0.30 between A. djedaba and A. vari, and 0.24 between A. kleinii and A. vari, while the genetic distance among them were 0.62, 0.70 and 0.76 respectively.***

Keywords-- Morphometric characteristics, DNA fingerprint, PCR-RAPD, Carangids fish, Iraq

1. INTRODUCTION

The carangidss are widely distributed in all tropical and subtropical seas. They are among the most economically important coastal pelagic fish of the world [1]. This family contains about 32 genera and 140 species worldwide [2]. Whose body shapes vary from elongate and fusiform to deeply ovate and strongly compressed [3]. Carangidss can be distinguished from other teleost groups by the presence of detached anal spines, lateral line scutes, cutaneous fleshy lateral keels, two dorsal fins are separate, the first moderate height or very low with four to eight spines, caudal fin forked with the lobes equal in most species, dorsal and ventral grooves on caudal peduncle, adipose eyelids [4, 5]. The genus *Alepes* Swainson, 1839 containing five species, *A. apercna*, *A. djedaba*, *A. kleinii*, *A. melanoptera* and *A. vari* distributed in the tropical to subtropical regions of the Indo-West Pacific region, their ranges overlap along the Indian, Asian, South Africa, northern Australia and the Red Sea [6].

DNA-based techniques offer an alternative approach to 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 [7]. The analysis of PCR-RAPD results has found a wide range of applications in gene mapping, population genetics and molecular evolutionary genetics. Thus could be attributed to their efficiency in generating large numbers of markers in a short period [8].

Previous studies differed in the number of species belonging to the genus *Alepes* in Iraq and the Arabian Gulf, some of which record the presence of one species [9, 10, 11, 12, 13], or two species [14], or three species [15], while, Bishop [16] recorded four species.

In this study, morphological and molecular methods were used to identify the species belonging to the genus *Alepes* in the Iraqi marine waters, northwest Arabian Gulf.

2. MATERIALS AND METHODS

A total of 104 specimens of three species of the genus *Alepes* (13 specimens of *A. djedaba*, 85 specimens of *A. kleinii* and six specimens of *A. vari*) were collected from Iraqi marine waters (29° 46' 50"N 48° 39' 46" E to 29° 78' 83"N 48° 75' 78"E) by using trawl net, and from commercial fishery in Al-Faw fish landings, 100 km south of Basrah city, northwestern Arabian Gulf (Fig. 1), during the period from January 2014 to June 2015. The specimens are deposited in the Marine Science Centre, University of Basrah, Iraq. Eight meristic characters were counted employing dissection microscope and twenty morphometric characters were measured to the nearest mm by fish measuring board and digital vernier following [15].

Genomic DNA was extracted from 20 mg of muscle tissues for *Alepes* fish, according to Invitrogen kit instructions (Pure linkgenomic DNA kit, USA). 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 [17]. 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 gels 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).

The genetic similarity (GS) between the species was computed based on pair comparison between them for primers using the following formula [18]: 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 [18].

Figure (1): Map showing sampling area in Iraqi marine waters

3. RESULTS

3.1 Morphological description

Tables 1 and 2 show the morphometric and meristic characteristics of the study species in Iraqi marine waters.

***Alepes djedaba* (Forsskål, 1775):** Body depth of *A. djedaba* (Fig. 2) ranged from 34.32 to 38.52% in standard length, and body compressed 9.20 - 13.56%. Dorsal and ventral profiles were almost evenly convex. Eye diameter ranged from 5.94 to 8.61%, about close to snout length, 7.28 - 9.07%, with adipose eyelid well developed on posterior half of eye only. Two separate dorsal fins, the first with seven to eight spines, and the second with 22 to 24 rays. Anal fin has two detached spines followed by 19 to 20 rays. Gill rakers ranged from 11 to 13 on upper limb and 27 to 31 on lower limb of first gill arch. The total vertebra was 24. The color was grayish green above, silvery to white below.

Figure (2): *A. djedaba* from Iraqi marine waters

***Alepes kleinii* (Bloch, 1793):** The body of *A. kleinii* (Fig. 3) was oval, body depth ranged from 43.27 to 46.06% in standard length, body compressed 11.55 to 13.95%, with ventral profile distinctly more convex than dorsal profile. Eye diameter was 8.74 to 9.13% slightly larger than snout length, 7.19 to 7.99%, with adipose eyelid well developed on posterior half of eye only. Species has two separate dorsal fins, the first with eight spines and the second with 25 to 26 rays. Anal fin has two detached spines followed by 21 to 22 soft rays. Gill rakers ranged from 10 to 11 on upper limb and 27 to 29 on lower limb of first gill arch. The total vertebra was 21 to 23. The color was bluish grey to green above, silvery below, with dark bands sometimes evident on sides above lateral line.

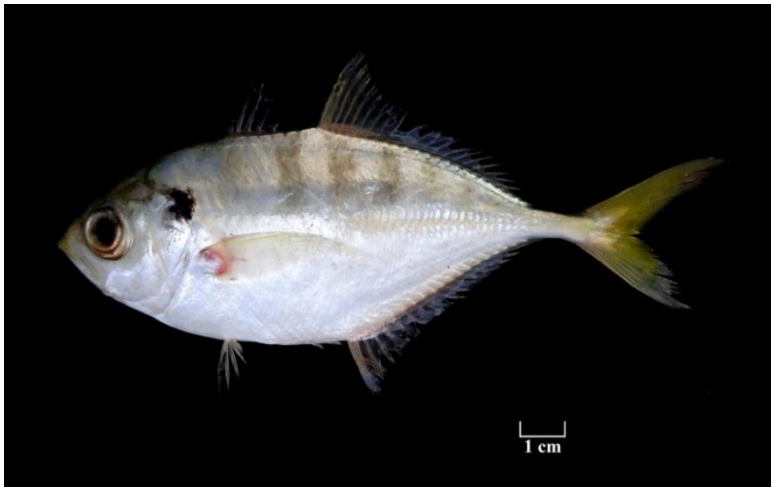

Figure (3): *A. kleinii* from Iraqi marine waters

***Alepes vari* (Cuvier, 1833):** *A. vari* (Fig. 4) was recorded for the first time in Iraqi marine waters. Body oblong and body depth ranged from 35.01 to 41.14% in standard length, body width was 9.51 to 15.64% with dorsal and ventral profiles almost evenly convex. Eye diameter was 5.3 to 9.7% close to snout length, 7.68 - 8.46% with adipose eyelid well developed on posterior half of eye only. Species has two separate dorsal fins, the first with seven to eight spines and the

second with 26 to 28 rays. Anal fin has two detached spines followed by 20 to 22 rays. Gill rakers ranged from 6 to 8 on upper limb and 22 to 23 on lower limb of first gill arch. The total vertebra was 23. The color was ash blue above, silvery to white below.

Figure (4): *A. vari* from Iraqi marine waters

3.2 Molecular analysis

The DNA fingerprints were identified of three species using Polymerase Chain Reaction-Random Amplified Polymorphic DNA (PCR-RAPD). In this study, six primers were selected to identify species showed evident banding patterns (Fig. 5) and distinguishable differences among them. The number of bands generated varied from 37 in *A. djedaba* (10, 0, 1, 5, 13 and 8 bands, respectively), 51 in *A. kleinii* (9, 11, 4, 7, 8 and 12 bands, respectively) and 49 in *A. vari* (12, 7, 7, 0, 13 and 10 bands, respectively). Size of bands ranged from 90 to1560 bp in *A. djedaba*, 130 to 1320 bp in *A. kleinii* and 90 to 1770 bp in *A. vari*. The genetic similarity was 0.38 between *A. djedaba* and *A. kleinii*, 0.30 between *A. djedaba* and *A. vari*, and 0.24 between *A. kleinii* and *A. vari*, while the genetic distance among them were 0.62, 0.70 and 0.76 respectively.

Table (3) shows distribution frequency of PCR-RAPD and polymorphic bands among *A. djedaba*, *A. kleinii* and *A. vari*. The genetic variation between them ranged from 38.24 % in P5 (265) molecular marker to 83.33 % in p3 (244) molecular marker and the mean was 54.01 %. *A. vari* revealed high number of polymorphic bands (31) while *A. djedaba* gave low number of polymorphic bands (14). P5 (265) molecular marker predominated on other primers as produced 34 bands with 13 polymorphic bands, regarding the genetic variation, a percent of 38.24% was reported. Whereas, P3 (244) and P4 (250) molecular markers revealed the lowest number of amplified bands which was 12 with a total of 10 and 8 polymorphic bands, and reported a percent of 83.33% and 66.67% as a genetic variation respectively.

Table (1): Morphometric characters of the genus *Alepes* from Iraqi marine waters

Morphometric characters	***A. djedaba***		***A. kleinii***		***A. vari***	
	Range	**Mean± SD**	**Range**	**Mean±SD**	**Range**	**Mean±SD**
Total length (mm)	130 - 248	207.88 ±48.63	122.57 - 158.0	136.21 ± 14.89	124 - 455	324.67 ± 122.18
Fork length (mm)	111 - 217	179.13 ± 42.71	103.43 – 133.0	115.09 ± 11.91	105 - 373	266 ± 96.37
Standard length [SL] (mm)	97 - 187	155.2 ± 36.74	90.71 - 117	100.14 ± 10.96	92 - 328	234.17 ± 85.87
Body depth % in SL	34.32 - 38.52	36.84 ± 1.34	43.27 - 46.06	44.28 ± 1.24	35.01 - 41.14	36.40 ± 2.37
Body width % in SL	9.2 - 13.56	11.63 ± 1.26	11.55 - 13.95	12.71 ± 1.01	9.51 - 15.64	11.87 ± 2.17
Head length % in SL	26.68 - 28.99	27.96 ± 0.82	27.70 - 29.10	28.27 ± 0.51	25.54 - 28.26	27.32 ± 1.00
Head depth % in SL	22.62 - 27.52	25.02 ± 1.54	27.91 - 29.65	28.51 ± 0.67	21.73 - 28.13	24.01 ± 2.23
Head width % in	10.74-	13.11 ±	13.67 -	13.94 ±	12.64 -	13.28± 0.60

SL	14.3	1.16	14.25	0.25	14.4	
Snout length % in SL	7.28 - 9.07	7.81 ± 0.59	7.19 - 7.99	7.64 ± 0.32	7.68 - 8.46	8.07± 0.31
Eye diameter % in SL	5.94 - 8.61	6.83 ± 0.92	8.74 - 9.13	8.90 ± 0.16	5.3 - 9.7	6.46 ± 1.72
Interorbital distance % in SL	7.56 - 9.43	8.20 ± 0.57	6.41 - 7.80	6.99 ± 0.56	6.11 - 8.1	7.56 ± 0.73
Predorsal length % in SL	33.67 - 37.42	35.53 ± 1.18	33.09 - 36.38	34.56 ± 1.40	32.32 - 36.04	34.42 ± 1.49
Postdorsal length % in SL	6.29 - 8.08	7.35 ± 0.68	6.76 - 8.30	7.69 ± 0.74	5.39 - 8.66	6.91 ± 1.06
1st Dorsal fin length % in SL	12.66 - 15.38	13.38 ± 0.85	13.78 - 14.56	14.29 ± 0.30	14.13 - 15.28	14.85 ± 0.39
2nd Dorsal fin length % in SL	44.7 - 47.01	45.82 ± 0.69	46.30 - 48.90	47.31 ± 1.00	45.75 - 48.87	47.32 ± 1.45
Anal fin length % in SL	38.04 - 42.63	40.05 ± 1.45	43.13 - 44.97	43.91 ± 0.79	38.4 - 41.77	40.16 ± 1.26
Pectoral fin length % in SL	27.34 - 36.33	33.22 ± 3.35	31.74 - 33.79	32.58 ± 0.77	33.24 - 36.64	35.11 ± 1.40
Pelvic fin length % in SL	11.69 - 13.43	12.55 ± 0.56	8.74 - 10.81	9.80 ± 0.80	10.33 - 12.66	11.61 ± 0.87
Caudal peduncle length % in SL	4.61 - 9.16	6.92 ± 1.48	7.37 - 8.83	8.04 ± 0.71	7.08 - 8.34	7.55 ± 0.52
Caudal peduncle depth % in SL	4.25 - 8.22	4.91 ± 1.34	4.59 - 4.87	4.77 ± 0.11	4.44 - 4.66	4.52± 0.09

Table (2): Meristic characters of the genus *Alepes* from Iraqi marine waters

Meristic characters	***A. djedaba***		***A. kleinii***		***A. vari***	
	Range	**Mean± SD**	**Range**	**Mean± SD**	**Range**	**Mean± SD**
1st Dorsal fin spines	7 - 8	7.13 ± 0.35	8 -8	8 ± 0	7 - 8	7.67 ± 0.52
2nd Dorsal fin rays	22 - 24	22.67 ± 0.82	25 - 26	25.33 ± 0.52	26 - 28	27.40 ± 0.89
Anal fin spines	2 -2	2 ± 0	2 -2	2 ± 0	2 - 2	2 ± 0
Anal fin rays	19 - 20	19.67 ± 0.52	21 - 22	21.17 ± 0.41	20 - 22	21.20 ± 0.84
Pectoral fin rays	18 - 22	20.80 ± 1.79	19 - 22	20.33 ± 1.03	20 - 21	20.80 ± 0.45
Pelvic fin rays	5 - 5	5 ± 0	5 - 6	5.67 ± 0.52	5 - 7	5.5 ± 0.84
Gill rakers (upper limb)	11 - 13	11.83 ± 0.98	10 - 11	10.25 ± 0.50	6 - 8	7.0 ± 1.15
Gill rakers (lower limb)	27 - 31	28.75 ± 2.06	27 - 29	28.25 ± 0.96	22 - 23	22.50 ± 0.58
Vertebrae	24 - 24	24 ± 0	21 - 23	22.0 ± 0.89	23 - 23	23 ± 0

Table (3): Frequency distribution of RAPD-PCR bands in polymorphic loci among species of the genus *Alepes* from Iraqi marine waters

Primers	Amplified fragments				Polymorphic fragments				Polymorphic %
	Total	*A. djedaba*	*A. kleinii*	*A. vari*	Total	*A. djedaba*	*A. kleinii*	*A. vari*	
P1(212)	31	10	9	12	14	3	4	7	45.16
P2(239)	18	0	11	7	12	0	8	4	66.67
P3(244)	12	1	4	7	10	1	3	6	83.33
P4(250)	12	5	7	0	8	3	5	0	66.67
P5(265)	34	13	8	13	13	4	2	7	38.24
P6(347)	30	8	12	10	17	3	7	7	56.67
Total bands	137	37	51	49	74	14	29	31	54.01

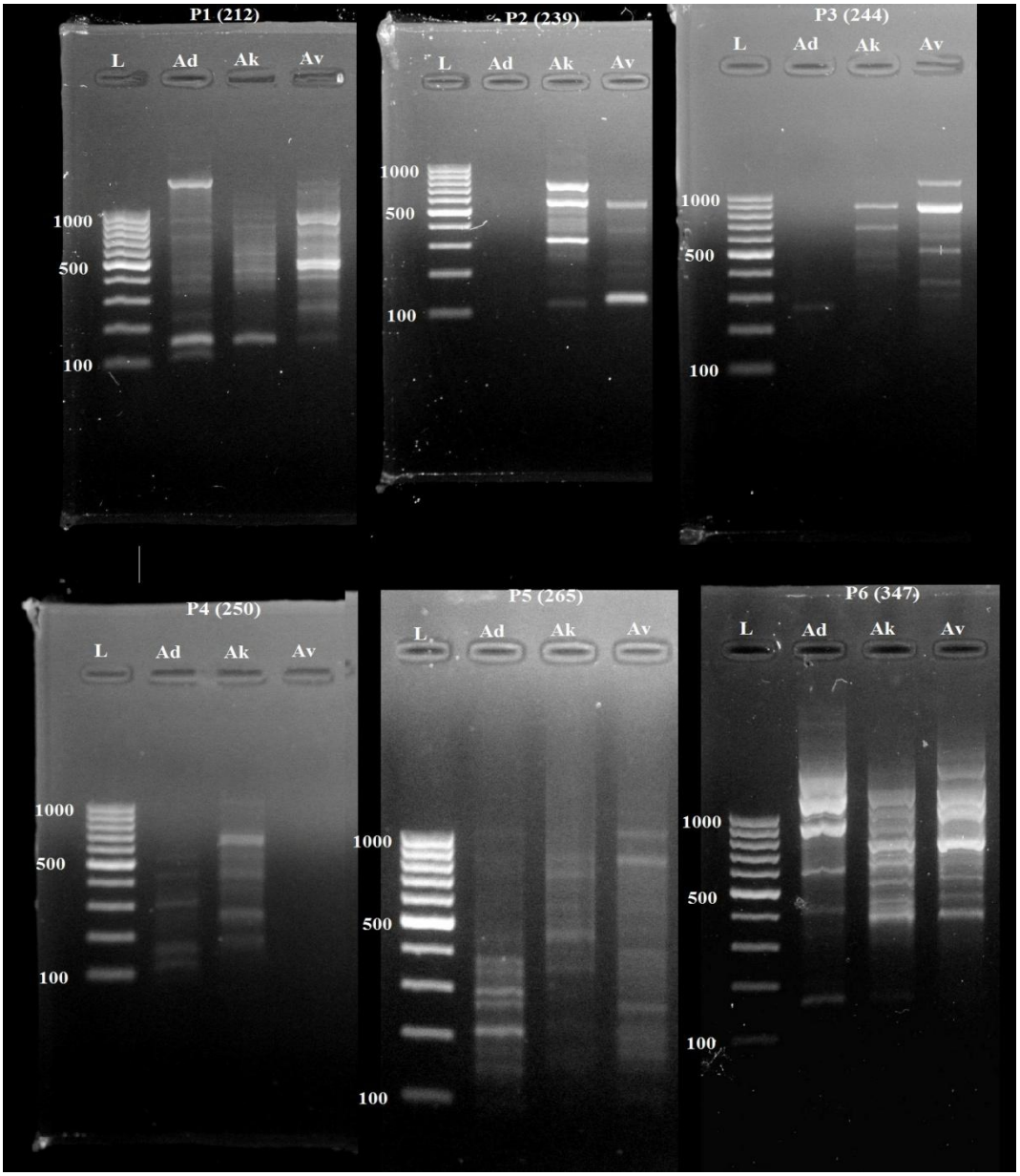

Figure (5): PCR-RAPD products of *A. djedaba* (Ad), *A. kleinii* (Ak) and *A. vari* (Av) DNA using P1 (212), P2 (239), P3 (244), P4 (250), P5 (265), and P6 (347) primers (L:100 bp ladder).

4. DISCUSSION

The morphological characteristics are still the most important evidence for defining the fish. Such analyses usually include two kinds of data, meristic and morphometric. Importance of meristic characters usually can be used as evidence for defining a new species [19]. Meristic characters of these three species of the genus *Alepes* from Iraqi marine waters exhibited variation in the count of dorsal fin rays, anal fin rays, gill rakers and vertebrae. In addition to, the differences in morphometric characters, such as body depth, snout length and eye diameter. The features of *A. djedaba*, *A. kleinii* and *A. vari* were in a good agreement with [15] and [4].

The results showed a new record of *A. vari* in Iraqi marine waters. The environment of these waters is quite different in compare with the remaining parts of the Arabian Gulf due to the discharge of freshwater from Shatt Al-Arab River which has potential sources of nutrients and organic materials [20].

The fingerprinting technique is important since it is relatively easy to obtain valuable data, reliable and simple to set up [21]. DNA fingerprinting of *A. djedaba*, *A. kleinii* and *A. vari* revealed a genetic variation between them which were evident by the number and size of amplified bands. It was noted that the markers which produced the lowest number of bands, had gave the highest values of polymorphism, as P3(244) and P4(250) molecular markers. Obtained results are in accordance with the results of [17], when they proved the genetic variation between two hybrids of carangids fish of the genus *Caranx* employing same primers. RAPD technique was successfully used to detect the genetic variation between different fish species. Faddagh *et al*. [22] used PCR-RAPD technique with seven decamere primers to identify eight cyprinid fish species from Shatt Al-Arab river, southern Iraq. Soufy *et al*. [23] evaluates common patterns of genetic variations among three species of tillapine in Egypt using the same technique.

5. CONCLUSION

It can be concluded that our results proved the reliability and viability based on morphological features and molecular technique to identify the species of the genus *Alepes*, in addition to the new record in Iraqi marine waters.

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