CYTOGENETIC STUDIES OF FISH SPECIES *HORABAGRUS NIGRICOLLARIS*, *PUNTIUS DENISONII* AND *PUNTIUS SARANA SUBNASUTUS* ENDEMIC TO THE WESTERN GHATS

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The Western Ghats of India is well known for its rich diversity of fish germplasm resources. The World Bank has identified the Western Ghats as one of the 25 globally recognized biodiversity hotspots with high levels of endemism as well as rich and varied species biodiversity including fresh water teleosts (18). Information on fish genetic resources is essential for undertaking fisheries conservation and management programme at ecoregional level (22). So far 287 freshwater fish species, belonging to 40 families have been reported from this region (8), which includes 153 species of cultivable food, sports and ornamental fishes endemic to this area, with 24 endemic species being listed as critically endangered (22).

Cytogenetic markers have been considered as authentic tools for characterization of species as well as to screen putative hybrids (1,16,17). Comparison of chromosome number and structure between different species reveals phylogenetic relationship between different species and throws light on their karyo-evolution. Cytogenetic studies can also be helpful in planning conservation strategies for endangered fish species. At present only 24 fresh water fish species have been cytogenetically studied from the Western Ghat region, with information on nucleolus organizer region (NOR) in fewer species (19). With this view, cytogenetic studies have been carried out in three endemic species of fresh water teleost namely *H. nigricollaris* (Pethiyagoda & Kottelat), *Puntius denisonii* (Day) and *P. sarana subnasutus* (Valenciennes) for which information is lacking (8). The brief descriptions of the three species are as follows:

Horabagrus nigricollaris (Pethiyagoda & Kottelat) commonly known as Majaletta or Manjakoori belongs to family Bagridae, is a critically endangered yellow catfish confined to the Chalakudy River in Kerala and enjoys a good market value as an ornamental fish. Due to over-exploitation for export purpose, the species has become extremely rare. Due to the restricted distribution and rare occurrence it has been listed as critically endangered based on International union for conservation of nature and natural resources (IUCN) categorization (2).

Puntius denisonii (Day) belonging to family Cyprinidae is a most attractive barb, endemic to the Kerala part of Western Ghats and

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commonly known as Denison's barb or *Red line torpedo* or "*chenkaniyan*" in vernacular. Owing to its restricted distribution only in a few rivers like Periyar, Chalakkudy, Kallada and Valapatanam and due to over-exploitation for export purpose as a highly valuable aquarium fish, this species has been enlisted as endangered (2).

Puntius sarana subnasutus (Valenciennes) is popularly called as 'peninsular olive barb' and vernacularly *'Kurichi'* or *'Kururva'* (family Cyprinidae). Based on detailed morphometric analysis four distinct sub-species of *P.sarana* have been reported namely *P.sarana sarana* (Hamilton), *P.sarana orphoides* (Valenciennes), *P.sarana spilurus* (Gunther) and *P. sarana subnasutus* (Valenciennes) (4). *P. sarana subnasutus* is endemic to Krishna and Cauvery River system and rivers of Kerala and attains a total length of 35cm. This is one of the highly preferred food fish of Central and Southern Kerala and its juveniles are exported as ornamental.

Materials and methods

Live specimens of *H. nigricollaris* (n=7), *P.denisonii* (n=7) and *P.sarana subnasutus* (n=6) were collected from Meenachil and Chalakudy rivers of Kerala, India. All the specimens were in juvenile stage with undifferentiated gonads. Fishes were administered intramuscularly with 0.05% colchicine (1.0 ml /100g body weight) to halt the nuclear division and maintained alive for two hours in a plastic tub. The specimens were then sacrificed and the kidney tissues were processed for chromosome preparations using hypotonic treatment - acetic acid - methanol fixation - flame-drying technique. The chromosome slides were stained with 4% Giemsa in phosphate buffer (pH 6.8). Ag-NOR banding was carried out according to the method of Howell and Black (9) with minor

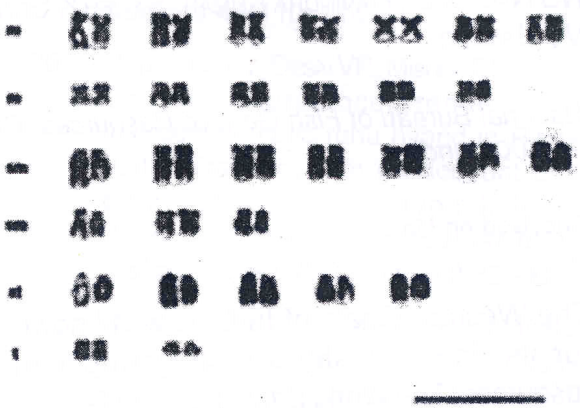

Fig 1. Karyotype of H. nigricollaris

modifications. NOR band pattern was determined in each species by studying a minimum of 25 metaphase spreads per specimen of each species. For karyotyping, chromosomes were grouped into metacentric (m), submetacentric (sm), subtelocentric (st) and telocentric (t) as per the classification proposed by Levan *et al.* (15).

Results and discussion

In *H. nigricollaris* the diploid chromosome number was found to be 2n=60 and the chromosome formula (C.F.) was derived for this species as 26m+20sm+10st+4t (FN=106) based on the chromosome morphology (fig 1). A similar chromosome number has been reported for another closely related species *H.brachysoma* but there existed a minor variation in karymorphology with C.F= 28m+ 20sm+ 8st+ 4t (FN=108) (19).

The other two species *P. denisonii* and *P. sarana subnasutus* were found to possess similar diploid chromosome number of 2n= 50; however, interspecies variation in karyomorphology has been observed between the two species with chromosome formula (CF) 4m+20sm+18st+8t (FN=74) and 12m+26sm+8st+4t (FN=88), respectively (fig 2, 3). Our results are in

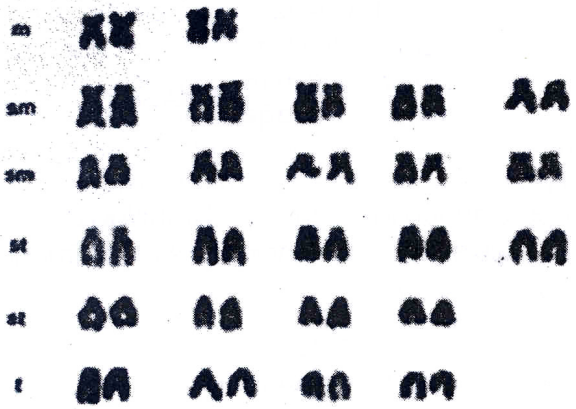

Fig 2. Karyotype of P. denisonii.

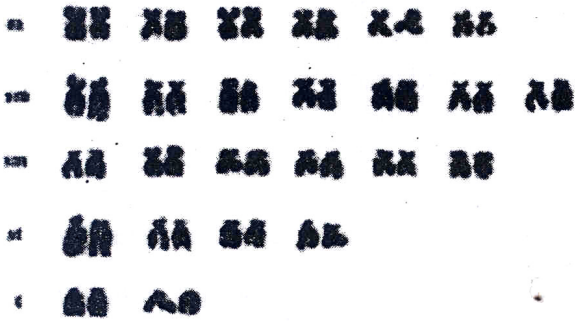

Fig 3. Karyotype of P. sarana subnasutus

agreement with chromosome number reported for another subspecies *P.sarana* (Hamilton) (13, 21). However, the C.F. (12m+14sm+12st+12a) reported for this subspecies (21) was in variation with our findings. Thus, the results of present investigation supported the existence of genetically distinct separate subspecies of *P. sarana.*

The nucleolus organizer regions (NORs) are the chromosomal sites of genes, which transcribe for 18S and 28S ribosomal RNA, which were presumably transcribed at preceding interphase and are important in view of their intimate relationship with protein synthesis (9,10) .The development of silver staining technique (7,11) to detect metaphase chromosome sites of NORs has

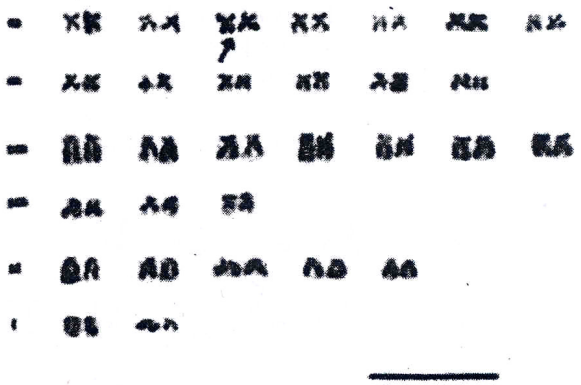

Fig 4. Karyotype of H. nigricollaris *showing Ag-NOR banding* (→)

greatly facilitated comparative studies of NOR variation within and between species and in studying cyprinid phylogenetics and systematics (5).

Ag-NOR staining in *H. nigricollaris* revealed presence of NORs on one pair of chromosome (3rd metacentric) (fig 4), while in *P. denisonii* and *P. sarana subnasutus* NORs were found to be localized terminally on shorter arms of 4 pairs of chromosomes with inter-species variation in their position. In *P. denisonii* prominent Ag-NORs were found to be localized on 1st submetacentric, 1st st, 4th st and 7th subtelocentric chromosomes (fig 5), while in *P. sarana*

Fig 5. Karyotype of P. denisonii *showing Ag-NOR banding* (→)

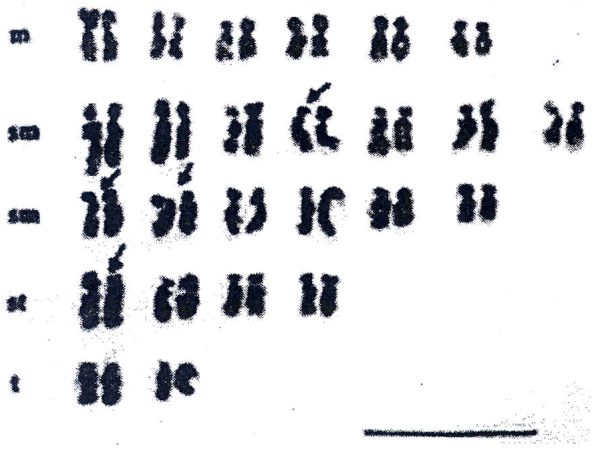

Fig 6. Karyotype of P. sarana subnasutus *showing Ag-NOR banding* (→)

subnasutus 4th, 8th, 9th submetacentric and 1st subtelocentric chromosomes exhibited Ag-NORs terminally (fig 6). In fishes, presence of single and small NOR on chromosome was considered as fundamental and original distribution of NOR, while multiple and large NORs are derived type (6). Thus, *H.brachysoma* can be considered as ancestral fish species as compared to *Puntius*, which exhibited large and multiple NORs. Presence of multiple NORs seems to the characteristic of *Puntius* species since *P. filamentosus,* another endemic species from Western Ghats, also possessed NORs on four pairs of chromosomes (19). Takai and Ojima (23) pointed out that multiple NORs could have been formed through partial translocation of NOR to another chromosome site or through polyploidization.

Information on cytogenetic markers has been found to be valuable in resolving taxonomic ambiguities between morphologically alike, closely related species (14). Since occurrence of interspecific and intrageneric hybrids is relatively common in fishes as compared to plants and animals (3,12,20), cytogenetic markers can aid in identification of putative hybrids. This is the first report on cytogenetic characterization of these three freshwater species endemic to the Western Ghats.

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Summary

Cytogenetic studies have been undertaken in three endemic fish species of the Western Ghats viz., *Horabagrus nigricollaris, Puntius denisonii* and *Puntius sarana subnasutus* by conventional karyotyping and nucleolus organizer regions (NOR) banding. Among them *Horabagrus nigricollaris, Puntius denisonii* have been reported to be endangered species. In *H. nigricollaris* the diploid chromosome number was 60 with

Table 1 *Karyomorphology and Ag-NOR banding in three endemic species from Western Ghats*

Species	Common name	2n	Chromosome formula	FN	Chrs. with Ag-NORs
H.nigricollaris	Yellow cat fish	60	26m+20sm+10st+4t	106	1 pair (3rd m)
P.denisonii	Denison's barb, Red line torpedo	50	4m+20sm+18st+8t	74	4 pairs; 1st sm, 1st st, 4th st, 7th st
P.sarana subnasutus	Peninsular olive barb	50	12m+26sm+8st+4t	88	4 pairs; 1st st, 4th sm, 8th sm, 9th sm

CF = 26m+20sm+10st+4t (FN=106). Both *P. denisonii* and *P. sarana subnasutus* possessed similar diploid chromosome number 2n= 50, however, interspecies variation in karyomorphology was noticed, with chromosome formula (CF) 4m+20sm+18st+8t (FN=74) and 12m+26sm+8st+4t (FN=88), respectively. Ag-NOR staining revealed presence of NORs on 3rd metacentric chromosome in *H. nigricollaris*, while in *P. denisonii* and *P. sarana subnasutus* NORs were localized terminally on 4 pairs of chromosomes, with inter-species variation in their position .In *P. denisonii* the Ag-NORs were found on 1st submetacentric and 1st, 4th and 7th subtelocentric chromosomes, while in *P. sarana subnasutus* 4th, 8th, 9th submetacentric chromosome and 1st subtelocentric chromosomes exhibited Ag-NORs terminally.

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