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Note

Cytogenetic characterization of two marine ornamental fishes, *Chaetodon collare* and *Stegastes insularis*

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Abstract

The results of the Cytogenetic studies carried out in two marine ornamental fishes, *Chaetodon collare* and *Stegastes insularis*, occurring in the south-eastern Arabian Sea, by Giemsa staining and nucleolar organizer regions (Ag-NOR) staining are reported. The diploid chromosome number (2n) in *C. collare* and *S. insularis* was found to be 48. Based on the karyomorphology, the karyotype formula was derived as 48t (FN=48) and 14m + 24 sm + 6st + 4 t (FN=86), respectively for these two species. Silver staining revealed presence of one pair of NORs in both the species. This is the first description of karyotypes in these species using conventional staining and chromosome banding techniques.

The rich fish diversity of India is reflected in its large number of documented 2163 fish species of which 1370 (63%) are from the marine ecosystem. So far, marine fishes have not received much deserved attention towards their genetic characterization and only about 40 of them have been cytogenetically characterized (Rishi and Haobam, 1984; Chakrabarty and Kagwade, 1989; Lakra and Rishi, 1991; Singh *et al.*, 1997; NBFGR 1998). Cytogenetic markers have been considered as authentic tools for characterization of fish species as well as to screen putative hybrids (Manna and Khuda-Bukhsh,1974; Amemiya and Gold, 1988). In addition, these markers have also been found useful for detection of intraspecific stocks and populations in some fish species (Phillips *et al.*, 1988) and in resolving taxonomic ambiguities between some species (Kushwaha *et al.*, 2001). Further, karyotypic information can throw light on phylogenetic relationship between different species and karyotype evolution in fish species. In the present scenario of WTO and IPR regime, genetic characterization of fish fauna could be of prime importance. In view of the above, cytogenetic characterization has been carried out in two marine fish species viz., *Stegastes insularis* and *Chaetodon collare*. The brief description of these fishes is as follows.

Chaetodon collare Bloch, 1787 (Butterfly fish)

Chaetodontids form one of the most colourful fishes of the coral reefs with a fantastic range in colour and pattern. These are very popular as marine aquarium fishes. Commonly known as butterfly fish, *C. collare* is distributed in Indo-West Pacific region from Persian Gulf and Maldives to Japan and Indonesia and inhabits in depths ranging from 03-15 meters. The species is recognized by its bright red coloration on the tail, which is green in juveniles. They are seen in pairs or several aggregations in coral reefs and juveniles are exported in large numbers to Western countries for aquarium trade, even though acclimatizing these fishes in aquarium conditions is often difficult.

Stegastes insularis Allen & Emery, 1985 (Damsel fish)

S. insularis form one of the colourful fishes of the coral reefs with pale blue-grey to charcoal body colour and dusky grey scale margins appearing as series of narrow transverse bands. The posterior most part of body, caudal peduncle, caudal fin and posterior part of dorsal fin are bright yellow, bluish or lavender. The fish belongs to the family Pomacentridae under the order Perciformes. These are very popular as marine aquarium fishes and are distributed in Eastern Indian Ocean and in India it is reported from Vizhinjam (Kerala), Mandapam and Tuticorin (Tamil Nadu). The species was identified as a distinct one by Allen & Emry only in 1985 from Indo-Pacific and there is no information on the karyomorphology of the species till date. This occasionally schooling species inhabits shallow rock and coral reefs. It is one of the most sought and widely exploited species by aquarists.

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).

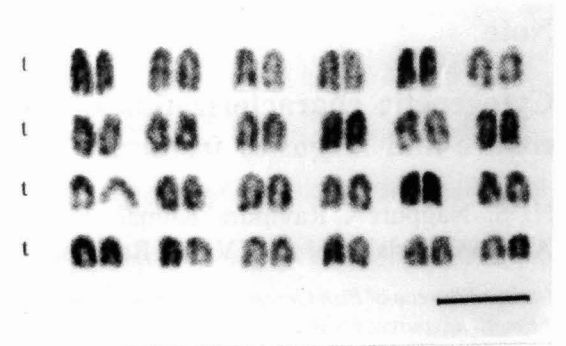

Fig. 1. Karyotype of *C. collare* (Bar = 10 μm)

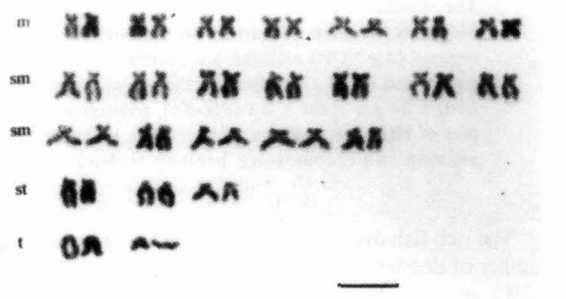

Fig. 2. Karyotype of *S. insularis* (Bar = 10 μm)

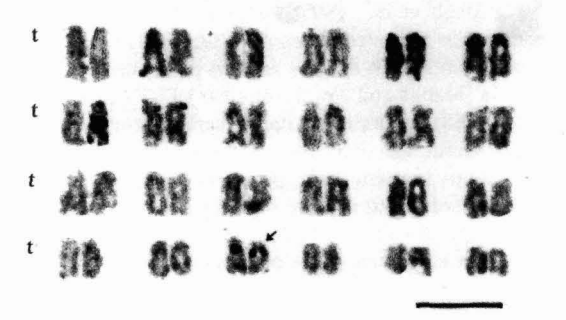

Fig. 3. Karyotype of *C. collare* showing Ag-NOR banding (Bar = 10 μm)

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

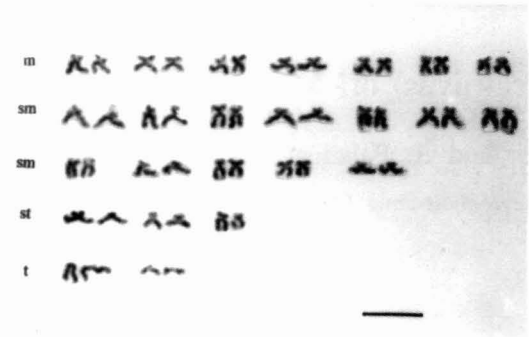

Fig. 4. Karyotype of *S. insularis* showing Ag-NOR banding (Bar = 10 μm)

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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