Mesopotamia Environmental Journal **ISSN 2410-2598**
Mesop. environ. j. 2016, Spicial Issue A.;106-114
(proceding of 6th International conference for Environmental Sciene –University of Babylon).

New record of the carangid fish from the Iraqi marine waters, northwest Arabian Gulf

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To cite this article:

Al-Faisal,A.J.; Mohamed,A.M.; Jaayid,T.A. New record of the carangid fish from the Iraqi marine waters, northwest Arabian Gulf. *Mesop. environ. j*., 2016, Spicial Issue A.;107-114.

Abstract:

Two species of carangid fish, *Alectis ciliaris* (Bloch, 1787) and *A. indica* (Rüppell, 1830) were collected from Iraqi marine waters, northwest Arabian Gulf from January to November, 2014. *A. ciliaris* was recorded as a first time from Iraqi marine waters. *A. ciliaris* could be distinguished by nape, head profile broadly rounded, and gill rakers on lower limb of first arch ranged from 14-15, while the nape and head profile something angular, and gill rakers on lower limb of first arch ranged from 18-25 in *A .indica*. The DNA fingerprints were identified of two species 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 number of bands generated by primers varied between 46 in *A. indica* to 48 in *A. ciliares*. The genetic similarity and genetic distance between two examined species were 0.42 and 0.57, respectively.

Keywords: New record, *Alectis ciliaris*, morphology, DNA fingerprints, PCR-RAPD Iraq.

Introduction

Carangidae forms one of the largest families of bony fishes, There are 140 species belong to 32 genera distribution 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]. Carangids 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 4-8 spines, caudal fin forked with the lobes equal in most species, dorsal and ventral grooves on caudal peduncle, adipose eyelids [3], [4].

The first fish in the genus, *Alectis* to be described was *Alectis ciliaris* under the genus name of *Zeus* part of the dory family (Zeidae). In 1815, Rafinesque proposed the name *Alectis*, into which three species were eventually placed; *A. alexandrina, A. ciliaris, and A. indica* [5].

The previous studies about fishes of Iraqi marine waters appeared availability one species of genus *Alectis* which is *A .indica* [6], [7], [8], [9]. In addition, this species was recorded in Kuwait marine waters and Arabian Gulf [10], [11], [12], [13], [14]. *A. ciliaris* was found in tropical waters worldwide, western and eastern Atlantic, western Indian Ocean, and eastern Pacific [5].

In this study, *A.* ciliaris, as new record in Iraqi marine waters and *A. indica* were described by morphological characteristics and DNA fingerprints by using PCR-RAPD technique.

Material and Methods

A total of 30 specimens of genus, *Alectis* were collected from commercial fishery in Al-Faw town, 100 km south of Basrah city, northwestern Arabian Gulf (figure 1), during the period from January to November 2014. The specimens is deposited in the Marine Science Centre, University of Basrah, Iraq. Eight meristic characters were counted employing dissection microscope and nineteen morphometric characters were measured to the nearest mm by fish measuring board and digital vernier following [13].

Genomic DNA was extracted from 20 mg of muscle tissues for *Alectis* genus, 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 andP6 (347): TTG CTT GGC G [15]. 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 were calculated based on pair comparison between them for primers using the formula [16]:

$$GSxy = 2\,Nxy / (Nx + Ny)$$

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:

$$D = 1 - GS$$ [16].

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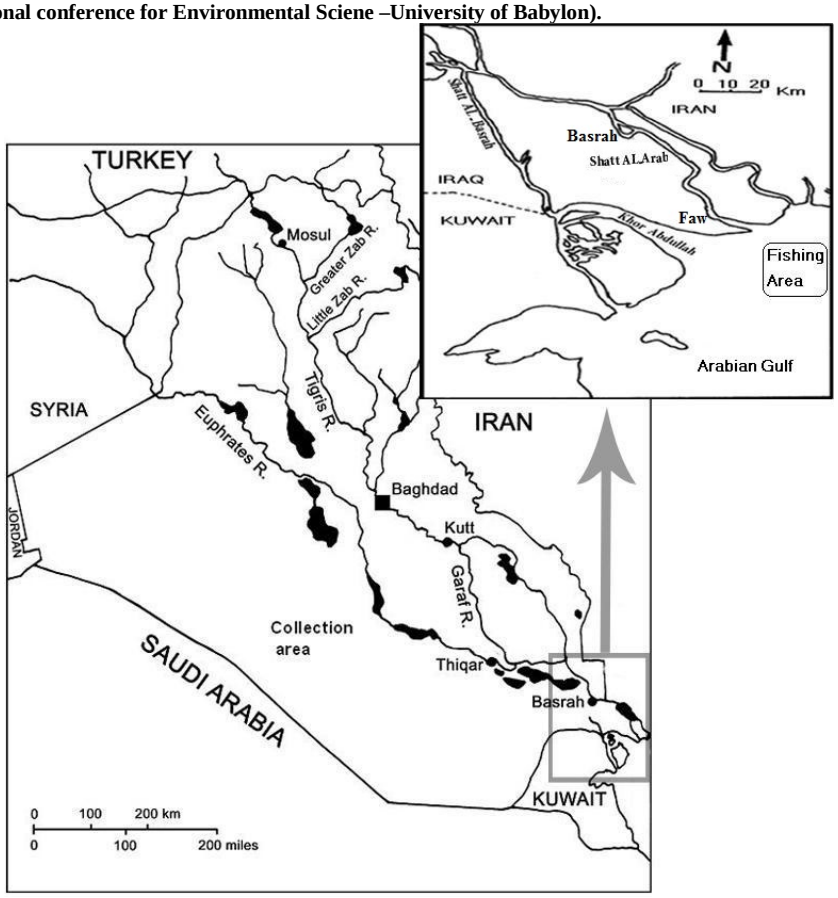

Fig. (1) Map elucidating sampling locations from Iraqi marine waters.

Results and Discussion

The following taxonomic key distinguish between the species of genus, *Alectis* in Iraqi marine waters.

1a. Nape and head profile broadly rounded, suborbital depth relatively narrow and gill rakers on lower limb of first arch are 14-15 …..… *A. ciliaris*

1b. Nape and head profile something angular, suborbital depth relatively broad and gill rakers on lower limb of first arch are 8-25……..…*A. indica*

***Alectis ciliaris* (Bloch, 1787)**

Our results showed that the meristic and morphometric characteristics of two specimens of *A. ciliaris*. The first dorsal fin contains four spines, second dorsal fin with 20-21 rays, anal fin contains 17 rays, anterior soft rays of dorsal anal fins extremely long and filamentous. Gill rakers were six inupper raw while 14-15 in lower raw. Total length of fish ranged from 216-245 mm. The body was deep, 68.63-71.29 % in standard length and very compressed10.8-12.97 %. Nape and head Profile were nearly rounded, suborbital depth relatively narrow, head length ranged from 34.98-35.42 % and head depth ranged from 37.46-39.28 %. The body color was silvery with some dark (tables 1-2 and figure 2).

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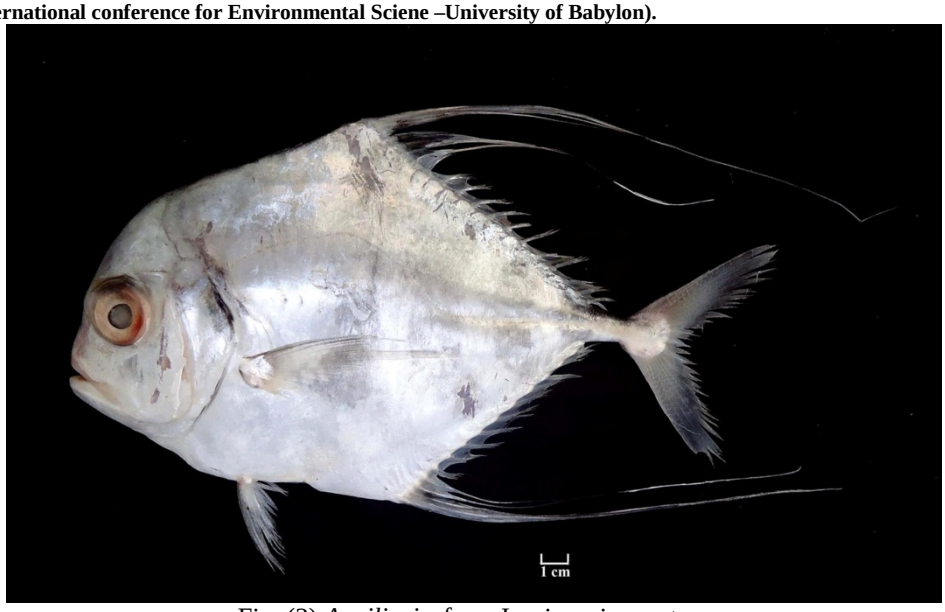

Fig. (2) *A. ciliaris* from Iraqi marine waters

Table (1). Meristic characters of *A. ciliares* isolated from Iraqi marine waters.

	Meristic characters	Range	Mean	SD
	1st Dorsal fin spines	4 - 4	4	0
	2nd Dorsal fin rays	20 - 21	20.5	0.71
Anal fin:	spines	2 - 2	2	0
	rays	17 - 17	17	0
	Pectoral fin rays	19 - 19	19	0
	Pelvic fin rays	6 - 6	6	0
Gill rakers:	upper raw	6 - 6	6	0
	lower raw	14 - 15	14.5	0.71
	Vertebrae	23-23	23	0

Table (2). Morphometric characters of *A. ciliares* from Iraqi marine waters.

Morphometric characters	**Range**	**Mean**	**SD**
Total length (mm)	216 - 245	230.5	
Fork length (mm)	183 - 205	194	
Standard length [SL] (mm)	162 - 182	172	
Body depth % in SL	68.63 – 71.29	69.96	1.88
Body width % in SL	10.8 – 12.97	11.89	1.53
Head length % in SL	34.98 – 35.42	35.20	0.31
Head depth % in SL	37.46 – 39.28	38.37	1.29
Head width % in SL	14.67 – 15.4	15.04	0.52
Snout length % in SL	10.96 – 13.53	12.25	1.82
Eye diameter % in SL	10.57 – 11.17	10.87	0.42
Interorbital distance % in SL	7.01 – 8.86	7.94	1.31
Predorsal length % in SL	38.75 – 45.26	42.01	4.60
Postdorsal length % in SL	7.29 – 7.5	7.40	0.15
fin length % in SL 2nd Dorsal	55.46 - 52.28	53.87	2.25
Anal fin length % in SL	46.12 – 46.24	46.18	0.08
Pectoral fin length % in SL	38.67 – 42.06	40.37	2.40
Pelvic fin length % in SL	20.25 – 21.23	20.74	0.69
Caudal peduncle length % in SL	9.01 – 10.09	9.55	0.76
Caudal peduncle depth % in SL	4.35 – 4.41	4.38	0.04

***Alectis indica* (Rüppell, 1830)**

Table (3) demonstrated meristic characters of *A. indica* (figure 3). The first dorsal fin spines ranged fromfour to five and second dorsal fin rays ranged from 18-21. Total anal fin rays 16-18. Gill rakers were six to nine in upper raw and 18-25 in lower raw. Table (4) showed morphometric characters of *A.indica*. Total length ranged from 125-442.5 mm. Body depth ranged from 59.16-87.11 % in standard length,body width 9.77-13.12 %.Nape and head profile somewhat angular, suborbital depth relatively broad, head length 32.94-43.1 % andhead depth 35.64-57 %. The body color mostly silvery with dusky green tinge dorsally or with dark bars (table 4 and figure 3).

Fig. (3) *A. indica* from Iraqi marine waters

Table (3). Meristic characters of *A. indica* from Iraqi marine waters

	Meristic characters	Range	Mean	SD
	1st Dorsal fin spines	4 - 5	4.71	0.47
	2nd Dorsal fin rays	18 - 21	19.93	0.70
Anal fin:	spines	2 -2	2	0
	rays	16 - 18	17.13	0.50
	Pectoral fin rays	16 - 19	18.08	1.0
	Pelvic fin rays	6 - 7	6.29	0.47
Gill rakers:	upper raw	6 - 9	7.75	1.22
	lower raw	18 - 25	22.46	2.18
	Vertebrae	23 - 23	23	0

Table (4). Morphometric characters of *A. indica* from Iraqi marine waters

Morphometric characters	Range	Mean	SD
Total length (mm)	125 – 442.5	267.22	
Fork length (mm)	105 – 377.5	221.96	
Standard length [SL] (mm)	90 – 344.0	198.1	
Body depth % in SL	59.16 – 87.11	70.99	10.44
Body width % in SL	9.77 – 13.12	11.27	1.13
Head length % in SL	32.94 – 43.1	37.24	3.85
Head depth % in SL	35.64 – 57.0	45.42	7.34
Head width % in SL	9.83 – 13.46	11.54	1.35
Snout length % in SL	15.19 – 18.72	17.20	1.31
Eye diameter % in SL	7.62 – 10.38	9.01	1.21
Interorbital distance % in SL	6.41 – 7.41	6.68	0.39
Predorsal length % in SL	45.7 – 51.86	48.94	2.36
Postdorsal length % in SL	6.46 – 9.26	8.15	0.95
fin length % in SL 2nd Dorsal	53.33 – 60.6	57.03	3.39
Anal fin length % in SL	45.58 – 54.71	48.74	3.48
Pectoral fin length % in SL	38.1 – 44. 49	41.90	2.60
Pelvic fin length % in SL	16.62 – 44.98	35.89	11.33
Caudal peduncle length % in SL	9.19 – 11.45	10.26	0.88
Caudal peduncle depth % in SL	4.14 – 5.11	4.62	0.32

DNA fingerprinting of *Alectis* species

RAPD-PCR technique was applied to analyze the genetic variation between *A. ciliares* and *A. indica* from Iraqi marine waters. In this study, six primers were selected to identify two species showed evident banding patterns (figure 4) and distinguishable differences between them. The number of bands generated varied between 48 in *A. ciliares* (7, 10, 9, 5, 6 and 11 bands, respectively) and 46 in *A. indica* (8, 4, 6, 6, 9 and 13 bands, respectively). Size of bands ranged from 110-1520 bp in *A. ciliares*, and 170-1650 bp in *A. indica*. The genetic similarity between *A. ciliares* and *A. indica* was 0.42 while the genetic distance between them was 0.57.

Table (5) showed distribution frequency of PCR-RAPD and polymorphic bands between *A.ciliaris* and *A.indica*. The genetic variation between them ranged from 45.5 % in P4 (250) molecular marker to 73.4 % in p1 (212) molecular marker and the mean was 57.6 %. *A. ciliars* revealed high number of polymorphic bands (28) while *A. indica* gave low number of polymorphic bands (26). P6 (347) molecular marker predominated on other primers as produced 24 bands with 12 polymorphic bands, regarding the genetic variation, a percent of 50% was reported, whereas, P4 (250) revealed the lowest number of amplified bands which was 11 with a total of five polymorphic bands, and reported a percent of 45% as a genetic variation.

Table (5). Frequency distribution of RAPD-PCR bands in polymorphic loci between *A. ciliaris* and *A. indica*

Primers	Amplified fragments			Polymorphic fragments			Polymorphic
	Total	*A. ciliaris*	*A. indica*	Total	*A. ciliaris*	*A. indica*	%
P1(212)	15	7	8	11	5	6	73.4
P2(239)	14	10	4	8	7	1	57.2
P3(244)	15	9	6	9	6	3	60.0
P4(250)	11	5	6	5	2	3	45.5
P5(265)	15	6	9	9	3	6	60.0
P6(347)	24	11	13	12	5	7	50.0
Total bands	94	48	46	54	28	26	57.6

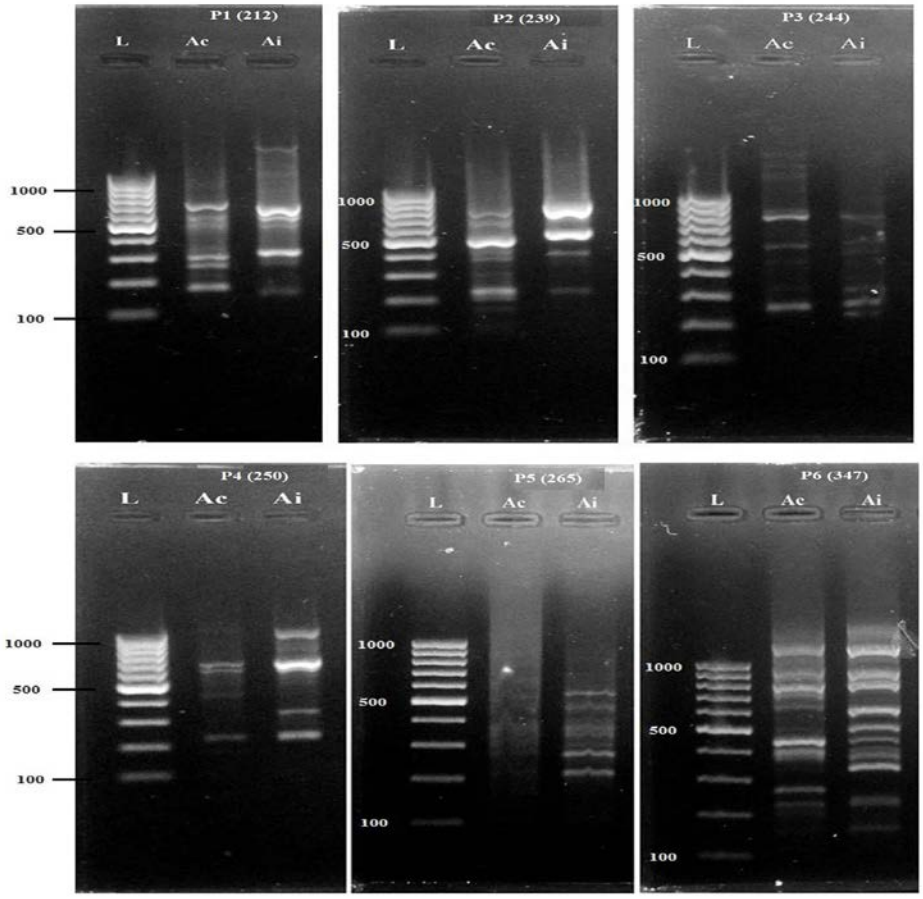

Fig. (4) PCR-RAPD products of *A. ciliares* (Ac) and *A. indica* (Ai) DNA using P1 (212), P2 (239), P3 (244), P4 (250), P5 (265), and P6 (347) primers (L:100 bp ladder).

The present study showed a new record of *A. ciliaris* in Iraqi marine waters, northwest of Arabian Gulf as a first time. The marine environments of the north part of Arabian Gulf and the environments of Iraqi marine waters are quite different in compare with the remaining parts of the Arabian Gulf. The main source of north part for freshwater is the Shatt Al-Arab river, which is formed by the confluence of Euphrates and Tigris rivers, in addition to Karun river. The Shatt Al-Arab river and its associated marshes present potential sources of nutrients, organics and pollutants [17].

The features of *A. ciliaris*and *A. indica* which showed in our study were in a good agreement with the results of [13] and [3], when they showed that *A. ciliaris* could be characterized by four to six gill rakers on upper limb and 12-17 on lower limb

of first gill arch, second dorsal fin with 19-21 rays, anal fin with two spines followed by 16-18 rays, anterior soft rays of dorsal and anal fins extremely long and filamentous in young, lateral line anteriorly with a strong and moderately long arch. While *A. indica* has 8–11 gill rakers on upper limb and 21-26 on lower limb of first gill arch, second dorsal fin has 19-21 rays, anal fin with two spines followed by 16-to 18 rays, anterior soft rays of dorsal and anal fins extremely long and filamentous in young, lateral line anteriorly with a strong and moderately long arch.

The analysis of 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 [18]. The fingerprinting technique is important since it is relatively easy to obtain valuable data, reliable and simple to set up [19]. DNA fingerprinting of *A. ciliaris* and *A. indica* revealed a genetic variation between them which were evident by the number and size of amplified bands. A significant relationship between the number of bands and the percentage of polymorphic was found, with an exception of P6 (347) molecular marker, which showed highest number of bands with a low polymorphism. Obtained results are in accordance with the results of [15], when they proved the genetic variation between two hybrids of carangid fishes of the genus *Caranx* employing same primers. RAPD technique was successfully used to detect the genetic variation between different fish species, Faddagh *et al*. [20] used PCR-RAPD technique with seven decamere primers to identify eight cyprinid fish species from Shatt Al-Arab river, southern Iraq. Soufy *et al*. [21] evaluates common patterns of genetic variations among three species of tillapine in Egypt using the same technique. It can be concluded that our results proved the reliability and viability based on morphological features and molecular technique to identify the species of *Alectis* as new records in the marine water of Iraq.

Acknowledgment

The authors are grateful to the College of Agriculture, Basrah University, Iraq, for their funding and supporting during the present study.

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