Materials and methods

Live specimens of *C. collare* (n=6) and *S. insularis* (n= 3) were collected from Kovalam Beach area in Tiruvananthapuram. All the specimens were immature and the sex could not be identified. Fishes were administered intramuscularly with 0.05% colchicine (1.0 ml / 100g body weight) to stop the nuclear division and maintained alive for two hours in a plastic tub. The specimens were then sacrificed and the kidney and gill 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 (*p*H 6.8). The chromosome slides were also stained by silver staining technique for detection of nucleolar organizer region (NORs). Ag-NOR banding was carried out according to the method of Howell and Black (1980) with minor modifications for getting crisp and clear banding effects. 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.* (1964).

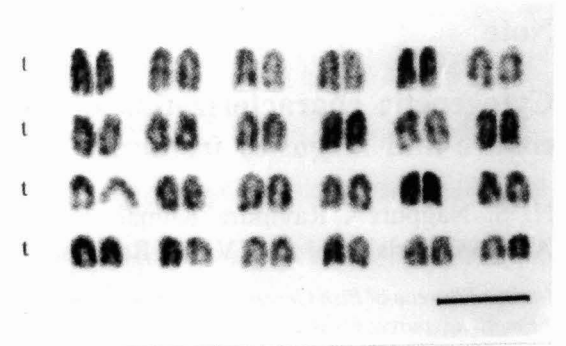

Fig. 1. Karyotype of *C. collare* (Bar = 10 μm)

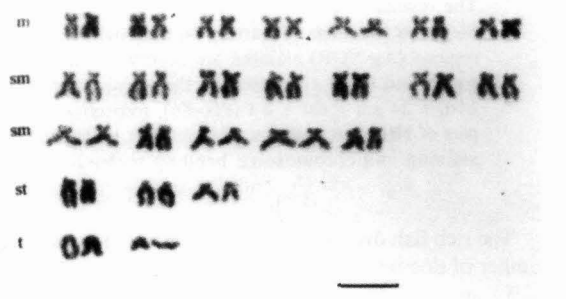

Fig. 2. Karyotype of *S. insularis* (Bar = 10 μm)

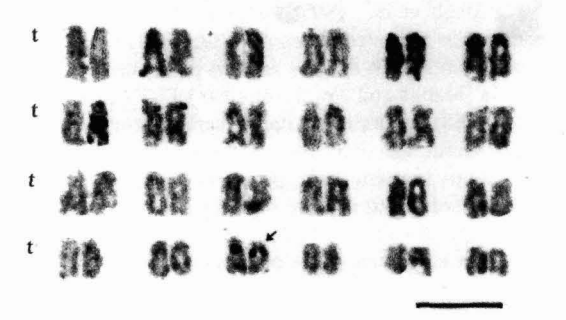

Fig. 3. Karyotype of *C. collare* showing Ag-NOR banding (Bar = 10 μm)

Results and discussion

The diploid chromosome number was found to be 48 in both the species with presence of variation in the chromosome morphology between them. In *C. collare*, all the chromosomes were telocentric in morphology (Fig.1), the karyotype formula was KF= 48t (FN= 48). The karyotype of *S. insularis* consisted of 14 metacentric, 24 submetacentric, 6 subtelocentric and 4 telocentric chromosomes (Fig.2), thus the karyotype formula was derived as KF= 14m+24sm+ 6st +4t with fundamental arm number (FN) as 86. The chromosome number of 2n=48 as found in these two species is similar to majority of marine fish species so far studied in India (Rishi and Haobam, 1984; Chakraborty and Kagwade, 1989; Lakra and Rishi, 1991). Presence of chromosome number 2n= 48, with all telocentric chromosomes seems to be characteristic of majority of marine species (Singh *et al.*, 1994). Among the non native fishes, approximately 60 % of the perciform species so far studied show a karyotype of characterized by 48 uni-armed (telocentirc) chromosomes (Galetti Jr. *et al.*, 2000). Presence of all uni-armed chromosomes has been considered as primitive character (Gold and Amemiya, 1986; Ozouf-costaz *et al.*,1997), hence *C. collare* can be considered as primitive in the evolution order owing to the presence of 48 telocentric chromosomes. On the contrary, *S. insularis* seems to be in advanced stage of karyoevolution as it possessed more bi-armed chromosome similar to fresh water fishes.

The nucleolus organizer regions (NORs) are the chromosomal sites of genes, which transcribe for 18S and 28S ribosomal RNA, that were presumably transcribed at preceding interphase and are important in view of their

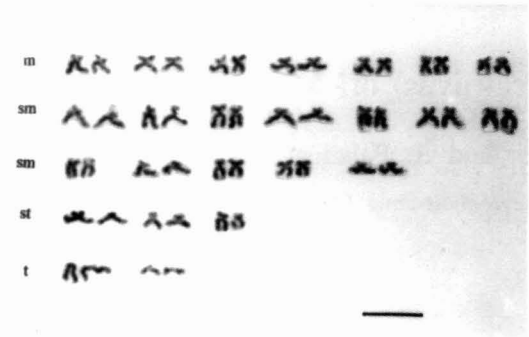

Fig. 4. Karyotype of *S. insularis* showing Ag-NOR banding (Bar = 10 μm)

intimate relationship with protein synthesis (Howell, 1977, 1982). The development of silver staining technique (Goodpasture and Bloom, 1975; Howell and Black, 1980) to detect metaphase chromosome sites of NORs has greatly facilitated comparative studies of NOR variation within and in between species and in studying cyprinid phylogenetics and systematics (Gold, 1984).The silver staining revealed the presence of NORs on interstitial region of 21st pair of telocentric chromosome in *C. collare* (Fig.3). The position of NORs in this species is rarely found and could be the result of pericentric inversion during the course of evolution (Klinkhardt, 1998). In *S. insularis*, Ag- NORs were present terminally on one pair of short arm of 1st subtelocentric chromosome (Fig.4) which is predominant NOR location in hitherto studied fish species. Such studies are useful in resolving taxonomic ambiguities among closely related fish species and can also throw light on karyo-evolution and speciation of the marine species. Cytogenetic markers have found useful for identification of intra-specific stocks and populations in some fish species (Phillips *et al.*, 1988) and can also aid in identification of putative hybrids between closely related species. Information on the cytogenetic markers can also be used for molecular cytogenetic assignment of genes on chromosomes (Donate *et al.*, 2003). To date, there seems to be no information on karyomorphology and chromosome banding studies. This study describes, for the first time, the karyotype and the localization of NORs in the two marine fish species from the Western Coast of India.

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