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KAYROTYPE OF SOL (*CHANNA MARULIUS*) FROM INDUS RIVER, PAKISTAN

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ABSTRACT

Sol (*Channa marulius*, family: Channidae, order: Perciformes) is an important fish species indigenous to Indo-Pakistan sub-continent, and has a commercial value, adapted to survive in low dissolved oxygen. Three populations (Indus, Indian and Thailand) appear isolated and significant difference between Indus and Indian population has appeared in mansural characters. Karyological studies on *Channa marulius* suggest a diploid number of 44 for the species but the Indian population and Thailand population are different in number of metacentric and telocentric chromosomes. Sol samples (n =7, 15-20 cm) were collected from Head Tounsa (river Indus) and their gill tissues were removed, torn apart and left in hypotonic solution, fixed and spread over a glass slide, stained with aceto-orcein and studied under microscope (100 X). Study of 45 well spread metaphase suggested a diploid number of 44, 8 metacentric having arm ratio of around 2 and 36 telocentric. Present population shares 2n number of 44 with the stocks of the species present in India and Thailand, yet is different from two other stocks in respect of chromosome morphology (Indian: 40 metacentric + 4 telocentric; Thailand: 4 metacentric + 4 submetacentric + 36 telocentric), suggesting intraspecific differences probably caused by isolation.

INTRODUCTION

Fish karyotyping is commonly exploited to confirm taxonomic identification and inter-population variations and can play an important role in sex control, better performance, rapid production of inbred lines (Thorgard and Allen, 1987; Lin and Peter, 1991), detecting environmental mutagens and genotoxic pollutants (Kligerman *et al.*, 1975; Manna, 1989). A sizable literature on karyotype studies on Cyprinidae is available from different parts of the world (Al-Sabti, 1985; Zhang and Reddy, 1990; Fister, 1989; Boro, 2001; Khuda-Bukhsh and Tiwary1994; Khuda-Bukhsh, 1996; Khuda-Bukhsh and Chakrabarti, 1996, 1999) including Pakistan (Rab, 1981; Rafique, 1992; Rafique *et al.*, 2000).

(This paper is a part of Ph.D. thesis of principal author)

Indian snakehead or Sol (*Channa marulius*, family: Channidae, order: Perciformes) is an important fish species indigenous to Indo-Pakistan sub-continent, and has a commercial value, better adapted in low dissolved oxygen and is a potential fish for introduction in the aquaculture system of Pakistan. The species is distributed throughout the subcontinent from Thailand to the river Indus. Different populations of the species are isolated, yet no study is available about sub-specific variations. Comparative studies on the mansural (morphometric and meristic) characters are limited to decide upon sub-specific variations, though there are indications of the population surviving in the Indus river is significantly different in some mansural (morphometric and meristic) variables (Bhatti, 2012). Karyological studies suggest that the species has a diploid number of 44, however different populations differ in the types of chromosomes. Donsakul (1991) and Donsakul and Wichian (1991) recorded that the stock of *C. marulius* has 4 metacentric, 4 submetacentric and 36 telocentric chromosomes for the Thailand stock, while the Indian stock has 40 metacentric and 4 telocentric chromosomes. Present study is based upon the hypothesis that stock of *Channa marulius* inhabiting the Indus river has remained isolated from the stocks surviving in river systems of central and eastern parts of the Indian subcontinent. Isolation may have caused variation in mansural variables as well as in karyology. Present report pertains to the results of studies on karyology of stock inhabiting the Indus river.

MATERIALS AND METHODS

Preparation: Specimen of *C. marulius* (n =7, 15-20 cm long), collected from the River Indus (in/ around Head Tounsa, southwestern Punjab, Pakistan) identified to species level (Mirza and Sandhu, 2007), brought to the laboratory at Pakistan Museum of Natural History, Islamabad in stress free conditions and maintained in aquarium (30’ X 18’ X 12). These specimens were used for karyological studies using the technique employed by Oellermann and Skelton (1990) and Rafique (1992). Each fish was caught from aquarium by a scoop net, weighed using top loading balance (Sartorius, minimum count 0.1 g) and given 0.01 ml/ g body weight of 0.1% colchicines (100 mg/ 100 ml distilled water) solution through intramuscular route using 27 gauge syringe. Fish such treated were maintained in a separate aquarium under normal condition for 4–10 hours, when the gill arches

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were removed and gill tissue was torn in to minute pieces physically using a needle. Gill tissue thus removed was placed in 0.4% potassium chloride hypotonic solution for 40 minutes, allowing cells to swell up which result in better spreading of the chromosomes (Manna, 1989). Hypotonic solution was decanted and tissue rinsed in 25 ml of freshly prepared fixative (ethanol/methanol: glacial acetic acid, 3 : 1) and left in fixative overnight for complete fixation.

Glass slides were cleaned by boiling in detergent for 20 minutes, rinsed thoroughly in running tap water, passed through two changes of 90% ethanol, washed with distilled water and oven dried at 50 ^{o}C. Clean and dry slides were placed on a slide warmer adjusted at 60 ^{o}C. Fixed tissue was dropped on a clean and dry glass slide, allowed to dry, stained with aceto-orcein and covered with clean and dry cover slip. Prepared slides were examined under light microscope (Olympus BH-2, phase contrast, 100X oil emersion lens) and good preparations labeled and maintained for photography.

Cells having good metaphase spreads were photographed using 35 mm camera fitted with automatic film advance and exposure systems with built-in microcomputer on light microscope. Negatives were developed in pepitol developer for 2-3 minutes at 3,000 X. Photographs were then placed in fixative (250 g sodium thiosulphate + 50 g potassium meta-bisulphide + 5 ml glacial acetic acid + I L distilled water) for 30 minutes. Photographs were thoroughly washed in tap water to remove traces of developer/ fixative and dried on a photographic drier.

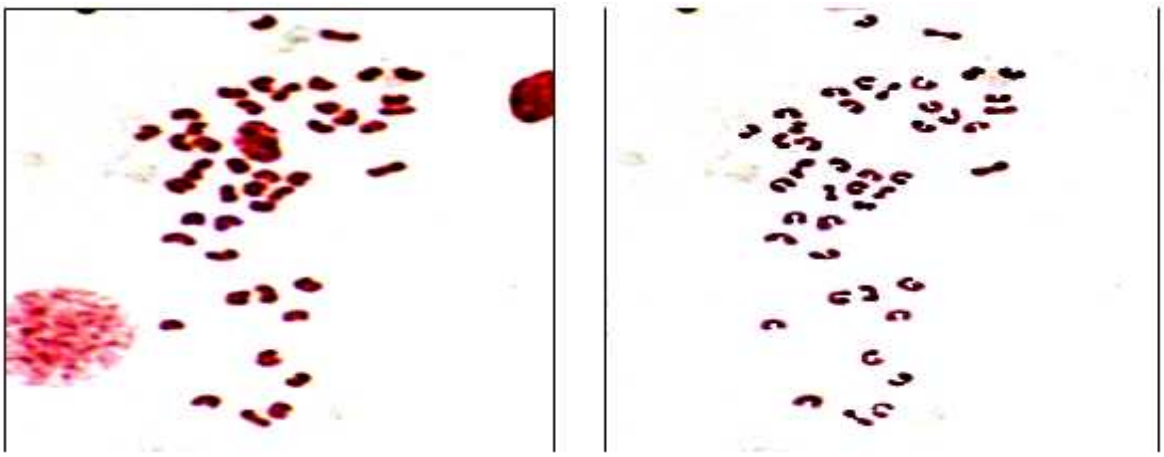
Analysis: Diploid chromosome number was determined by examining 45 photographs of well spread metaphases. Maximum number of chromosomes inmajority of the spreads was taken as diploid chromosome number of the species.

Homologous chromosomes were arranged on the basis of morphological similarity (size, position of centromere and secondary constriction). Homologous chromosome pairs were then arranged in decreasing order of their sizes, and location of centromere to develop karyotype for the species, and assigned number in consecutive order, the largest metacentric regarded as pair 1. Each chromosome of the karyotype was also assigned a specific number in consecutive order of its arrangement. Individual chromosome and homologous pair were classified as metacentric, submetacentric or telocentric, based upon the relative centromeric location.

RESULTS

Plate 1 presents microphotograph of the mitotic chromosome spread of *C. marulius* collected from Tounsa Barrage (river Indus). Count of chromosomes of 45 good spreads of chromosome suggested a modal 2n number of 44, which could be organized into 22 pairs (n = 22).

Karyotype developed for the present population of the species (Fig. 1) suggests that 4 homologous pairs (8chromosomes) are metacentric, with an arm ratio of around 1, and 18 pairs (36 chromosomes) are acrocentric chromosomes. The fundamental arm number for the population under present analysis has been calculated as 52 (2 X # metacentric + # acrocentric chromosomes).



(a) **(b)**

Plate No. 1: Mitotic metaphase spreads of *Channa marulius* captured from Head Taunsa (southern Punjab, Pakistan) a. without clearing, b. after clearing

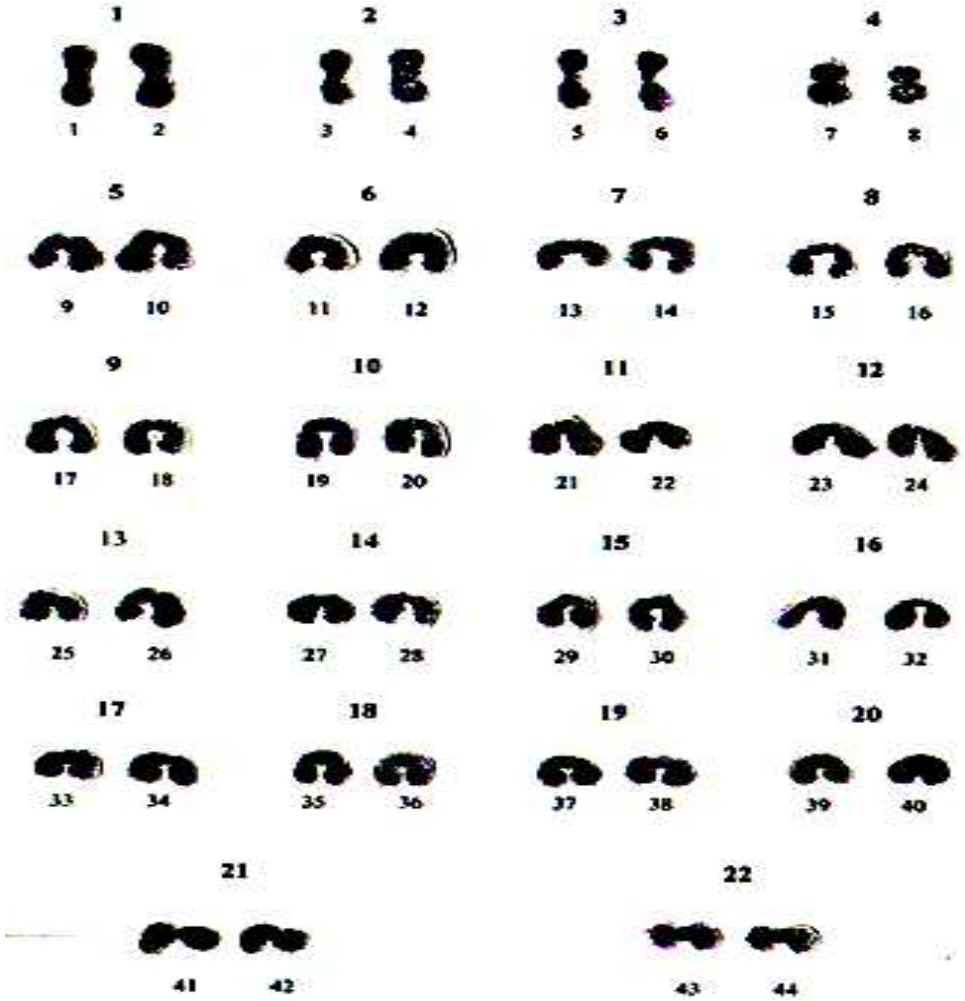


Figure 1: Karyotype of *Channa marulius* captured from Head Taunsa (southern Punjab, Pakistan). (bold numbers above denote the numbers assigned to homologous pair and small numbers below denote the numbers assigned to individual chromosome).

DISCUSSION

Two previous studies available on the karyology of *C. marulius* (Donsakul, 1991; Donsakul and Wichian, 1991; NBFGR, 1998) are in line with the present results in suggesting that the species has a 2n number of 44 which indicates that the species over the range of its distribution maintains a diploid number of 44 (n = 22). On the other hand this also supports the taxonomic identification of the stock of fish in this analysis as *C. marulius*, as has been suggested by our previous observations on general morphology along with morphometric and meristic analyses revealing that the sample exhibited a good degree of similarity in majority of variables to justify its assignment to *C. marulius* (Bhatti, 2012).

Present results on the types of chromosomes present in the karyotype of this stock of *C. marulius* are not in conformity to two previous studies available on this species. Present analysis reveals 8 metacentric chromosomes (arm ratio around 1) and 36 acrocentric chromosomes (arm ratio around 0). This part of present results is not in conformity on any previous study. No study is available on the population of sol inhabiting on river Indus waters. Studies on Thailand stocks suggests predominance of telocentric chromosomes (36) with 4 submetacentric and 4 metacentric ones (Donsakul, 1991; Donsakul and Wichian, 1991). Karyological studies on the population of Indian species revealed 40 metacentric and 4 telocentric chromosomes (NBFGR, 1998). Population of *C. marulius* inhabiting the Indus river seems therefore closer to its Thailand counterpart, compared to Indian population. Intrapopulation differences have also been indicated between the Indian and Indus populations in different mansural characters (Bhatti, 2012), which appears confirmed through the karyological variation between populations. No study is available on mansural variables of Thailand population

and hence possible level of morphometric / meristic similarities with Indus population. A number of studies are available on different species, where diploid chromosome number remains same, yet the relative number of different types of chromosomes is different for different populations (Rishi, 1976; Tripathi and Sharma, 1987; Rafique, 1992). Though such discrepancies can be ascribed to excessive chromosomal contraction as a consequence of higher dose or over-exposure to cholchicine, improper fixation of tissue, methods of chromosomal preparation and classification (Zhang and Ruddy, 1990), yet these variations may also arise from chromosomal aberrations/rearrangements followed by population isolation during evolutionary process. Chu and Bender 1961; Campos and Cuevas 1997).

Channidae is one of the advanced families of freshwater teleost fishes. This family originated in the south Himalayan region of Indian subcontinent some 50 million years ago, during the early Eosine epoch (Bohme, 2004). This advancement of family is well reflected by its karyotype to have all 40 metacentric chromosomes, as against 4 telocentric chromosomes in Indian population, considered to be most primitive (Morescalchi, 1975; Campos and Cuevas, 1977). The presence of majority of telocentric chromosomes in karyotype of Indus and Thailand populations of *C. marulius* (Donsakul, 1991; Donsakul and Wichian, 1991) is difficult to explain, except that these populations may have secondarily derived from more advanced populations present in central parts of the Indian subcontinent, from where this species may have originated.

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