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Heteromorphic NORs in Two Indian Catfishes Becoming Endangered Teleostei: Siluriformes)

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Abstract: Somatic chromosomes and localization of nucleolar organizer regions in two vulnerable Indian catfishes, belonging to order Siluriformes have been analyzed. The diploid chromosome number in *Mystus gulio is* 58 (22m + 36t) with FN=80 and *Ompok bimaculatus is* 42 (12m + 12sm + 6st + 12t) with FN=66. Both the species showed a single pair of heteromorphic NORs at the terminal regions. However, in *M. gulio* these were present on the telomeric regions of the long arms of acrocentric pair, which is a unique feature. This suggests that this species is not closely related to any of other. The heteromorphic of NORs found during the present investigations in both the species, may either be functional or may even be structural.

Key words: NORs, catfishes, karyotype.

Introduction: The NOR-Silver staining method of the mammalian chromosomes were applied for the first time in the case of chromosomes of a fish, *Fundulus* (Howell and black, 1980). The visualization of nucleolus organizer regions (NORs) in the chromosomes is an important parameter adding to the structural details of the karyotype. NORs are species specific and showed marked diversity in different worked out fish species. Closely related species with very similar karyotype also may show different NOR sites. Therefore, it is quite clear that NORs can serve as an important aid for analyzing species differentiation and evolution of particular group of animals. Moreover, variation in NORs within a species helps is studying the nature of NORs themselves (structure, function and evolution of ribosomal genes) and genetic polymorphism.

In Indian fishes, some work on the distribution of NORs has been done earlier (Rishi and Thind, 1992; 1994; Rishi *et al.*, 1994; 1995; Barat *et al.*,1990; Neeru, 2014, Neeru *et al.* 1018; Verma *et al.* 2020). But there is a need to analyze more fish species for NORs. Presently silver-staining for observing NORs was successfully employed in two Indian catfishes, *Mystus gulio* and *Ompok bimaculatus.* Both these species becoming endangered and show rare presence in fish catches.

Materials and Methods: Nine specimen of *M. gulio* (5 males and 4 females) were obtained from Poonthura estuary near Trivendrum in Kerala state. Ten specimens of *Ompok bimaculatus* (5 males and 5 females) were collected from Jyotisar near kurukshetra in Haryana state. The preparation of mitotic chromosomes were obtained from total kidney cells by well-known colchicine-hypotonic-acetic methanol - air drying method. Ag- staining was performed by the method of Howell and Black (1980).

Results: ***M. gulio:*** Majority of the somatic metaphases revealed 2n=58 in both the sexes. Their karyotype comprised 22 metacentrics and 36 telocentrics with FN=80. Clear dark and large sized NORs were observed in Ag-stained metaphase plate (Fig. 1). The NOR bearing chromosomes placed at 4th position in the karyotype in both the sexes. These NORs were present at the telomeric region of the acrocentric pair, one of them was larger in size in both the sexes (Fig. 4 & 5).

O. bimaculatus: Both the sexes of this species revealed a 2n=42. The karyotype consisted 12 metacentrics, 12 submetacentrics, 6 subtelocentrics and 12 telocentric with FN=66. Silver-staining revealed the presence of NOR-active sites only on a single pair (Fig. 2 & 3). The NOR bearing chromosomes are 3rd acrocentric pair, however one of the elements in this pair showed remarkably larger NOR in both the sexes (Fig. 6 & 7).

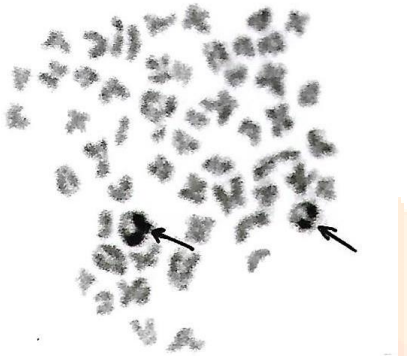

Fig 1: Ag-stained chromosomes of male *Mystus gulio*

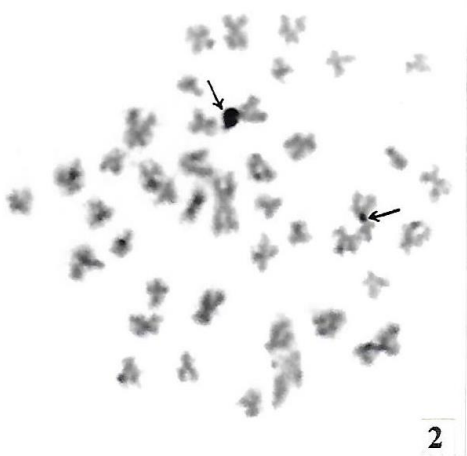

Fig 2: Ag-stained chromosomes of male *Ompok bimaculatus*.

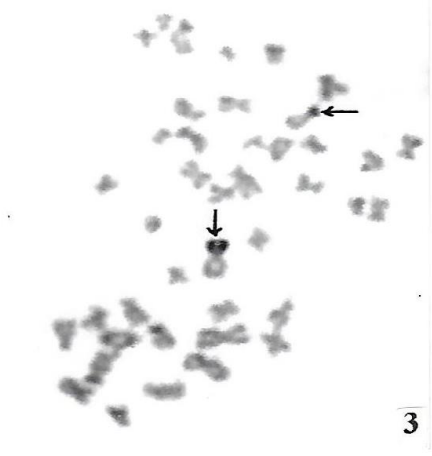

Fig 3: Ag-stained chromosomes of female *Ompok bimaculatus*.

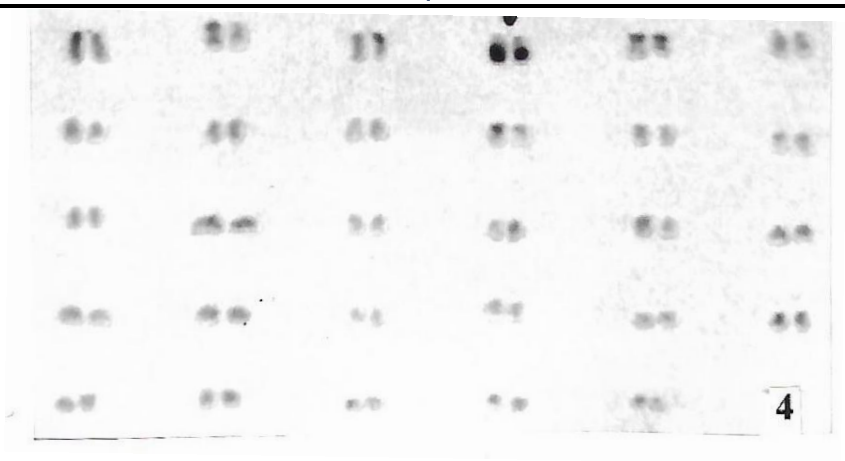

Fig. 4 Ag-stained chromosomes of female *Mystus gulio*.

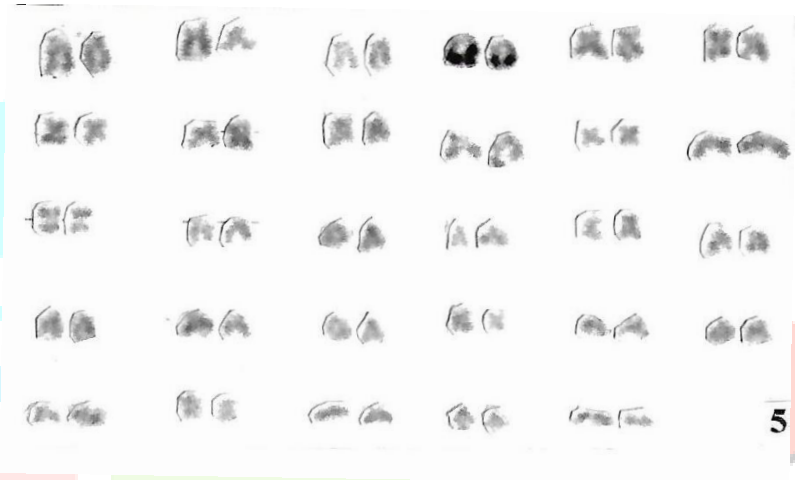

Fig. 5 Ag-stained chromosomes of male *Mystus gulio*.

Fig. 6 Ag-stained chromosomes of female *Ompok bimaculatus*.

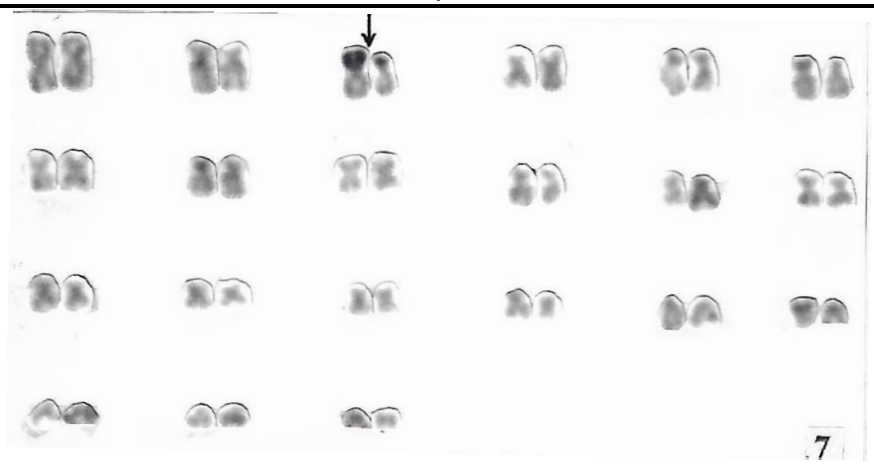

Fig. 7 Ag-stained chromosomes of female *Ompok bimaculatus*.

Discussion: Most species of fishes have only small NORs on a single chromosome pair (Amemiya and Gold.,1990; Das and khuda-Bukhsh, 2003; Gold and Amemiya, 1986; Rab et el., 2002). This usual condition, therefore, has been considered as fundamental and original in fishes by Takai and Ojima (1986). The larger NORs found in some Cyprinid forms and Gymnotiform species (Rabova *et al*. 2003; Rishi and Thind, 1992.) This could appear as a result of an increase in DNA content by accidental translocation, duplication or other mechanism. Takai and Ojima (1986) observed that the NOR bearing chromosomes showed various forms among different species but most of them belong to acro or subtelocentric type (54%) and some to submetacentric type (35.7%). During present investigations NORs were found to be located on the single acrocentric pair in both the species (*M.gulio* and *O. bimaculatus)*.

Different workers explained the varied locations of NORs on the chromosomes of various species. Presently both the species revealed the terminal location of the NORs on the chromosomes, however *M. gulio* have been observed to possess a unique location of NORs at telomers of the long arm of the acrocentric pair. The frequency of the occurrence of this type was observed 4% (Takai and Ojima, 1986). The unequal size and telomeric location of NORs in *M. gulio* suggest that this species is not closely related to any of other.
In *O. bimaculatus* NORs have been found to be located on short arm of acrocentric. The frequency of the occurrence of this type was found to be highest 64% (Takai and Ojima, 1986).

Heteromorphism of NORs seems to be very common in fishes (Gold, 1984; Amemiya and Gold, 1986; Phillips et. al., 1986) Polymorphism may be due to the variable distribution and activity of ribosomal cistrone (Takai and Ojima, 1986; Foresti *et. al.*, 1989). As stated by some earlier workers this heteromorphism may either be functional (when one NOR show more activity) or it may even be structural due to the presence of more - DNA cistron on one of the two homologues.

Conclusion: The presence of darkly stained heteromorphic NORs on the telomeric region of the long arms of the chromosome as detected in *M. gulio* by Ag- NOR staining reveals the distant relation with other species. NORs found in *O. bimaculatus* also showed heteromorphism but their presence on short arm of acrocentric pair is a common feature found in other species also. Study of fish Chromosome is significant as it provides basic data for chromosomal manipulation and hybridization techniques, which are commonly used to get the hybrid vigor to increase fish production.

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