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Chromosomal studies of three vulnerable marine fishes from west coast of India

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Cytogenetic profiling was carried out in three vulnerable marine ornamental fishes, namely *Thalassoma lunare, Zanclus cornutus* and *Arius subrostratus*, using silver nitrate, chromoycin A3 CMA_3 staining and C- banding techniques to study the variation in localization of NORs and C-bands. Karyotype analyses of these species revealed a diploid chromosome number of 48, all acrocentrics, in *T. lunare* and *Z. cornutus*. In *A. subrostratus*, however, the diploid chromosome number was found to be 58 consisting of 22 metacentric, 16 submetacentric, 10 subtelocentric and 10 telocentric chromosomes with fundamental arm number of 96. The silver stained NORs were observed on 3 pairs of chromosomes in *T. lunare*, whereas other two species possessed NOR on single pair of chromosome. Within the species, there was complete concordance in number and position of NORs as detected by $AgNO_3$ and CMA_3 staining. Prominent constitutive heterochromatic bands were detected on 4, 2 and 3 pairs of chromosomes, respectively, in *T. lunare*, *Z. cornutus* and *A. subrostratus*. There was variation found in the number and position on NORs and C-bands among these species, which could be used as species-specific markers. This study describes for the first time the cytogenetic profiling in *Z. cornutus* and *A. subrostratus*.

[Keywords: Ag-NORs; C-bands; Chromomycin A_3; Cytogenetics; Marine fishes].

Introduction

Though India has rich diversity of ichthyofauna, merely about 40 species from marine ecosystems have been cytogenetically characterized[1]. In the present scenario of WTO and IPR regime, genetic characterization of fish fauna has gained utmost importance. The karyotypic information can throw light on genetic characterization, phylogenetic relationships among species and karyotype evolution in fishes. In view of the above, the present cytogenetic studies have been carried out in three marine fish species viz., *Thalassoma lunare, Zanclus cornutus* and *Arius subrostratus* so as to facilitate conservation and management of natural stocks of these species.

Materials and Methods

Six live specimens each of *T. lunare* and *Z. cornutus* were collected from Kovalam area, Kerala whereas specimens of *A. subrostratus* (n=5) were collected from Vypeen Island, Cochin, Kerala. The average wet weight of the specimens of *T. lunare* was 92 g (range 75-100) whereas those of *Z. cornutus* and *A. subrostratus* were 21.8 g (range 20-25) and 23 g (range 20-25), respectively. All the specimens were in juvenile stage and the sex was unidentifiable by visual examination.

For preparing chromosome slides, the specimens were administered intramuscularly with 0.05% colchicine @1.0 ml/100 g body weight and were sacrificed after 2 h for dissecting out the kidney tissue. The tissue was homogenized in 0.56% KCl and further processed for chromosome preparations using fixation (acetic acid-methanol fixation) and flame drying technique. The chromosomes were stained with 4% Giemsa in Phosphate buffer (pH 6.8). Approximately, 200 chromosome complements were analyzed for establishing the modal chromosome number and characteristic chromosome morphology of each species. For karyotyping, chromosomes were grouped into metacentric (m),

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submetacentric (sm), subtelocentric (st) and telocentric (t) as per the classification proposed by Levan *et al.*[2]

Localization of nucleolar organizer regions (NORs) was studied by both silver nitrate ($AgNO_3$) impregnation and chromoycin A3 CMA_3 staining techniques. Ag-NOR staining was performed according to the method of Howell and Black[3]. For CMA_3 staining, the method described by Ueda *et al.*[4] was followed. C-banding was carried out as per the technique described by Sumner[5] with staining of slides by propidium iodide (5µg/ ml in DDW) instead of Giemsa as described by Fontana *et al.*[6] A particular band pattern was determined by studying a minimum of 20 metaphase spreads per specimen.

Results

Chromosome number was conserved in both the perciform species, viz. *T. lunare* and *Z. cornutus*, as evidenced by the model diploid number of 48 acrocentric chromosomes (FN= 48) (Figs. 1a, b). In the catfish *A. subrostratus*, however, the diploid chromosome number was 58 with karyotype formula 22m +16sm+10st+10t (FN= 96) (Fig. 1c).

The silver-stained NOR indicates the transcriptional activity of NORs. The Ag-NOR staining of chromosomes revealed variation in number and position of NORs among these species. In *T. lunare*, the silver stained NORs were present on terminal portions of 3 pairs of chromosomes, i.e. p arm of 4th, 12th and 18th chromosomes (Fig. 2a). In both *Z. cornutus* and *A. subrostratus*, the NORs were present on single pair of chromosome (Figs. 2b, c) and the position was terminal on 10th chromosome in the former and on 5th submetacentric pair in the later.

The CMA_3 staining of metaphase complements in these marine species showed bright fluorescent signals at the same position (Figs. 2a-c), which were positive for $AgNO_3$ staining, thus, confirmed the position of active transcribing zones of NOR-bearing chromosomes. In *Z. cornutus*, a prominent CMA_3 band present on pericentric region of a chromosome showed likely association with C-band.

The C-banding technique revealed presence of constitutive heterochromatin (CH) blocks on few chromosomes in these species and the pattern was found to be species-specific. In *T. lunare*, the C-bands were present on 4 pair of chromosomes at centromeric positions (Fig. 2a), while in *Z. cornutus*, C-bands were located at centromeric position on one pair of chromosomes (Fig. 2b). In *A. subrostratus*, prominent C-bands were present at the p arm of the three pair of chromosomes (Fig. 2c). The variation in positions of heterochromatic blocks could be used as species-specific marker for identification of these species.

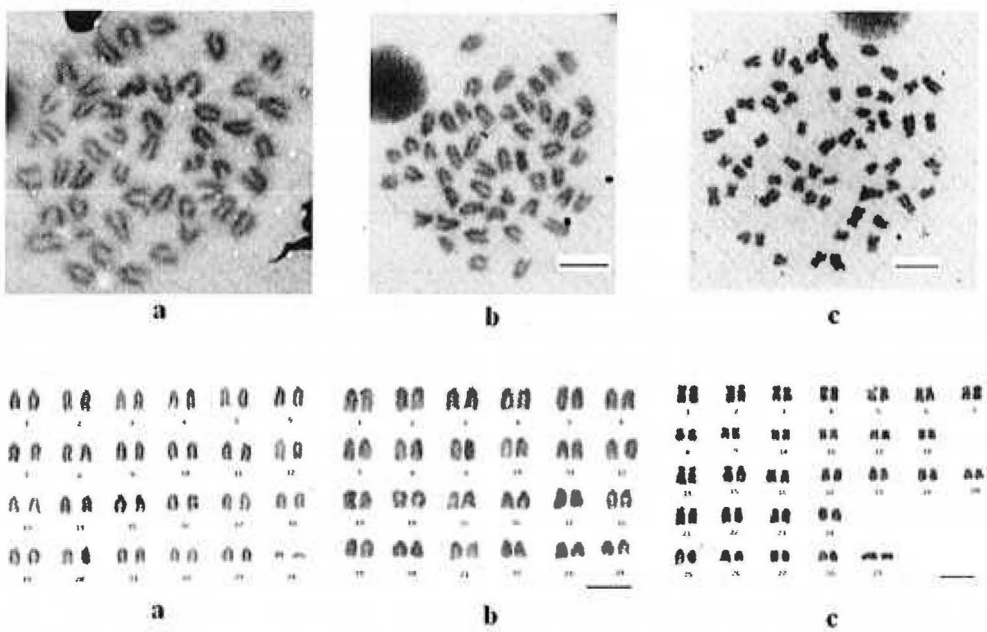

Fig. 1—Giemsa stained metaphase spreads and karyotypes of: (a) *T. lunare*, (b) *Z. cornutus* and (c) *A. subrostratus*. Bar size: 5 µm.

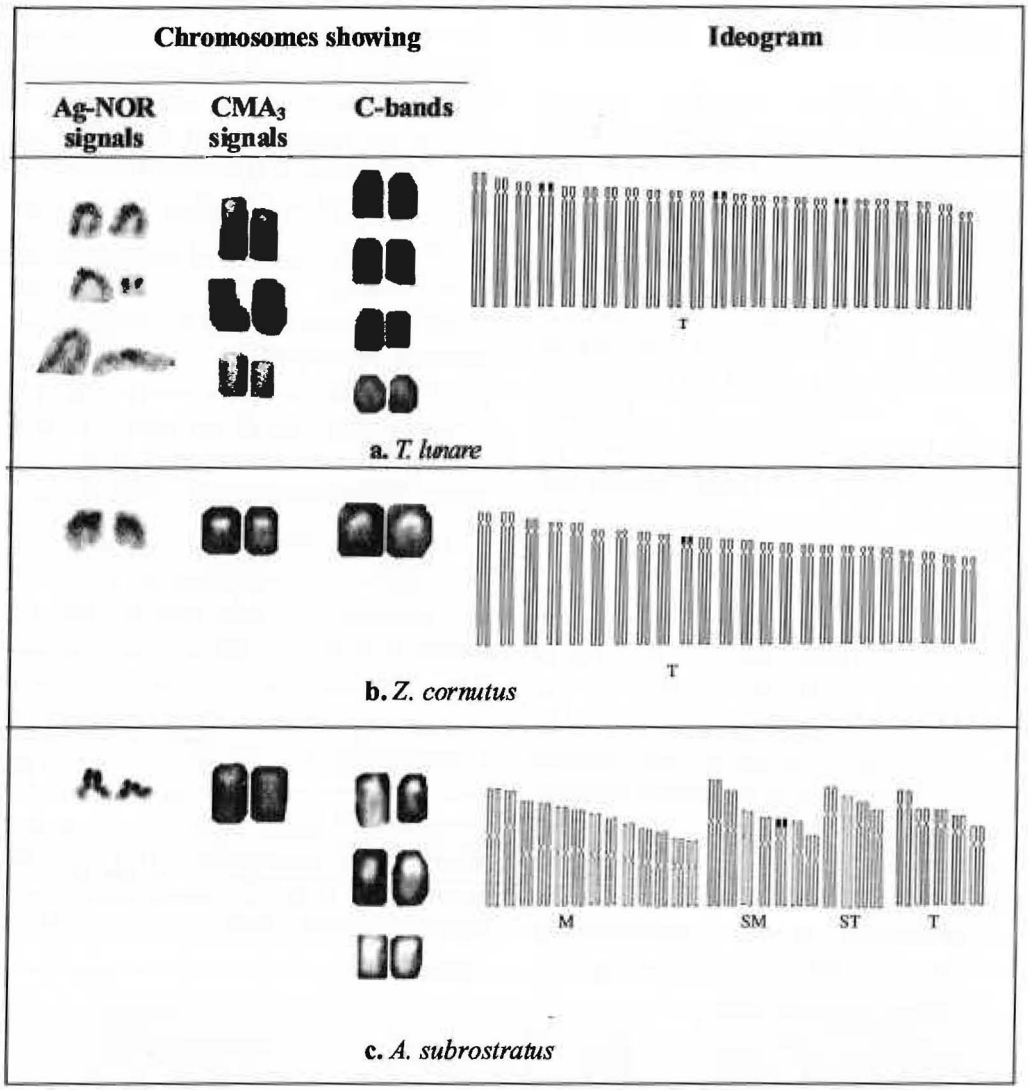

Fig. 2—Chromosomes showing silver and CMA_3 stained NORs, C-bands and ideogramatic representation in: (a) *T. lunare*, (b) *Z. cornutus* and (c) *S. subrostratus*. Dark regions in ideogram indicate the NOR-bearing chromosomes

Discussion

The family Labridae (Wrasses) contains approximately 500 species distributed into 60 genera, and so far only 47 species (belonging to 21 genera) of them are cytogenetically studied[7-12]. Under genus *Thalassoma*, around 62 species are listed (www.fishbase.org, version 4/2009) and only 6 of them were karyotyped. In all the earlier reports on *T. lunare*, the diploid chromosome number was found to be 48[11,13].

To date, no cytogenetic investigation has been undertaken in *Z. cornutus*. The species also possessed 2n= 48, with all acrocentric chromosome, thus, carrying the characteristics of the higher group. So far, chromosomal studies are available only for 9 species (7 genera) of Ariidae family[7-9,14-17].

Evolutionary stasis has often been referred to as a common phenomenon, whether at a higher or a lower degree, in the karyotypic evolution of Perciform species[18]. In family Labridae, the degree of karyotypic differentiation (number of changes in FN values) is inversely related to the dispersive potential provided by the extent of pelagic larval duration of each species[18-20].

In the family Labridae, although most species have been reported with 2n=48, the number of chromosome arms (FN) is often higher (48–90), indicating a predominant occurrence of pericentric

inversions within this group[21]. The representative fish species of the family Ariidae show a karyotypic pattern of 2n=54–58 and high chromosome arm numbers, FN>80[22,23], which is very similar to the most probable ancient karyotype of the Siluriformes[24].

The presence of single NOR pair in fishes, in general, was considered to be plesiomorphic or primitive condition and multiple NORs as apomorphic or derived condition[25]. The remarkable karyotypic conservativeness detected in the present study is similar to that observed in other perciform species previously studied, regarding both the number of acrocentric chromosomes and NOR location.

The GC-rich active regions of NORs are revealed by CMA_3 staining technique and a positive correlation was observed between $AgNO_3$ and CMA_3 staining in these undertaken marine species that indicated presence of active NOR in these species.

Positive C-bands identify regions of heterochromatins, which consist of repetitive DNA sequences and are not transcriptionally active[26]. No earlier report on the C-banding pattern in these undertaken marine species is available. In the present study, high genetic diversity with respect to the heterochromatic bands was observed in the genome of three marine fishes. In *T. lunare*, the bands were located on 4 pair of chromosomes at their centromeric positions, whereas the other perciforme fish *Z. cornutus* possessed prominent C-band on only one pair of chromosome at centromeric position. The centromeric positions of C-bands have been reported in large number of fish species[27,28]. Large variations in number and localization of C-bands have also been reported in our study on six freshwater fish species belonging to the genus *Tor* (unpublished results).

The distribution of C-bands on the different chromosomes may be due to the pericentric inversion[29]. C-band positive segments in the terminal and centromeric positions have been observed in flatfishes[30] and in *Labeo rohita* and *Cirrhinus mrigala*[31]. Almeida-Toledo *et al.*[32] and Uwa[33] observed such type of observations in other fish species and suggested that the large C-banded heterochromatic region could have been formed by tandem duplication of heterochromatic DNA that may be considered as species specific marker chromosome. Similar to our finding, heterochromatin blocks were also detected at the centromeric position on most of the chromosome pairs in Brazilian marine fishes belonging to the Sciaenidae and Sparidae groups[34]. Positive correlation between C-bands and NOR bearing chromosomes were observed in all species under study which seems to be a common feature in other fish species[35-37].

The distribution of C-banded heterochromatin allows for a more accurate pairing of homologous chromosome of the metacentric, submetacentric and subtelocentric series in *A. subrostratus*. Another characteristic about the observed C-bands were their localization at centromeric regions in both the perciformes species and at telomeric position in *A. subrostratus*. This indicated that the heterochromatic regions in *A. subrostratus* might have evolved due to breaking and rejoining of chromosomes during karyo-evolution.

Detailed information related to the differences in chromosome morphology and the position as well as number of NORs and C-bands in the undertaken species suggested that these cytotaxonomic markers could be utilized in identification and characterization of genetic variability between the species and their hybrids. Moreover, the karyological data supplemented with technological progress in molecular cytogenetics will offer important evidences for the classification of these fish species.

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