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MOLECULAR STUDY ABOUT *CYPRINUS CARPIO* THAT IMPLANT IN STATIONS OF MARINE SCIENCE CENTER

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ABSTRACT

The common carp (*Cyprinus carpio*) may be distinguished by its small eyes, thick lips, two barbells at each corner of the mouth, large scales, and strongly serrated spines in the dorsal and anal fins. Thirty five specimens of *cyprinus carpio* were collected from the implant stations of Marine Sciences Center, Basrah University, Iraq. Standard procedures for chromosomal preparation from the head kidney tissue were used. The DNA extractions from tissue, and the cytochrome oxidase subunit I (COI) was amplified. The diploid chromosomes number of implant *cyprinus carpio* were found to be 2n=100. Acetocarmine stained metaphase spread showing a diploid set of 100 chromosomes is presented in11pairsof metacentric chromosomes,16 pairs of submetacentrie chromosomes, 6 pairs of telocentric homologous pair and17acrocentric chromosomes. Sexual dimorphisms of the chromosomes were not detected in somatic karyotypes of male and female *Cyprinus carpio***.** The amplification of COI genes for the common carp revealed the expected PCR products of 655 bp, in all samples**.** Although the *Cyprinus carpio* specimens that used in this study appear in different morphs (wobbled among color, speared of scales and body architecture) but the cytogenetic and genetic results fixed that all the specimens of fish were under same species

INTRODUCTION

Fish as a source of protein for human eating into tropical and subtropical water systems is continuing apace. The common carp has been implanted and is also a popular angling and attractive fish; is the third most frequently introduced species in the world. The common carp (*Cyprinus carpio*) may be distinguished by its small eyes, thick lips, two barbells at each corner of the mouth, large scales, and strongly serrated spines in the dorsal and anal fins. The color of carp varies; in the natural, they are usually olive green to bronze or silvery in color with a paler underside (NSW Department of Primary Industries, 2005). Carp are typically found in still or slowly flowing waters, lakes and stable marshs, usually with silt bottoms (Environment ACT Undated). They are found at low altitudes (Reynolds, 1983, Driver *et.al*., 2005, Jones & Stuart, 2006), especially in areas where there is rich aquatic vegetation; they are also found in salty lower reaches of some rivers and coastal lakes (NSW Department of Primary Industries, 2005). Common carp live in many microen- vironments they typically inhabit the benthopelagic zone of fresh to salty waters within a pH range of 7.0 to 7.5 and temperates of 3°C to 32°C within latitudes of 60°N to 40°N(DeVaney *et.al*., 2009). Part of the cause why common carp are successful freshwater invaders is their aptitude to make the most of a range of available habitats and take benefit of corrupted habitats (Jones & Stuart, 2009). They have a greater tolerance of low oxygen levels, pollutants and turbidity than most native fish, and are often associated with degraded habitats, including stagnant waters (NSW Department of Primary Industries, 2005). They are most abundant in streams enriched with sewage or substantial runoff from agricultural land and are rarer in clear, cold waters and streams of high gradient. In fact, they are cultured in rural southern Asia in rice fields, which are reported to be the richest habitats of aquatic organisms (Saikia & Das, 2009). Changes to water flows, declining water value and other changes to river habitats over the past few decades have negatively affected many native fish while favouring carp (NSW Department of Primary Industries, 2005). Brown *et al*., (2005) have suggested that exotic species such as carp benefit from floodplain inundation

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The aim of study

Cyprinus carpio, usually called common carp, is a extensively circulated freshwater fish and a ordinary inhabitant of rive ring systems of Asia and Eastern Europe. The species entered the carp polyculture system in Iraq as one of important food source and thereafter the species has become an essential part of the compound fish culture system in the country. The acceptance of rigorous rural practices, free use of inputs and inbreeding in hatcheries, has led to increased in appearance of different morphed carp; so this study was done to identified the *Cyprinus carpio* according to chromosomes numbers and present of COI fish gene.

METHODOLOGY

Thirty five specimens of cyprinus carpio were collected from the implant stations of Marine Sciences Center, Basrah University, Iraq. Standard procedures for chromosomal preparation from the head kidney tissue were used (Sugiyama, 1971) (figures: 1, 2, 3&4)

DNA Isolation

DNA extractions from tissue. Briefly, tissue samples were homogenized separately in incubation buffer (10 mM Tris-HC1 and 10 mM ethylenediaminetetraacetic acid (EDTA), pH8.0, centrifuged at 10000 rpm at 4 °C , after which the supernatants were digested with lysis buffer (l0mM Tris-HC1, 10 mM ethylenediaminetetraacetic acid (EDTA), pH8.0, 0.5% SDS and 50 ug/ml Proteinase K). After incubation at 37 °C for overnight, the digests were deproteinized by successive phenol / chloroform and iso-amyl alcohol extraction and DNA was recovered by child ethanol precipitation, drying and resuspension in TE buffer. The DNA was visible under UV light.

Amplification of the COI fish gene

The cytochrome oxidase subunit I (COI) was amplified in a 50 µl volume with 5 ul of 10X Taq polymerase buffer, 2µl of $MgCl_2$ (50 mM), 0.25µl of each dNTP (0.05 mM), 0.5µl of each primer (0.01 mM), 0.6 U of Taq polymerase and 5µl of genomic DNA. The primers used for the amplification of the COI gene were FISH F5'TCAA CCAA CCAC AAAGA CATTG GCAC3'and FISH R-5'TAGA CTTCT GGGTG GCCAA AGA ATCA3' (Ward *et.al*., 2005). The PCR condition were an initial step of 2 minutes at 95 °C followed by 35 cycles of 40 seconds at 94 °C, 40 seconds at 54 °C and 1 minute 10 seconds at 72 °C followed by final extension of 10 minutes at 72 °C. The PCR products 655 bp were visualized on 1.2 % agarose gels

RESULTS AND DISCUSSION

The diploid chromosomes number of implant cyprinus carpio were found to be 2n=100. Aceto- carmine stained metaphase spread showing a diploid set of 100 chromosomes is presented in11pairs of metacentric chromosomes, 16 pairs of submetacentrie chromosomes, 6pairs of telo- centric homologous pair and 17 acrocentric chromosomes. Sexual dimorphism of the chromosomes were not detected in somatic karyotypes of male and female Cyprinus carpio (figure .4 & 5)

Figure.1

Figure.2

Figure.3

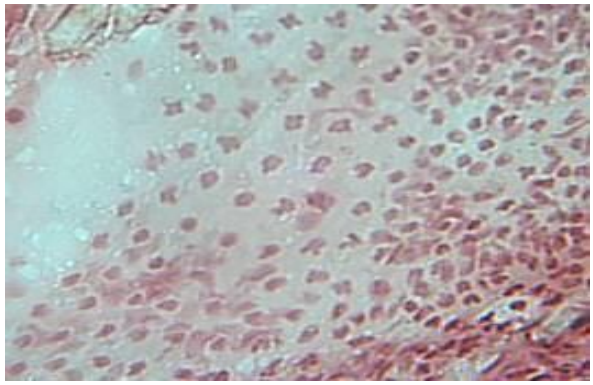

Figure.4 Metaphase spread of common carp (2n =100)

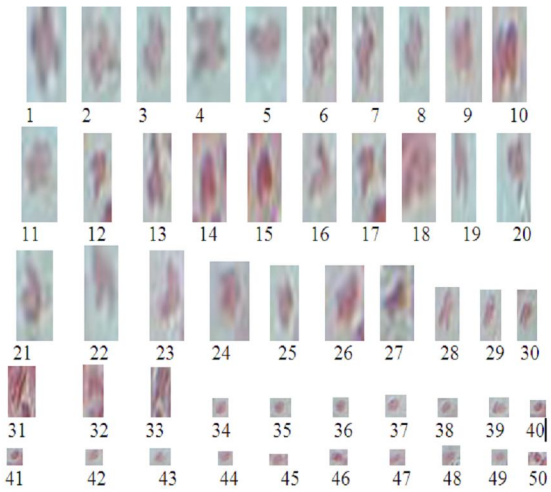

Figure.5 Karyogram of metaphase spread of common carp

The amplification of COI genes for the common carp revealed the expected PCR products of 655 bp, in all samples (figure.6).

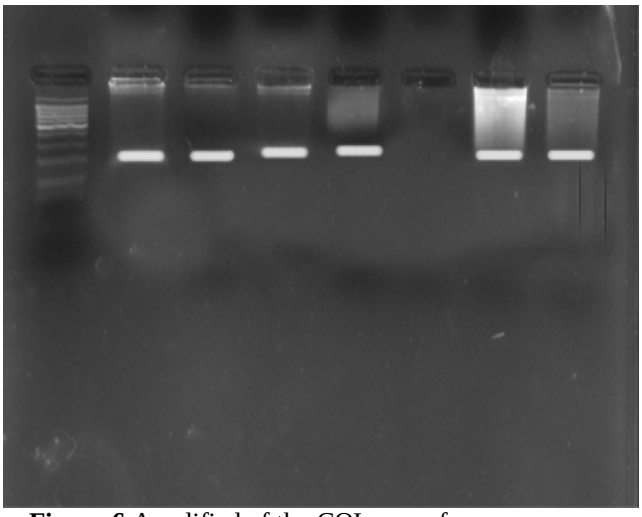

Figure.6 Amplified of the COI gene of common carp Lane 1 ladder , lane 6 (Negative) lane 2, 3, 4, 5,7&8 PCR product(655bp)

Cyprinus carpio is a teleostean species having a tetraploid origin. Inspite of the fact that carp possesses alarge number of very small chromosomes, it has been fairly well studied by the cytogenetic researchers. In a majority of these studies, a diploid chromosome number has been reported to be 2n=100 in common carp that is agree with Raicu *et al*., (1972);Denton, (1973); Zan & Song, (1980); Blaxhall, (1983); Labat *et.al.*, (1983); Rab *et.al*., (1989); Larka & Rishi, (1991); Anjum &Jankun, (1994) and Anjum, (1995). Diploid chromosome number of native carp from Amur river has been divided into eight well-defined groups on the basis of their morphology and a standard karyotype has also been proposed (Rab *et.al*.,1989). Karyotypic configuration also varied in several cases, mainly because of very small size of carp chromosomes which are very often very difficult to arrange in separate lasses. Silver-NOR staining has revealed an existence of two sub-metacentric chromosomes (belonging to two different pairs) having different sizes and bearing Nucleolus organizer regions (NORs) on their entire upper shorter arms. Similarly an expression of two functionally active NOR banding chromosomes has previously been detected by some researchers through the use of silver staining. (Takai &Ojima, 1982; Ruifang *et al*., 1985; Mayr *et al*., 1986; Sola *et.al*., 1986; Anjum, 1995).A number of diagnostic techniques have been developed and optimized for the differentiation of fish species in a variety of product types This study discusses the use of DNA-based techniques in the authentication of *Cyprinus carpio* fish; commercial applications of these techniques (PCR); online resources that provide support for fish and seafood species identification; and future trends in this field. The detection of species substitution has become an important topic within the food industry and there is a growing need for rapid, reliable, and reproducible tests to verify species in commercial fish. Increases in international trade and fish consumption, along with fluctuations in the supply and demand of different fish and seafood species, have resulted in intentional product mislabeling. The effects of species substitution are far-reaching and include economic fraud, health hazards, and illegal trade of protected species. Although the *Cyprinus carpio* specimens that used in this study appear in different morphs (wobbled among color, speared of scales and body architecture) but the cytogenetic and genetic results fixed that all the specimens of fish were same species.

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