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EXPLORING FISH MISCELLANY FROM BIODIVERSITY HOTSPOT REGION OF NORTH-EAST INDIA THROUGH CHROMOSOME PROFILING

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ABSTRACT

North Eastern states of India are blessed with extensive ecoclimatic conditions and much of India's endemic flora and fauna. This region is rich in fish diversity with plenty of species of ornamental importance, including model species zebrafish. Among the potential ornamental fishes, *Ctenops nobilis* is designated as Near Threatened and *Danio dangila* is included in the Least Concern list by IUCN. Cytogenetic profiling was undertaken here to provide complementary data for precise species identification and to study genetic inheritance, genome organization and evolution of the species. The cytogenetic analyses revealed diploid chromosome numbers 44 and 50 with karyotype formula 8m+20sm+10st+6t (FN=72) and 20m+24sm+6st (FN=94), respectively, in *C. nobilis* and *D. dangila*. The staining of nucleolar organizer regions revealed the presence of Ag-NORs, CMA_3 sites and 18S rDNA probe signals on one pair sub-telocentric chromosome and one pair sub-metacentric chromosome in *C. nobilis* and *D. dangila*, respectively. These biomarkers are an important resource for molecular taxonomy, evolutionary/phylogenetic studies and conservation genetics of *C. nobilis* and *D. dangila*.

How to Cite

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INTRODUCTION

India is one of 17 mega-diverse countries with 4 biodiversity hotspots. The North Eastern states of India are recognized globally as biodiversity hotspots under Indo-Burma region (Goswami et al., 2012). This region of India is bestowed with more than 267 fish species belonging to 114 genera, 38 families and 10 orders (Dutta et al., 2018). Assam is the largest among the North Eastern states and endowed with 180 fish species of ornamental value and 131 food fishes, of which 25 species are reported as Threatened (https://asbb.assam.gov.in/information-services/biodiversity-of-assam). *Ctenops nobilis* has importance as aquarium fish, found in fresh and brackish inland wetlands in India, Nepal and Bangladesh. It is categorized as Near Threatened ver. 3.1 in the International Union for Conservation of Nature (IUCN) Red List Category & Criteria. *Danio dangila* is a potential aquarium fish found in freshwater inland wetlands in India, Bangladesh, Nepal, Bhutan and Myanmar. It is categorized as Least Concern ver. 3.1 in the IUCN Red List Category & Criteria. The concern towards the biodiversity and sustainability of these two species ascended, as there is a decreasing trend in the current population of both species due to continuous decline in area and habitat quality. Both species have good ornamental value due to their small size, colour, peaceful nature, calm behaviour, harmlessness, etc. (Neeru et al., 2018).

Every fish species contributes to balancing the ecology of the water body, and thus it is necessary to precisely identify and protect them. Accurate species identification is very important from a conservation point of view, especially for overlapping characters among the species. The cytogenetic tools are robust and provide reliable as well as useful taxonomic markers to supplement species identification for specific conservation programmes. The description of karyomorphology, possible trends of rDNA compartmentalization through nucleolar organizing regions (NOR) using staining and rDNA probes on metaphase chromosomes are reported as additional reliable tools for the identification of species, putative hybrids, stocks, etc. (Castro et al., 1996; Singh et al., 2009; Kumar et al., 2019). Other genetic information also supports the conservation and management of threatened fishes (Sola et al., 1992).

As *C. nobilis* and *D. dangila* are valuable and potential ornamental species and are regularly caught by aquarium traders for commercial trade, their genetic characterization is deemed necessary from a conservation genetics point of view. Thus, the present study was conducted with the focus to generate baseline comprehensive cytogenetic profiles, including chromosome banding and 18S ribosomal deoxyribose nucleic acid (rDNA), fluorescence in situ hybridization (FISH), in these fishes of northeastern regions.

MATERIALS AND METHODS

Individual collection, chromosome preparation and staining

Live specimens of *C. nobilis* (n= 5) and *D. dangila* (n= 5) at their juvenile stage were obtained from the Fish Live Gene Bank, maintained by the Department of Zoology at Gauhati University, Jalukbari, Guwahati, Assam, India, and transported to the laboratory in a plastic container in live condition with proper oxygenation. These species were initially collected from the Manas River, Brahmaputra River drainage Assam, India, and maintained in the above-mentioned live gene bank as germplasm resource. Taxonomic identification of the individuals of both species was done using the taxonomic keys described by Talwar and Jhingran (1991) and Kullander (2015). Measurements were made on the left-hand side of the specimens following Hubbs and Lagler (1964) and Fang (1997), and the morphometric data are presented in Table 1. The guideline of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), constituted by the Ministry of Environment, Forest and Climate Change, Government of India, was followed strictly while handling the fish specimens.

The metaphase chromosome spreads were attained by harvesting the gill and kidney tissues, after injecting 0.05% colchicine intramuscularly @1ml/100 gm of body weight. The tissues were treated with hypotonic solution (0.56% KCl), and the cells were fixed with fresh fixative, containing 3:1 methanol and acetic acid, after 20 minutes followed by centrifugation at 132 g for 10 minutes, removal of supernatant and addition of fresh fixative. The cells were further washed three times at a minimum time interval of 30 minutes and then chromosomes were prepared by air-drying technique (Kumar et al., 2019). The slides containing metaphase spreads were then stained with 6% Giemsa dye and fixed permanently using Dibutylphthalate Polystyrene Xylene (DPX) mountant. The stained slide was screened under the fluorescence microscope (Leica, Germany). All well-spread metaphase plates were photographed at 100x magnification. The parameters, such as centromeric position, short/long arm lengths, etc., were recorded in both species after pairing homologous chromosomes and estimating arm ratio (q/p), total chromosome length (p+q), centromere index and relative chromosome length (%). The arm ratios were used to group the chromosomes as per standard nomenclature (Levan et al., 1964). The ideograms were constructed as per arm lengths. The NORs (Nucleolar Organizing Regions) were localized on the chromosomes by staining, using silver nitrate ($AgNo_3$) 0.5-1.0 g/ml in water and 1.0 g gelatin in 100 ml of water containing 1% formic acid for 5-10 min incubations under room temperature (Howell and Black, 1980). The Chromomycin A_3 (CMA_3) was performed as per the methods described earlier (Ueda et al., 1987).

Table 1. Morphometric data of *Ctenops nobilis* and *Danio dangila*

Ctenops nobilis				***Danio dangila***			
Measurements	**Range**	**Mean**	**SD**	**Measurements**	**Range**	**Mean**	**SD**
Standard Length (SL) (in mm)	48.0-51.6	49.4	1.1	Standard Length (SL) (in mm)	51.6-59	55.7	2.9
In %SL				**In %SL**			
Depth of body at pelvic-fin origin	27.3-27.5	27.4	0.2	Body depth	32.4-32.7	32.5	0.2
Predorsal length	73.3-74.0	73.7	0.5	Head length	24.9-25.8	25.5	0.5
Prepectoral length	40.3-41.7	41.0	1.0	Snout length	6.8-7.4	7.0	0.3
Prepelvic length	37.8-39.6	38.7	1.3	Head depth	19.8-20.7	20.3	0.4
Preanal length	49.0-50.8	49.9	1.3	Head width	16.8-18.0	17.4	0.6
Depth of caudal peduncle	15.2-16.1	15.6	0.6	Upper jaw length	4.5-5.0	4.7	0.3
Length of dorsal fin	27.7-36.8	32.3	6.4	Lower jaw length	5.1-6.0	5.6	0.5
Length of dorsal-fin base	19.6-20.0	19.8	0.3	Orbital diameter	6.6-6.9	6.8	0.2
Length of pectoral fin	16.0-17.4	16.7	1.0	Interorbital width	10.3-11.2	10.8	0.5
Length of pelvic fin	40.7-41.7	41.2	0.7	Caudal peduncle length	12.6-13.2	12.9	0.3
Length of anal-fin base	50.8-51.6	51.2	0.5	Caudal peduncle depth	13.4-14.5	13.9	0.6
Lateral head length	38.2-39.8	39.0	1.1	Dorsal-fin base length	18.5-22.5	20.9	2.1
Head depth	23.3-24.2	23.7	0.6	Anal-fin base length	23.4-27.2	25.8	2.1
Head width	17.1-17.7	17.4	0.5	Predorsal length	57.6-61.2	59.6	1.8
Snout length	13.3-14.1	13.7	0.6	Preanal length	64.1-66.5	65.7	1.3
Interorbital width	12.5-13.0	12.7	0.3	Prepelvic length	46.8-47.3	47.0	0.3
Eye diameter	9.5-9.6	9.5	0.1	Pectoral-fin length	26.2-26.9	26.5	0.4
				Pelvic-fin length	16.8-18.2	17.6	0.7
				Rostral barbel length	16.3-17.8	16.9	0.8
				Maxillary barbel length	24.8-29.2	27.1	2.2
Meristic Counts				**Meristic Counts**			
Dorsal fin ray	vi, 6 ½			Dorsal fin ray	ii, 9 ½ - 10½		
Anal fin ray	29			Anal fin ray	iii, 13½ - 14½		
Pectoral fin ray	ii, 6, v-ii,7,4			Pectoral fin ray	i, 10 - 11		
Pelvic fin ray	i,5			Pelvic fin ray	i,7		
Caudal fin	6+5			Caudal fin	10+9		
Lateral line scales rows	38			Lateral line scales	33-34		

In short, the fixed metaphase chromosome slides were stained first with 0.5 mg/ml CMA_3 in the presence of 2.5 mM $MgCl_2$ for one hour and rinsed with McIlvaine's buffer (0.15 M phosphate-citrate), having pH 6.8. The rinsed chromosomes slide was finally stained with 2 µg ml/4',6-diamidino-2-phenylindole (DAPI). The preparations were mounted in a 1:1 mixture of glycerol and McIlvaine's buffer. The model chromosome morphology and a particular band pattern were determined after analysing 50 metaphase spreads in both species. Out of 50 metaphase spreads, 30 good-quality images were used for karyotyping.

DNA extraction and 18S rDNA amplification

The genomic DNA extraction for both species was done from 25-mg muscle tissue using the standard phenol–chloroform–isoamyl alcohol method (Sambrook and Russell, 2001) with partial modification in tissue lysis overnight at 37°C. Precipitated DNA was dissolved in TE buffer (10 mM Tris–HCl, 0.1 mM EDTA, pH 8). The quality and concentration of extracted DNA were assessed by 0.7% agarose gel electrophoresis (Bangalore Genei, India) and spectrophotometrically on NanoDrop 2000 (Thermo Scientific, USA), respectively.

The 18S rDNA amplification was done in a 50-µl reaction mixture using 10× PCR buffer (having 15 mM MgCl2), 10 mM dNTPs mix, 10 pmol of primers (forward: 5'-TTGGTGACTCTCGATAACCT C-3' and reverse: 5'-CCTTGTTACGACTTTTACTTCCTC-3'), 5U *Taq* polymerase and 200 ng of template DNA. The thermal cycling condition was: initial denaturation at 94°C for 10 min, followed by 35 cycles of denaturation at 94°C for 60 sec, annealing at 55°C for 60 sec and primer extension at 72°C for 120 sec, with a final extension at 72°C for 10 min. Amplicons were then checked in 1% agarose gel with a 1 kb DNA ladder (Fermentas, Waltham, Massachusetts, USA) and visualized in the gel documentation unit (Syngene, USA).

18S rDNA probe construction and hybridization

The 18S rDNA amplicon was purified using a gel extraction kit (GeneJET, Thermo Scientific, USA) and 1 µg purified 18S rDNA was labelled with Tetramethyl-rhodamine 5-dUTP (Roche) using the nick translation method for probe construction (Kumar et al., 2020) with slight modification in the quantity of DNase (0.45 µl of 0.02 U/ µl concentration). A pellet of labelled 18S rDNA was dissolved in 10 µl ultrapure water and stored at -20°C till further use. Single-colour fluorescence *in-situ* hybridization (FISH) was performed to determine the location of 18S rDNA on metaphase chromosomes. The probe hybridized chromosomes were counter-stained with VECTRASHIELD mounting medium (Vector Labs, Burlingame, USA), containing DAPI and antifade for 60 minutes. The slides were then examined under the fluorescence microscope (Leica, Wetzlar, Germany) for visualization of two colours with two band filters, *i.e.* DAPI filter (excitation 340-380 nm and absorption 461 nm) for chromosome visualization in blue colour and N2.1 filter (excitation 515-560 nm and absorption 595-605 nm) for DNA probe visualization in red colour. The images of the chromosomes and signals of the probes were acquired and then superimposed using KARYO4000 software (Leica, Germany) to simultaneously visualize the location of 18S rDNA on the metaphase chromosome complements.

RESULTS

The analysis of Giemsa stained metaphase chromosome spreads of *C. nobilis* and *D. dangila* revealed diploid chromosome numbers (2n) as 44 and 50, with karyotype formula 8m+20sm+10st+6t (Fig. 1a), 20m+24sm+6st (Fig. 2a) and fundamental arm number (FN) 72, 94, respectively. The ideogram of *C. nobilis* (Fig. 1b) represents metacentric (1–4), submetacentric (5–14), subtelocentric (15–19), telocentric (20–22) chromosomes. In *D. dangila*, the ideogram (Fig. 2b) characterizes metacentric (1–10 pairs), submetacentric (11–22 pairs) and subtelocentric (23–25 pairs) chromosomes.

The mean values of homologous chromosome measurement, *viz*. short/long arm lengths, arm ratio, centromeric index (%) and relative length (%) of both species, are presented in Tables 2 and 3. The chromosome length ranged from 1.375 (telocentric, 22nd pair) to 4.787 µm (submetacentric, 5th pair) in *C. nobilis*, and 3.464 (submetacentric, 25th pair) to 5.286 µm (sub-metacentric 11th) in *D. dangila*. The contribution of individual chromosomes, estimated from relative length to haploid genome length, ranged from 5.55 to 2.46% in *C. nobilis* and from 4.03 to 3.94% in *D. dangila*.

The metaphase spreads stained with $AgNO_3$ exhibited the presence of a single prominent NOR on p-arms of the submetacentric 9th chromosome pair in *C. nobilis* (Fig. 1c) and of the submetacentric 19th chromosome pair in *D. dangila* (Fig. 2c). The CMA_3 stained heterochromatin fluorescence signals were also observed as single bright zones on the same chromosomes in *C. nobilis* (Fig. 1d, *i.e.* p-arms of submetacentric 9th chromosome pair) and in *D. dangila* (Fig. 2d, i.e. p-arm of submetacentric 19th chromosome pair), as observed through $AgNO_3$. Repetitive DNA, particularly the ribosomal DNA 18S, 5S, 5.8S and 28S, are important segments of the chromosome which play role in protein synthesis and are usually used as markers for chromosomal mapping for the characterization of species, comparative genomics and karyo-evolutions. The screening of chromosome spreads containing hybridized 18S rDNA probe also revealed the presence of single pair FISH signals on the same chromosome and region in *C. nobilis* (submetacentric 9th chromosomes pair, Fig. 1e) and *D. dangila* (submetacentric 19th chromosomes pair, Fig. 2e). Strong FISH signals indicated the presence of highly repeated rRNA genes on the chromosomes. Thus, there was co-localization of all $AgNO_3$, CMA_3 and 18S rDNA probe signals on the same chromosome and region in both species.

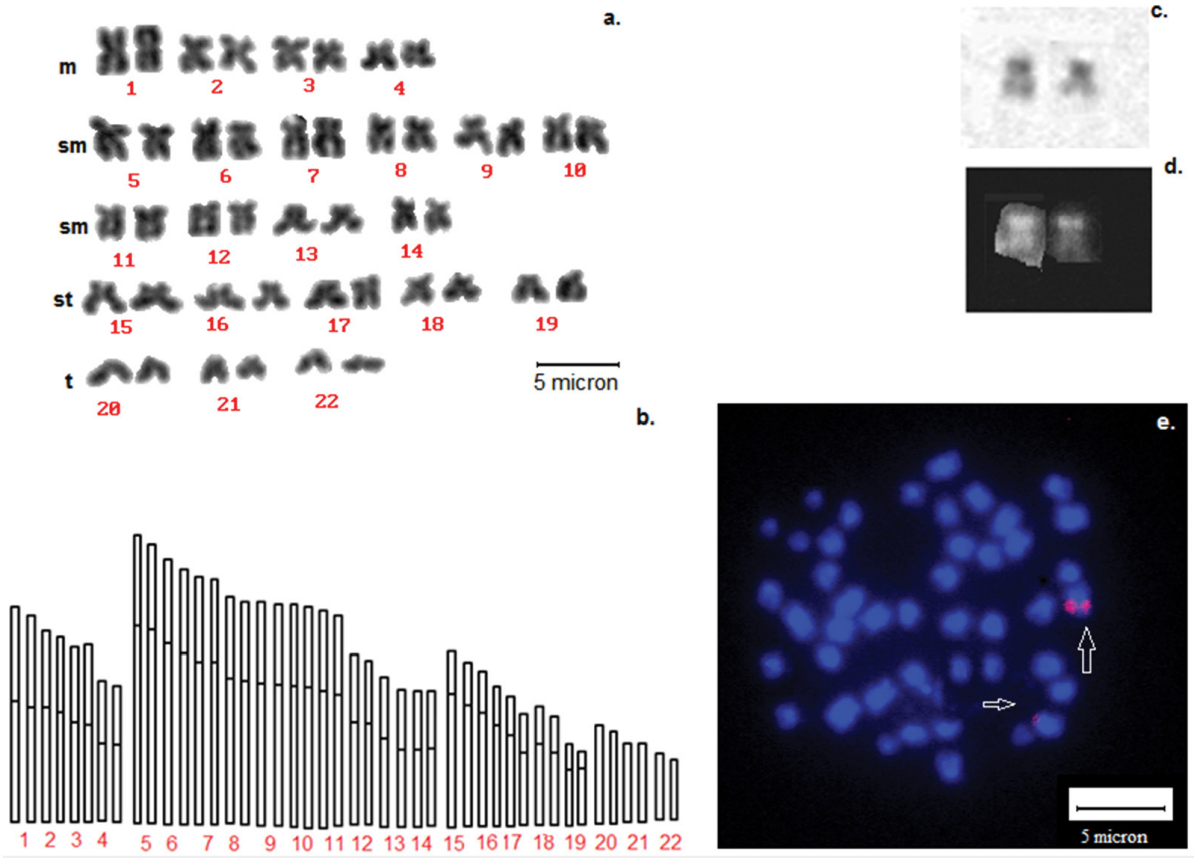

Fig 1. Giemsa-stained karyotype (a), model ideogram (b), $AgNO_3$ stained NOR bearing chromosome pair (c), CMA_3-stained NOR bearing chromosome pair (d) and FISH mapped 18S rDNA probe (e) of *C. nobilis*

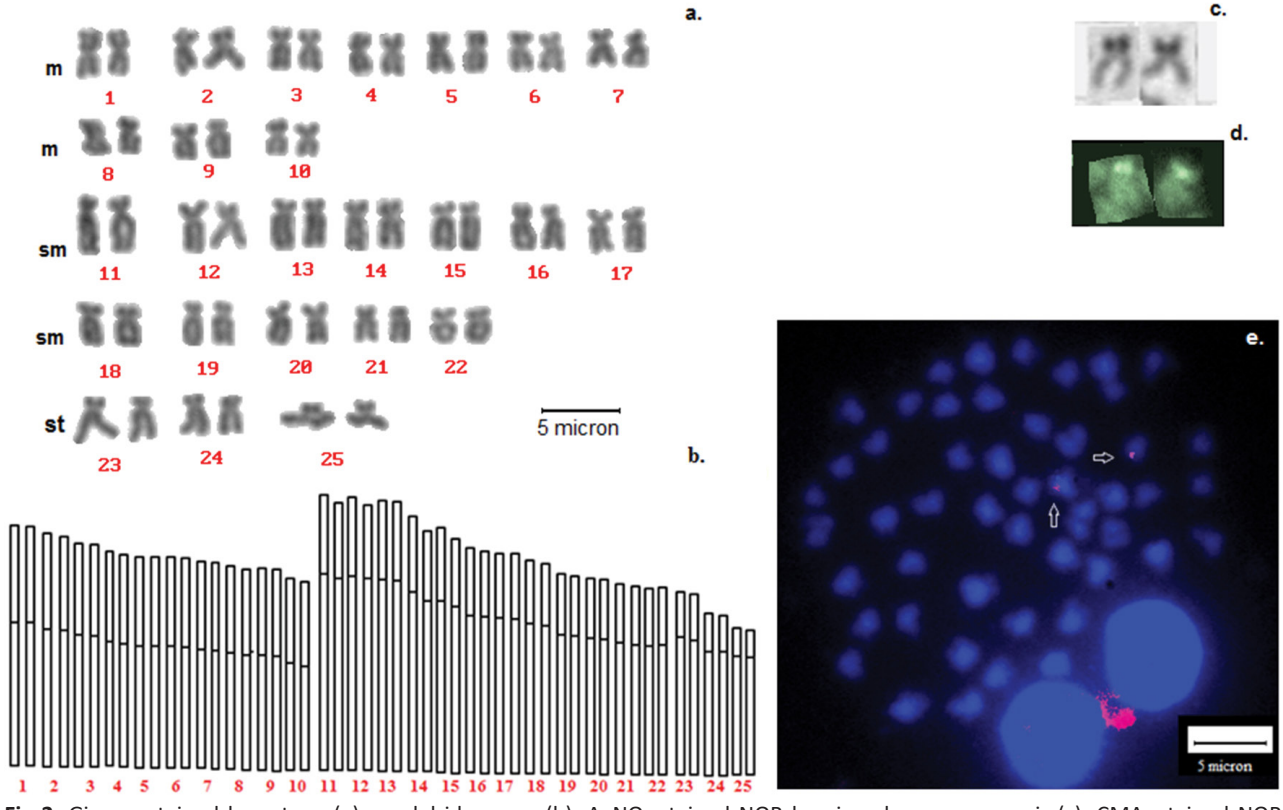

Fig 2. Giemsa-stained karyotype (a), model ideogram (b), $AgNO_3$ stained NOR bearing chromosome pair (c), CMA_3-stained NOR bearing chromosome pair (d) and FISH mapped 18S rDNA probe (e) of *D. dangila*

Table 2. Details of somatic metaphase chromosomes of *C. nobilis*

Chromosome pair	Length (in µm)		Arm ratio (q/p)	Centromeic index (%)	Relative length (%)	Chromosome type
	p arm	q arm				
1	1.338	2.041	1.526	39.592	5.205	m
2	1.082	1.636	1.514	39.788	4.187	m
3	1.098	1.437	1.309	43.299	3.906	m
4	0.882	1.125	1.276	43.925	3.091	m
5	1.282	3.506	2.736	26.761	7.377	sm
6	1.197	3.251	2.715	26.922	6.857	sm
7	1.206	3.022	2.505	28.528	6.515	sm
8	1.154	2.765	2.396	29.440	6.038	sm
9	1.159	2.731	2.355	29.805	5.996	sm
10	1.152	2.713	2.354	29.814	5.956	sm
11	1.134	2.641	2.327	30.053	5.817	sm
12	0.949	2.159	2.296	30.336	4.775	sm
13	0.865	1.912	2.209	31.158	4.278	sm
14	0.825	1.799	2.183	31.419	4.044	sm
15	0.706	2.348	3.325	23.119	3.397	st
16	0.599	2.319	3.865	20.557	4.497	st
17	0.574	2.038	3.552	21.971	4.026	st
18	0.554	1.674	3.029	24.858	3.432	st
19	0.526	1.579	3.005	24.973	3.248	st
20	0	1.813	0	0	2.793	t
21	0	1.594	0	0	2.456	t
22	0	1.375	0	0	2.119	t

DISCUSSION

The karyotype analysis of a species based on the centromere position of chromosomes and their grouping as metacentric, submetacentric, subtelocentric and telocentric/acrocentric along with the ideogram have largely and regularly been used for characterizing the species and assessing the inter-relationships among the species. As the number of somatic diploid chromosomes greatly varies in fishes (https://www.fishbase.de/search.php) from 12 (*Sigmops bathyphilus*) to 288 (*Acipenser transmontanus*), the cytogenetic investigations are arduous due to the small size and high number of chromosomes. The cytogenetic descriptions of *C. nobilis* and *D. dangila* for these species are unavailable.

C. nobilis also known as frail gourami is a single species reported under the genus *Ctenops* in India. As reported by Rishi (1996), *C. nobilis* exhibited 2n=44 with 8 metacentric, 8 sub-metacentric and 28 acrocentric chromosome types (Table 3), with no morphologically distinguishable sex chromosomes and no information on chromosome banding. In the present study, we report the same 2n of 44 with precise *C. nobilis* karyotype of 8m+20sm+10st+6t chromosomes along with NOR banding and 18S rDNA FISH. The precise karyotypic information is also useful in identifying different cytotypes of this species.

Table 3. Details of somatic metaphase chromosomes of *D. dangila*

Chromosome pair	Length (in µm)		Arm ratio (q/p)	Centromeic index (%)	Relative length (%)	Chromosome type
	p arm	q arm				
1	1.875	2.794	1.489	40.163	4.447	m
2	1.835	2.665	1.453	40.779	4.286	m
3	1.777	2.564	1.443	40.946	4.135	m
4	1.745	2.415	1.384	41.957	3.963	m
5	1.717	2.373	1.382	41.995	3.896	m
6	1.717	2.364	1.377	42.082	3.887	m
7	1.699	2.310	1.359	42.379	3.819	m
8	1.682	2.263	1.345	42.651	3.758	m
9	1.677	2.246	1.339	42.760	3.738	m
10	1.642	2.075	1.263	44.202	3.540	m
11	1.499	3.787	2.526	28.361	5.035	sm
12	1.485	3.764	2.534	28.299	4.999	sm
13	1.525	3.726	2.443	29.046	5.002	sm
14	1.417	3.435	2.423	29.222	4.622	sm
15	1.376	3.303	2.399	29.424	4.456	sm
16	1.294	3.054	2.359	29.771	4.141	sm
17	1.249	3.017	2.413	29.301	4.064	sm
18	1.218	2.903	2.384	29.554	3.926	sm
19	1.164	2.689	2.309	30.217	3.671	sm
20	1.149	2.629	2.288	30.418	3.599	sm
21	1.130	2.540	2.248	30.796	3.497	sm
22	1.117	2.512	2.247	30.806	3.457	sm
23	0.880	2.662	3.025	24.853	3.431	st
24	0.725	2.417	3.333	23.084	3.336	st
25	0.535	2.303	4.304	18.863	3.299	st

D. dangila, known as moustached danio, is the largest of the true *Danio* species. There are 26 valid species enlisted under the *Danio* genus (https://www.fishbase.se/search.php). Karyomorphological and other genetic information is reported only for *D. rerio. D. dangila* possesses 2n=50 with karyomorphology of 10 pair metacentric, 12 pair submetacentric and 3 pair subtelocentric chromosomes. The numbers of metacentric chromosomes are higher in *D. dangila* in comparison to the reports for *D. rerio* (Table 4). The present study has generated the first comprehensive information on the chromosomal profile of *D. dangila*, which can serve as baseline information for studying the genome organization of this species. Based on the karyotype, *C. nobilis* has trimodal and asymmetrical karyotype with three separate chromosome groups and lower metacentric chromosomes, hence evolved earlier than that of *D. dangila* which also possesses trimodal but symmetrical karyotype due to a high number of metacentric and submetacentric chromosomes.

Table 4. Comparison of karyo-morphological parameters in *C. nobilis* and *D. dangila*

Species name	2n	KF	FN	Reference
Ctenops nobilis	44	8m + 8sm + 28a	60	(Rishi et al., 1972)
	44	8m + 20sm + 10st + 6t	72	Present study
Danio rerio	50	10m + 12sm/st +28a	-	(Rishi, 1976)
	50	16m + 32sm + 2a	-	(Schreeb et al., 1993)
	50	12m + 26sm + 12st	-	(Pijnacker and Ferwerda, 1995)
	50	4m + 16sm + 30st	-	(Daga et al., 1996)
	50	7m + 7sm + 36st (female) 6m + 8sm + 36st (male)	-	(Sharma et al., 1998)
	50	12m + 26sm + 12st	-	(Ueda and Naoi, 1999)
	50	4m + 30sm + 16st	-	(Amores and Postlethwait, 1999)
	50	4m + 16sm + 30st	-	(Phillips and Reed, 2000)
	50	-	-	(Sola and Gornung, 2001)
D. devario	50	12m+24sm+10st+4T		(Barat A,1985)
D. dangila	50	20m + 24sm + 6st	94	Present study

The non-sequential $AgNo_3$ and CMA_3 staining of metaphase slide revealing one pair NORs in these species are generally visualized in transcriptionally active rDNA sites. The NOR positive sites with AT-rich active rDNA are stained with $AgNo_3$ inorganic salt, while the GC-rich active rDNA are stained through CMA_3 fluorescent dye. The NORs are expressed during the preceding interphase by the presence of acidic proteins associated with nucleolus and pre-RNA. The $AgNo_3$ stains the NOR-associated protein, producing a dark area wherein the silver is deposited and denotes the rRNA gene activity within the NOR. The CMA_3 binds reversibly to G-C base pairs in the minor groove of DNA and inhibits RNA synthesis. These tools are also useful in the analysis of structural chromosome abnormalities with double satellites (Rab et al., 1989). These markers could be able to detect the transposable element associated with the chromosomal rearrangement (Pearson et al., 2005).

The majority of the eukaryotic genomes contain a sizable proportion of repetitive elements Howe et al. (2013) reported the repeat content of 52.2% in the zebrafish genome in the Zv9 assembly), either in the form of transposable elements or tandem repeats. A total of 116,915 tandem repeats were detected in the Zv8 assembly of the zebrafish genome (Rouchka, 2010). The tandemly repetitive 18S rDNA has widely been used for the characterization and karyo-evolution of the species. A genome typically contains over 100 copies of tandemly repeated rDNA genes on one or many chromosomes to meet the rRNA transcript demands for ribosome assembly during developmental stages. Agrawal and Ganley (2018) reported around 200–600 rDNA copies in the human genome that varies among as well as within the species at different developmental stages. The rDNA copy number also relates to biological activities such as age, disease status, mitochondrial activity, etc. In zebrafish, the total rDNA copies were apparently affected by aging, ranging from ~327 copies in unfertilized eggs to ~49 copies at 2 years of age (Boxiang et al., 2020). Characterization of *C. nobilis* and *D. dangila* with 18S rDNA has not been attempted to date. The presence of single FISH signals of 18S rDNA probe on one chromosome pair in both species is a potential chromosomal marker for identification. A noticeable difference in 18S probe signal size was observed between the homologous chromosome pair in *C. nobilis*, but such difference was very small in *D. dangila*. This difference in signal size may be due to the gain of the chromosome segment during the crossing over in one chromosome or the loss of the same part in another chromosome, resulting in the difference in the size of the mapped DNA segment. Such differences in the rDNA signal size between homologous chromosome pairs are common in fishes (Wasko et al., 2000; Singh et al., 2009). The location of 18S rDNA is the same as of $AgNo_3$ and CMA_3 stained positive NORs sites in both species. Such type of positional correlation between active rRNA genes was also reported earlier (Kushwaha et al., 2008; Kumar et al., 2019, 2020). Giemsa stained karyotyping, NOR staining by $AgNo_3$ and CMA_3 and 18S rDNA localization are a broad range of cytogenetic tools for the

characterization of stocks/species and also distinguishing hybrids, phylogenetics and evolutionary relationships and population structures (Kumar et al., 2019; Silva et al., 2018). The study explored the cytogenetic diversity of these two ornamental fishes from the northeastern region of India.

CONCLUSION

A first-hand comprehensive cytogenetic information, including karyotype information, NOR staining and 18S rDNA localization, has been generated for *C. nobilis* and *D. dangila*. The generated information is useful in species characterization and phylogenetic/ evolutionary/ population genetics studies, cytotaxonomy and ultimately for the genetic conservation program of these two species of northeast India.

ISTRAŽIVANJE RIBA IZ ŽARIŠTE BIORAZNOLIKOSTI SJEVEROISTOČNE INDIJE KROZ PROFILIRANJE KROMOSOMA

SAŽETAK

Sjeveroistočne države Indije obiluju ekstenzivnim ekoklimatskim uvjetima te sadrže veliki dio indijske endemske flore i faune. Ova regija obiluje ribljom raznolikošću s mnoštvom vrsta akvarijskih riba, uključujući zebricu. Među potencijalnim ukrasnim ribama, *Ctenops nobilis* označena je kao gotovo ugrožena, a *Danio dangila* uključena je na popis najmanje zabrinutosti IUCN-a. Ovdje je poduzeto citogenetsko profiliranje kako bi se osigurali komplementarni podaci za preciznu identifikaciju vrsta i proučavanje genetskog nasljeđa, organizacije genoma i evolucije vrste. Citogenetskim analizama utvrđen je diploidni broj kromosoma 44 i 50 s formulom kariotipa 8m+20sm+10st+6t (FN=72) odnosno 20m+24sm+6st (FN=94) u *C. nobilis* i *D. dangila*. Bojanje nukleolarnih regija otkrilo je prisutnost Ag-NORs, CMA_3 mjesta i signala gena 18S rDNA na jednom paru subtelocentričnog kromosoma i jednom paru submetacentričnog kromosoma u *C. nobilis* i *D. dangila*. Ovi biomarkeri važan su izvor za molekularnu taksonomiju, evolucijske/filogenetske studije i genetiku očuvanja *C. nobilis* i *D. dangila*.

Ključne riječi: 18S rDNA, Ag-NOR, *Ctenops nobilis*, *Danio dangila*, FISH, kariotip

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