Basrah J. Agric. Sci., 29 (1): 25-33, 2016

Genetic Diversity of Kattan *Luciobarbus xanthopterus* Heckel, 1843 (Pisces: Cyprinidae) in Four Mesopotamean Inland Waters, Iraq

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Abstract

Genetic diversity of fish species in Iraqi waters studies are rare, the study design to invistigate the genetic diversity of Kattan *Luciobarbus xanthopterus* Heckel, 1843 among four ecosystems; Shatt Al-Arab River southern Iraq, Dokan reserviour in Sulaimanya province, Tigris near Kut city and Euphrates near Kerbala'a city. Couple RAPD decamere primers have been selected. The profiles of RAPD-PCR products on agarose gel revealed 22 and 19 bands respectively. The C15 primer amplified seven, seven, one and four bands and C16 primer created four, eight, two and eight bands respectively. The study concluded that the *L. xanthopterus* genetically differentiated while adapting to various environments. whereas population in Tigris near Kut city was the most divers from the others. In the same time populations from Shatt Al-Arab river and Dokan reserviour were the most similar, while the Euphrates population near Kerbala'a varied from the two later most similar populations. The result of RAPD test revealed that this species acclimated with the ecological variation. The study recommoneds not to use each of the four stocks in artificial crossbreeding in order to conserve the *L. xanthopterus* genetic diversity.

Key words: Fish, Luciobarbus, Iraq, RAPD, genetic diversity.

Introduction

Mesopotamean Iraqi waters are including, Tigres, Euphrates, Shatt Al-Arab River, Ahwar marshes, lakes and reserviours. These various aquatic environments differ chemically, physically and eographically, accordingly they would affecting the fauna inhabiting there. Cyprinid fish species in addition to their economic importance, play a central ecological role in the aquatic ecosystems in Iraq [11], While genus *Luciobarbus* including six member of the most important fishe species from ecological and economical aspects in Iraq [10]. In the close nighbouring regions in Iran wich overlapped Mesopotamian basin, *L. xanthopterus* studied using RFLP technique [16]. While Tsignopoulos and Berrebi [29] proved that there was a high divergence among different *Barbus*

population and concluded that geographical site causes the genetic difference within genus copying with ecological alteration. Also three morphotypes of *Barbus gananensis* were distinguished using mitochonderial DNA in Ethiopia, Genele River [12].

All the previous studies on fish taxonomy rely on biometry [23; 24], osteology [25] and protein electrophoresis [2; 3]. While theses previous methods have limited value to discriminate among many similar fish species furthermore among populations belong to the same species [8].

Also Ahmed *et al.* [1] used RAPD protocol to distinguish among three fish genera: *Tilapia*, *Sarotherodon* and *Oreochromis* furthermore between two species (O. *aureus* and O. *niloticus*). While Ali *et al*. [4] followed RAPD markers to differentiate among families: Cichlidae, Mugilidae, Sparidae and Serranidae in Egypt costs. El-Zaeem *et al.* [14] invistigated the genetic similarity among four carp genera and between common carp and mirror carp and achieved fingerprints for them.

The geomorphology and changes of drainage channels are the essential factor that influences the distribution of the freshwater species [26]. However, climate has been changed so rapidly across last few decades (e.g. temperature) [22], so fish populations either adapt and evolve *in situ* or forced to move to suitable habitat. Furthermore the environment degradation such as desiccation of marshes (which consider as reproductive and raising area) in southern of Iraq forced many fish species to exploit other habitats [27].

Anthropogenic effects have impact on genetic structure and phenotype in freshwater fish species [9]. Mostly these changes such as dams, pollution and stream channel alteration can result in decreasing of population size [6]. Nevertheless more genetic divers population has more potential to respond to environmental disturbance and have a higher potential to evolve and survive [26]. While there are many genetic studies related with barbel species phylogeny using *Cyt b* sequence [13; 30]. The molecular studies on fish species using DNA techniques in Iraq started lately. Faddagh *et al.* [15] studied the DNA fingerprints using RAPD technique of eight freshwater cyprinids including six species of the genus *Barbus* that endemic Shatt Al-Arab River.

The present study aimed to invistigate the genetic diversity of Kattan *Luciobarbus xanthopterus* among four different habitats in Iraq; Shatt Al-Arab River in Basrah governerate, southern Iraq, Dokan reserviour in Sulaimanya northren Iraq, Tigris near Kut city in the East and in Euphrates near Kerbala'a in the West using RAPD technique.

Materials and Methods

Seventeen fish specemens of *Luciobarbus xanthopterus* were collected from Shatt Al-Arab River in Basrah Southern Iraq, Dokan reserviour in Sulaimaya, Northern Iraq, Tigris River in Kut city Eastren Iraq and in Euphrates River near Kerbala'a Westren Iraq, which clarified in table 1, The fish were classified according to Coad [11] and updating according to Froese and Pauly [17]. Fin tissues were cut and preserved in 95% ethanol and stored at 20°C until extraction time.

Table (1): sampling sites and number of specimens of *Luciobarbus xanthopterus.*

Sampling sites	Shatt Al-Arab River-Basrah	Dokan Resviour, Sulaimania	Tigris River-Kut	Euphrates River, Karbela'a
Number of specimens	6	2	5	4

Figure (1): Sampling sites of *Luciobarbus xanthopterus* from Shatt al-Arab River in Basrah, Dokan Reservoir in Sulaimanyia, Tigris near Kut city and Euphrates near Kerbela'a.

Genomic analysis

Fin tissues were cut and preserved in 95% ethanol until test time. Genomic DNA were extracted following Sambrook *et al*. [28] and tested for integration using electrophoresis on 0.8% agarose gel stained with ethidium bromid dye.

RAPD technique was followed using two decamere primers C15 and C16 according to Callejas and Ochando, [7] and purchased from Alpha Company. Thermocycler programmed as intial denaturation 95°C for 6 min. followed by 35 cycle of 95°C denaturation temperature for 1 min., 36°C annealing tempreture for 1 min. and 72°C extension temprature for 1 min. and the final extension 72°C for 6 min. The reaction volume of PCR was 25µl composed of 12.5µl mastermix (Promega) solution, 2µl primer, 4µl template DNA and 6.5µl free nuclease deionized distilled water.

The PCR products then electrophoresed on 2% agarose gel stained with ethidium bromide dye. Ladder of 100 bp (promega) was utilized with this test. The profiles were tested on UV light transilluminator and decomented by photographing by Canon Camera with gel decomentation tool.

Data analysis

The data of the RAPD patterns of four populations were transformed to the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm program [19]. RAPD patterns of four populations were compared based on the on the index of similarity between samples, providing a mathematical model by calculating a similarity matrix, transforms similarity coefficients into distance matrix (Distance Matrix value "0.000" indicating identical strains) and makes a clustering to construct a dendrogram from a set of variables, to study genetic variation especially with difficult or closely related RAPD patterns.

Results and Discussion

The RAPD profiles of the four *L. xanthopterus* populations in Shatt Al-Arab River, Dokan Reservoir, Tigris and Euphrates Rivers showed that there are 41 bands were created by using the couple of RAPD primers as in fig 2 and fig 3. The profiles of RAPD-PCR products on agarose gel of C15 and C16 revealed 19 and 22 bands respectively. The C15 primer amplified seven, seven, one and four bands and C16 primer created four, eight, two and eight bands respectively, while we excluded the fant bands. The size of bands ranged from 90 bp up to C15-1142 bp. While the band C15-640 bp can be considered as diagnostic marker to this barbel species due to presence in the four sampling areas. So from the RAPD profile the four populations of *L. xanthopterus* can be discriminated as shown. Whereas the figure 2 revealed the approximate value of RAPD band calculated by mathematic method according to straiteline equation four populations can be differentiated by RAPD technique. In the same time the similarity analysis by the Distance matrix

showed (Table 2), that the population of the Tigris-Kut is the most dissimilar with the other three populations. So the highest index between Tigris-Kut population and Euphrates- Kerbala'a was 2.078, while the lowest dissimilarity between Shatt Al-Arab population and Tigris-Kut population was 0.09. All the four populations responded to the used primers.

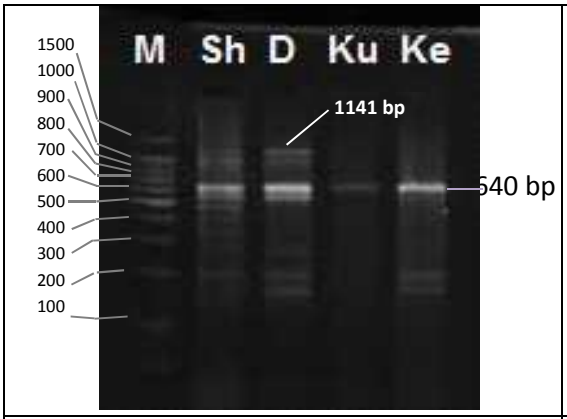

Fig. (2): RAPD DNA profile of *Luciobarbus xanthopterus* Populations using C15 primer electrophoresed on 2% agarose gel with 60 V. Lanes: M: 100 bp Ladder, Sh: Shatt Al-Arab River, D: Dokan Reservoir, Ku: Tigress in Kut City, Ke: Euphrates-Kerbla'a City.

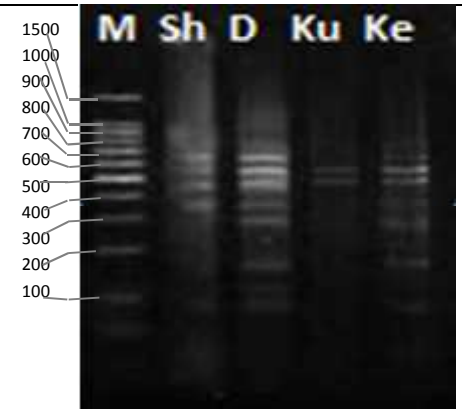

Fig. (3): RAPD DNA profile of *Luciobarbus xanthopterus* Populations using C16 primer electrophoresed on 2% agarose gel with 60 V. Lanes: M: 100 bp Ladder, Sh: Shatt Al-Arab River, D: Dokan Reservoir, Ku: Kut City, Ke: Kerbla'a City.

Table (2): UPGMA Distance Matrix of *Luciobarbus xanthopterus* populations

***Luciobarbus xanthopterus* Populations**	Shatt Al-Arab	Dokan	Tigris-Kut	Euphrates-Kerbel'aa
Shatt Al-Arab	0	1.447	0.090	1.811
Dokan		0	2.009	1.576
Tigris-Kut			0	2.078
Euphrates-Kerbela'a				0

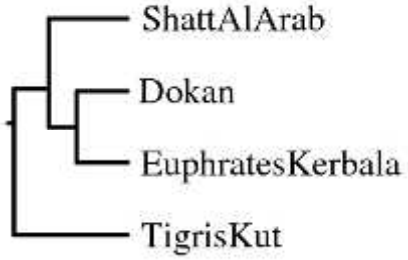

Figure (4) UPGMA dendrogram of *Luciobarbus xanthopterus* populations.

The freshwater habitats are divers according to geological variation, physical and chemical properties of water and climatic factors of the region. So fish have to adapt to variant curcumstances copying with the whole charcters of the habitat including feeding habits, color and temperature tolorence [ ].

The tree of the four populations of *L. xanthopterus* revealed that the Euphrates-Kerbala'a and Dokan populations clustered together and strapped with the Shatt Al-Arab population. The Tigris-Kut population branched alone. The four populations are divers which mean that this fish species is adapting to the different environments in Iraqi waters. So, *L. xanthopterus* Kattan is not in danger of declining till now because their populations adapting with the different environment.

The population of Tigris – Kut has the maximum distance value due to the isolation belong to dams, properties and hydrology of Tigris water. We recommend not crossbreeding these four populations to conserve their genetic diversity. We should act to rehabilitate the marshes in order to let all native *Luciobarbus* species to return back to their reproduction & rearing environment. On the other side continuous monitoring to the barbel species using genetic tools would be important for conservation programs.

The difference in DNA make-up in fish from various habitats also found in *Barbus (Luciobarbus) xanthopterus* using RFLP technique to differentiate among three population from different rivers and branches in Southern-West of Iran[16]. The genetic isolation among different populations belong to the same species due to many reasons while the essential one is the geographical distances or natural and artificial barriers like mountains or dams [20].

The fish population would adapt to different habitat in physical such as temperature, bottoms and vegitation in addition to chemical properties such as nuterients, salinity and pollution and all of these factors [5]. The inter-population differences can be explained as adaptation to local environment [18]. On the other side, RAPD profiles above revealed noticable polymorphism among *L. xanthopterus* populations [21].

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التنوع الجيني لجماعات سمكة القطان *Luciobarbus xanthopterus* Heckel, 1843 (Pisces: Cyprinidae) في أربع بيئات في بلاد مابين النهرين، العراق

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المستخلص: ان الدراسات حول التنوع الجيني للاسماك ضمن النوع في المياه العراقية قليلة جدا. لتحديد التنوع الجيني لسمكة القطان *Luciobarbus xanthopterus* في اربع انظمة بيئية هي نهر شط العرب جنوب العراق وخزان دوكان في محافظة السليمانية ونهر دجلة بالقرب من مدينة الكوت ونهر الفرات بالقرب من مدينة كربلاء. أختير بادئين عشريين عشوائيين لأستخدامهما في طر يقة التضخيم العشوائي للذخيرة الجينية. أن صورة ناتج الطريقة العشوائية للتفاعل البوليميريزي المتسلسل على هلام الأكروز بينت 19 و22 حزمة على التوالي من البادئين المستخدمين. ضخم باديء C15 سبع وسبع وحزمة واحدة واربع حزم بينما ضخم باديء C16 أربع وثمان واثنين وثمان حزم في جماعات القطان الأربعة على التو الي . استنتجت الدراسة ان سمكة القطان تمايزت جينيا خلال التكيف لبيئات مختلفة في المياه الداخلية. واوضحت ان جماعة القطان في نهر دجلة قرب مدينة الكوت هي الأكثر اختلافا عن بقية الجماعات، بينما كانت الجماعتين من شط العرب وخزان دوكان الأكثر تشابها جينيا، اما الجماعة في نهر الفرات بالقرب من مدينة كربلاء فقد ارتبطت معهما. وقد استنتجت الدراسة ان جماعات سمكة القطان تأقلمت مع التغيرات البيئية الداخلية وهي غير معرضة لخطر التناقص في الجماعات. توصي الدراسة بعدم استخدام المخازين الأربعة في التكثير الاصطناعي فيما بينها من اجل ان تحتفظ بخواص التنوع الجيني الذي يشكل قاعدة لتحمل وتجاوز التغيرات البيئية.