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Karyological and molecular diversity in three freshwater species of the genus *Channa* (Teleostei, Perciformes) from India

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Genetic variability was assessed among three species of genus *Channa*, namely *C. gachua*, *C. punctata* and *C. striata*, using karyological tools including karyomorphology, silver nitrate and chromomycin A_3 staining of nucleolar organizer regions (NORs) and C-banding. Additionally, sequence variation in partial 18S and divergent domain 1 of 28S ribosomal DNA was utilized for inter-species diversity assessment. Diploid chromosome numbers of 32, 40 and 52 in the three species were observed and the karyotype formula was derived as 18m + 12sm + 2st (fundamental arm number [FN] = 62); 6m + 2sm + 10st + 22t (FN = 48) and 12m + 10sm + 14st + 16t (FN = 86) in *C. punctata*, *C. striata* and *C. gachua*, respectively. Similarly, variations were also observed in number and location of silver and chromomycin A_3 stained NORs and C-banding patterns. DNA sequencing generated sizes for 18S of 243, 248, 245 bp and for 28S of 401, 392 and 393 bp in *C. gachua*, *C. punctata* and *C. striata*, respectively. The results indicated that the cytogenetic and molecular markers are useful for the assessment of genetic diversity among the fish species and could provide valuable information for germplasm evaluation and *ex situ* conservation.

Keywords: *Channa* spp; cytogenetics; Manipur; nucleolar organizer region; rDNA

Introduction

The genus *Channa* (family: Channidae and order: Perciformes) comprises around 30 valid species distributed worldwide and 11 species belong to India (http://www.fishbase.org, version 6/2011). Three species of this genus, namely *C. punctata* (Bloch), *C. striata* (Bloch) and *C. gachua* (Hamilton) are important food fishes, generally found in derelict and swampy water due to the presence of an external respiratory organ. Due to their morphological features, *C. punctata* and *C. striata* are commonly called 'spotted snakehead' and 'striped snakehead', respectively, whereas *C. gachua* is commonly known as 'walking snakehead'. *C. gachua* can tolerate very stagnant, poorly oxygenated, turbid and even very foul water (Rahman 1989). They are widely distributed in India, Pakistan, Bangladesh and Burma. All the species have commercial importance in terms of fisheries, aquaculture and aquaria. They are desirable to consumers because of their good flavor, high protein content and low numbers of intra muscular spines (Haniffa et al. 2003). Over the last 10 years, the wild population of these species has undergone a steady decline mainly because of over exploitation and various anthropogenic stresses. According to IUCN (2013, all three *Channa* species have been listed under the least concern (LR-lc) category and are understood as a species complex with no known major widespread threats. *C. striata* and *C. gachua* require taxonomic review and, therefore, need to be reassessed.

These species are identified on the basis of few morphomeristic keys, such as number of scales on the lateral line, number of dorsal and anal fin rays, and spots on the body. For the present study these species were precisely identified based on the morphomeristic keys described by Jayaram (1999) and Vishwanath et al. (2007). Efforts were earlier made to address the problems related to species identification and to assess the genetic variability by employing classical morphological criteria, karyomorphology, and protein and DNA markers (Dhar and Chatterjee 1986; Banerjee et al. 1988; Li et al. 2006). Except for a few karyological studies (Nayyar 1966a; Manna and Prasad 1973; Rishi and Rishi 1981; Dhar and Chatterjee 1986; Naorem and Bhagirath 2006), these species have not been investigated thoroughly using chromosome banding and DNA markers for deducing the evolutionary and phylogenetic relationships among them. Therefore, the present study was undertaken to investigate the genetic variation among three common *Channa* species using cytogenetic as well as DNA markers, to increase understanding of the taxonomy and to generate information useful for evolutionary and conservation genetics.

Materials and methods

Chromosome preparation, staining and banding

The representative specimens (n = 5) of *C. punctata*, *C. striata* and *C. gachua* were collected from Imphal and

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nearby areas in Manipur (24.8200° N, 93.9500° E), India. All the specimens were in juvenile stage and the sex was unidentifiable by visual examination. The average wet weight and length of *C. punctata* specimens were 26.9 g (range 20–35 g) and 170.4 mm (range 155–190 mm), whereas for *C. gachua* and *C. striata* specimens, it was 44.98 g (range 25.6–69.0 g) and 174.7 mm (range 139–225 mm) and 23.9 g (range 8.6–40.0 g) and 148.5 mm (range 110–182 mm), respectively.

The specimens were administered intramuscularly with 0.05% colchicine (1 ml/100 g body weight) to arrest the chromosomes in metaphase stage, and kept alive in a plastic bucket. After 2 h, the specimens were sacrificed, kidney tissues were dissected out and processed for chromosome preparation using hypotonic treatment – acetic acid – methanol fixation – flame-drying technique (Bertollo et al. 1978). The chromosomes were stained with 6% Giemsa in phosphate buffer (pH 6.8). The chromosome complements exhibiting the complete somatic chromosome number and characteristic chromosome morphology were analyzed for karyotypes. The homologous pairs were arranged on the basis of chromosome length and their centromere positions. Averages of the paired chromosomes were taken for total length, short (p) arm, long (q) arm, arm ratio, centromeric index and relative length. The ratio of the long arm to the short arm was used to group the chromosomes 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). Diagrammatic presentations of chromosomes, namely karyograms and ideograms were constructed according to arm ratio and relative length for the karyotypes of each species and were evaluated for overall symmetry versus asymmetry in terms of centromere position and relative size differences.

Silver nitrate (Ag-NO_3) staining of chromosomes for nucleolar organizer region (NOR) localization was done according to the method of Howell and Black (1980). Chromomycin A3 (CMA_3) staining of chromosomes for localization of NORs was performed according to the method of Ueda et al. (1987), with minor modifications. In brief, the fixed chromosomes were stained first with 0.5 mg ml^{-1} CMA_3 in the presence of 2.5 mM $MgCl_2$ for 60 min and rinsed with McIlvaine's buffer, pH 6.8 (0.15 M phosphate-citrate). The rinsed chromosomes were finally stained with 2 μg ml^{-1} DAPI. The preparations were mounted in a 1:1 mixture of glycerol and McIlvaine's buffer. The slide was observed under microscope in the dark and bands were determined in each species. For C-banding, the technique described by Sumner (1990) was followed; however, the slides were stained with propidium iodide (5 μg ml^{-1} in DDW) instead of Giemsa. The patterns obtained using the above methods was determined in each species by studying a minimum of 25 metaphase spreads per specimen per species.

DNA isolation, PCR amplification and sequencing

The genomic DNA was extracted from the whole blood using the standard phenol-chloroform-isoamylalcohol method described by Sambrook and Russell (2001). For amplification of partial 18S and divergent domain 1 of 28S ribosomal DNA (rDNA) in three species, a standard PCR reaction was performed using 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.0 mM $MgCl_2$, 200 μM dNTPs mix, 10 pmoles of each primer (forward 18S: 5′-CTCA-AAGATTAAGCCATGCA-3′, reverse 18S: 5′-GAG-GTTATCTAGAGTCACCA-3′ and forward 28S: 5′-CCCGCTGAATTTAAGCATATAAGTAAGCGG-3′, reverse 28S: 5′-AACGGTTTCACGCCCTCTTGAACT-3′), 1U *Taq* DNA polymerase (Fermentas) and 50 ng of genomic DNA in a final reaction volume of 50 μl. PCR cycling conditions were: initial denaturation at 94°C for 4 min; 35 cycles of denaturation at 94°C for 45 s, primer annealing at 55°C for 45 s, primer extension at 72°C for 1 min; with final extension at 72°C for 10 min. Amplified products were run on 1.5% agarose gel stained with ethidium bromide. The amplified products were commercially sequenced using custom automated sequencing facility.

The sequences obtained were aligned first using the ClustalW in MEGA software (version 5.05) with default settings and the resulting initial dataset was used for distance estimation as well as phylogeny reconstruction in the *Channa* species along with *Puntius chelynoides* (GenBank Acc. Nos. EU836888 for partial 18S and FJ182063 for D1 region of 28S) as an outgroup. The dataset was used for pairwise distance estimation and to construct phylogenetic trees using maximum parsimony (MP) and unweighted pair group method with arithmetic mean (UPGMA) methods. In MP, phylogeny was tested using the bootstrap method with 1000 replications. Gap/missing data treatment was given using all sites with the search method of close-neighbor-interchange (CNI) on random. For UPGMA, a phylogeny test was also done using the bootstrap method with 1000 replications. The Kimura 2-parameter (K2P) based gamma distributed model with transitions and transversions substitution was applied. Gap/missing data treatment was given with complete deletion.

Results and discussion

In the present study, the chromosomes were prepared using the method described above. In another study, Pradeep et al. (2011) obtained metaphase chromosomes from whole larval body of red hybrid tilapia after 4–6 h colchicine treatment at 0.01% concentration, 40 min hypotonic treatment, fixation in Carnoy's solution (methanol and acetic acid ration 3:1) and subsequent staining in Giemsa (10%) for 20 min. They developed the chopping method and avoided the dropping of cell suspension.

The cells of the present study exhibited normal mitotic metaphase complement in all the species. In *C. punctata*, the somatic diploid chromosome number was found to be 32 (Figure 1A, B). The karyotype formula (KF) was derived as 18m + 12sm + 2st and the fundamental arm number (FN) as 62. Our results are in complete agreement with the findings of Manna and Prasad (1973), while Dhar and Chatterjee (1986) reported different diploid numbers (32 and 34) in two geographical stocks of *C. punctata.* Intraspecific variations in diploid chromosome numbers and in fundamental arm number are not uncommon in fish species (Martins-Santos et al. 2005; Valente et al. 2012). Further, the specimens of the same species showed polymorphism for the presence of up to two supernumerary chromosomes (Machado et al. 2012). In *C. striata*, the diploid chromosome number was found to be 40 (Figure 2A, B). The KF in *C. striata* was derived as 6m + 2sm + 10st + 22t with FN of 48. In *C. gachua*, the diploid chromosome count was recorded as 52 and the karyotype consisted of six metacentric chromosomes, five submetacentric pairs, seven sub-telocentric pairs and eight telocentric pairs (Figure 3A, B).

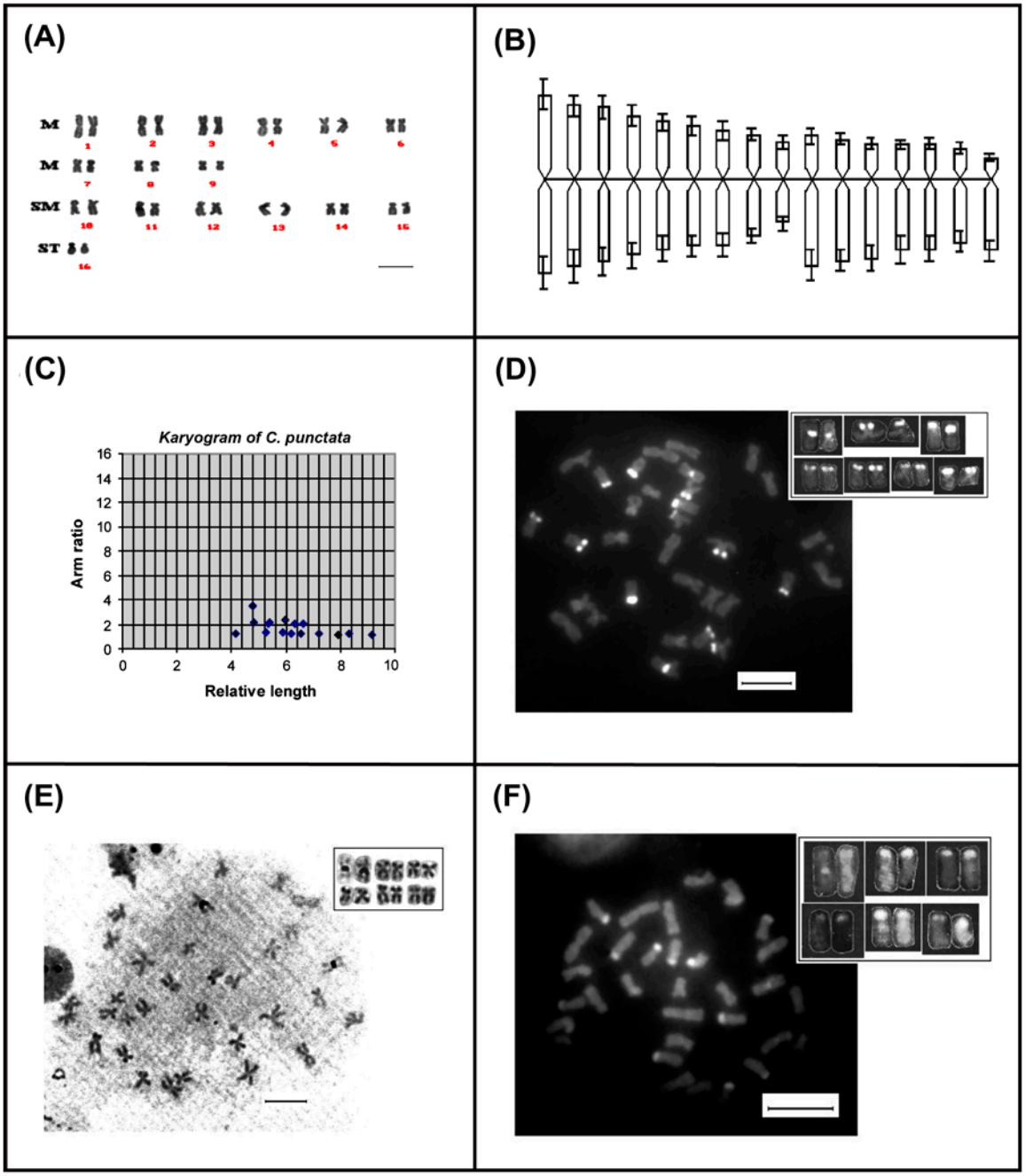

Figure 1. (Color online) Cytogenetic profile in *C. punctata*: (A) karyotype; (B) idiograms; (C) karyodiagram; and chromosomes showing (D) C-bands, (E) silver stained NORs, and (F) CMA_3 stained NORs. Bar = 5 μM.

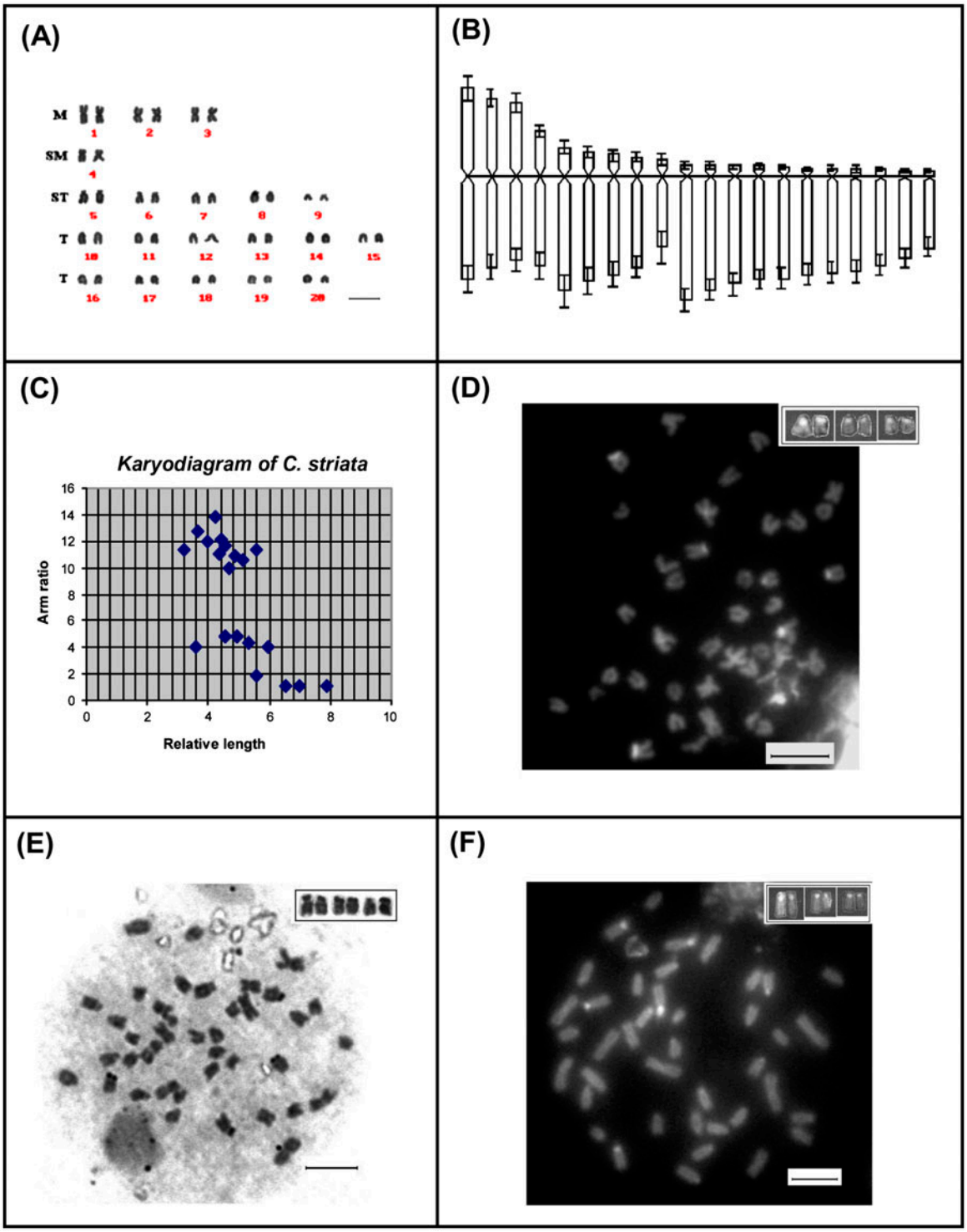

Figure 2. (Color online) Cytogenetic profile in *C. striata*: (A) karyotype; (B) idiograms; (C) karyodiagram; and chromosomes showing (D) C-bands, (E) silver stained NORs, and (F) CMA_3 stained NORs. Bar = 5 μM.

A morphology based taxonomic classification should be validated by cytogenetic data, which reflect genetic divergence and are least affected by environmental distortion. Further, comparative karyology has become a useful tool in fish systematic studies as chromosome number and morphology exhibit changes from the ancestral karyotype, evolving into new lines. Karyology is useful for addressing a variety of evolutionary, genetic and cytotaxonomic questions about the fishes. Comparison of cytogenetic profiles of the three *Channa* species in the present investigation with the earlier published literature revealed variation in karyomorphology within and between the species (Table 1). The chromosome number observed in *C. punctata* and *C. striata* was in

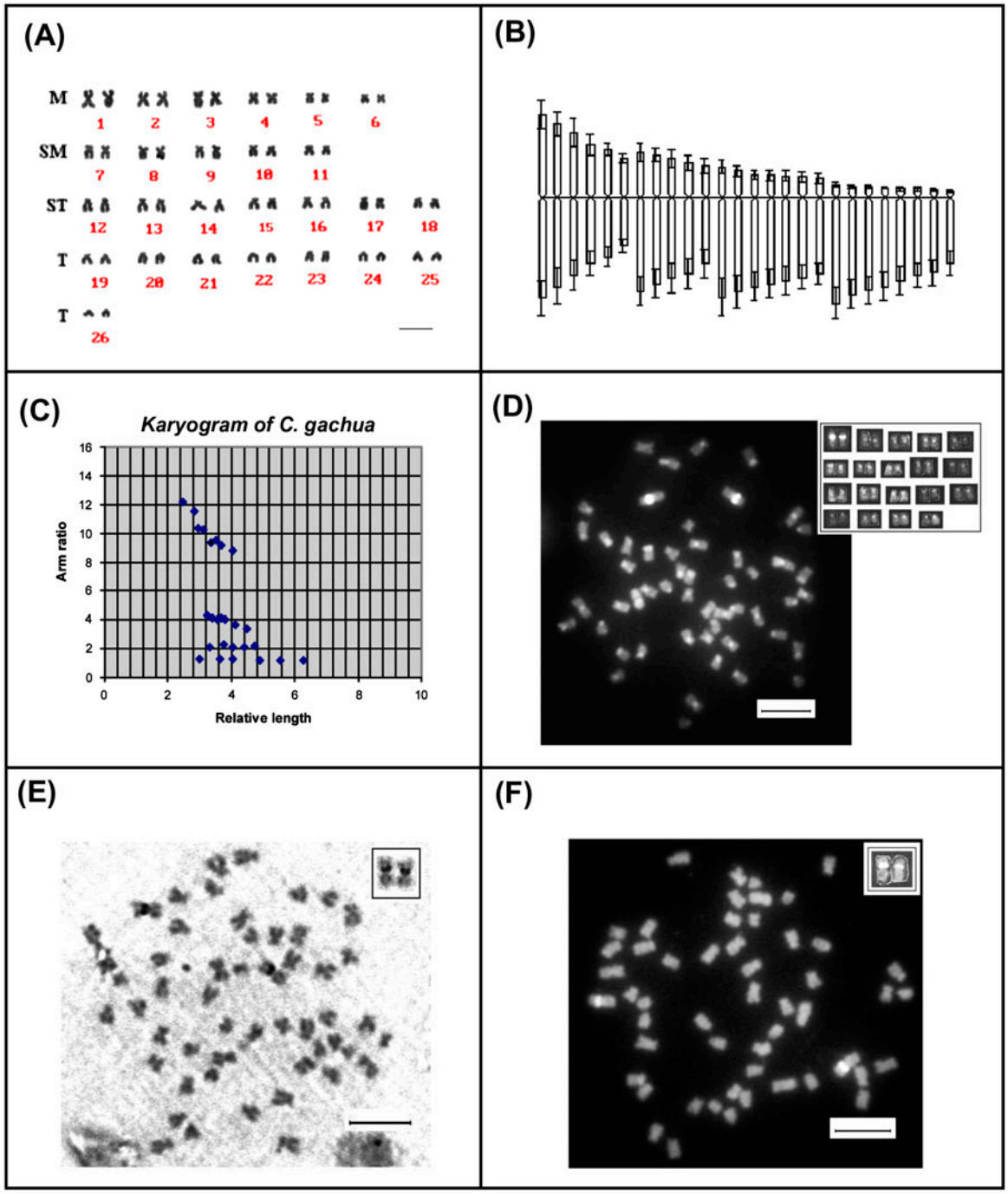

Figure 3. Cytogenetic profile in *C. gachua*: (A) karyotype; (B) idiograms; (C) karyodiagram; and chromosomes showing (D) C-bands, (E) silver stained NORs, and (F) CMA_3 stained NORs. Bar = 5 μM.

agreement with most of the earlier reports. Klinkhardt (1995) observed considerable variation in diploid chromosome number in perciformes (spanning a range of $2n$ = 16 to 62) with the majority of species having a diploid complement of 48 chromosomes and half of those with a fundamental arm number of 48. Both centric fission and fusion probably provide important mechanisms to explain diverse chromosome numbers in various animal taxa, where fusion is usually more common in evolution than fission. The variation in the chromosome numbers in genus *Channa* could be due to Robertsonian fusion of chromosomes from the common ancestor that possessed 48 to 50 acrocentric chromosomes (Nayyar 1966a; Banerjee et al. 1988). However, some species from India and Bangladesh have different diploid number (Supiwong et al. 2009). The reduction in chromosome number, especially in *C. punctata*, may probably be due to fusion of acrocentric chromosomes, which is evidenced from the presence of a higher number of metacentric chromosomes and absence of telocentric/

Table 1. Comparative karyomorphological variation in three species of genus *Channa.*

Species	2n	Chromosome formula	Fundamental arm number	Reference
C. punctata	34	16m + 8a	50	Nayyar (1966a)
	32	18m + 12sm + 2st	62	Manna and Prasad (1973)
	32	16m + 14sm + 2t/a	62	Rishi and Rishi (1981)
	32	14m + 12sm + 6st	58	Sharma and Agarwal (1981)
	34	16m + 14sm + 4t/a	64	Dhar and Chatterjee (1986)
	32	16m + 16sm	64	Dhar and Chatterjee (1986)
	32	24m + 8sm	64	Banerjee et al. (1988)
	32	18m + 14sm	—	Naorem and Bhagirath (2006)
	32	24m + 2sm + 6t	—	Ruma et al. (2006)
	32	18m + 12sm + 2st	62	Present study
C. striata	40	10m + 30t/a	50	Nayyar (1966a)
	40	—	54	Nayyar (1966b)
	40	8m + 2sm + 16st + 14t	50	Manna and Prasad (1973)
	40	8m + 2sm + 2st + 28t	54	Banerjee et al. (1988)
	44	—	50	Wattanodorn et al. (1985)
	40	8m + 6st + 26t	54	Chatterjee (1989)
	44	1m + 1sm + 20t	48	Donsakul and Magtoon (1991)
	40	8m + 2sm + 16st + 14t	50	Manna and Prasad (1973)
	40	8m + 10sm + 22st	—	Naorem and Bhagirath (2006)
	42	6m + 36t	50	Supiwong et al. (2009)
	40	6m + 2sm + 10st + 22t	48	Present study
C. gachua	78	12m + 12sm + 54t/a	102	Nayyar (1966b)
	78	16m + 10sm + 52t	104	Banerjee et al. (1988)
	112	2m + 1sm + 53t(a)	—	Donsakul and Magtoon (1991)
	78	10m + 8sm + 8st + 52t	104	Sharma and Agarwal (1981)
	78	12m + 12sm + 4st + 50t	102	Manna and Prasad (1973)
	52	12m + 10sm + 14st + 16t	74	Present study

Note: Acrocentric chromosomes reported by workers have been shown as telocentric.

acrocentric chromosomes. A similar reduction in chromosome number has also been reported in *Erythrinus* spp. (Bertollo et al. 2004). A decrease in 2*n* and FN in *C. striata* compared to *C. gachua* may be attributed to Robertsonian arrangements and pericentric inversion, as also opined by Choudhury et al. (1982). It was earlier hypothesized that *C. punctata* diverged early from the common ancestor and became specialized by attaining maximum bi-armed chromosomes, whereas *C. striata and C. gachua* diverged later (Banerjee et al. 1988). Manna and Prasad (1973) opined that polyploidy, in addition to the above mechanism, could also be responsible for the evaluation of the karyotype of other *Channa* spp.

Among the three subject species, the karyotype of *C. punctata* can be considered as relatively symmetrical with the larger chromosomes having median centromeres and the smaller chromosomes being median-submedian or submedian. Further, the karyodiagram of this species is comparatively kurtotic and relatively dispersed (Figure 1C). The karyotypes of *C. striata* and *C. gachua* are relatively asymmetrical compared to *C. punctata* and the chromosome points in the genomic diagram of *C. striata* and *C. gachua* are negatively skewed (Figures 2CFigures 3C). Thus, C. *punctata*'s karyotype can be regarded as a relatively advanced/specialized one, in which many structural changes have occurred. The observations of the present study favor the hypothesis of divergent evolution from a common ancestor.

The C-bands in the three *Channa* species were found to be variable both in number and position (Figures 1DFigures 2DFigures 3D). In *C. punctata*, C-bands were found to be localized on seven pairs of chromosomes. The majority of the C-bands (six pairs) were present on the telomeric region, whereas one pair of metacentric chromosomes exhibited C-bands on the pericentromeric region. In *C. striata*, prominent C-bands were found on four pairs of chromosomes, while the rest of the chromosomes possessed weak C-bands. In *C. gachua*, almost all the chromosomes exhibited prominent centromeric C-bands with the presence of a prominent constitutive heterochromatin (CH) block covering the centromeric and pericentromeric region of the p arm of the larger metacentric chromosome, which could be used as a marker for differentiation among these three species. Rishi and Rishi (1992) also reported a multi-site type of C-band distribution in different fish species and the majority of them were present at the centromeric position. Kornfield et al. (1979), in fishes, suggested that elimination of CH accompanies the phyletic evolution of fishes. Phillips and Zajicek (1982) opined that C-banding could be a better device for intraspecific comparisons since CH is one of the most rapidly evolving parts of the genome. Moreover, loss of heterochromatin may also be responsible for a smaller genome size during the course of evolution (Hinegardner and Rosen 1972). According to this hypothesis, the loss of heterochromatin in *C. punctata* would have reduced the genome size and this species

can be considered to be in the advanced stage of evolution. However, in several reports the comparative analyses of C-band patterns have led to a greater understanding of the genetic and evolutionary relationships within and between different groups and have contributed significantly to cytotaxonomic studies (Garcia et al. 1987; Galetti et al. 1991; Almeida-Toledo et al. 1996).

The chromosomal sites of the major rDNA region have been localized *in situ* using isotopes, fluorochrome dyes, N-banding, silver staining (most commonly used), immunofluorescence, and more recently by fluorescent *in situ* hybridization (FISH). So far, limited information is available on localization of NORs in these *Channa* species. Silver staining of chromosomes revealed little variation in location and number of NORs on chromosomes among the three species. In *C. punctata*, six pairs of silver stained NORs were observed with the presence of a prominent NOR pair on the pericentric region of the q arm on the metacentric chromosome, whereas other NOR pairs were comparatively less prominent, small and present on submetacentric chromosomes (Figure 1E). In *C. striata*, the NORs were present on three subtelocentric chromosome pairs (Figure 2E). In *C. gachua*, a single prominent NOR pair was present on the pericentric region of the p arm of the metacentric chromosome (Figure 3E). In these species, Naorem and Bhagirath (2006) reported three pairs of Ag-NOR in both *C. punctata* and *C. striata*, and single pair in *C. orientalis*. Supiwong et al. (2009) reported terminal Ag-NOR on chromosome pair 14 in *C. striata* in Thailand. In other perciforms, namely *Anabas testudineus*, *Colisa fasciatus* and *Zanclus cornutus*, AgNOR has been reported earlier on a single pair of chromosomes by Kushwaha et al. (2008, 2011), whereas multiple NORs have been reported in *C. fasciatus* (Rishi and Gill 1992) and *Thalassoma lunare* (Kushwaha et al. 2011). In fishes, the presence of multiple NORs is considered an apomorphic or derived condition and a single NOR pair on chromosomes as a plesiomorphic or primitive condition (Gold and Amemiya 1986), thus *C. punctata* and *C. striata* can be considered in a more advanced stage of karyo-evolution than *C. gachua*. This information on size, position and number of NORs is suitable for analyzing intraspecific and interspecific variations for tracing karyo-evolution (Klinkhardt 1998; Takai and Ojima 1986).

In *C. punctata*, the CMA_3 stained NORs were observed on six chromosome pairs (five pairs were located terminally on the p arm of submetacentric chro-

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Channa_punctata_18S         CTCAAAGATTAAGCCATGCCAGTCTAAGTACACACGGCCGGTACAGTGAA
Channa_striata_18S          CTCAAAGATTAAGCCATGCAAGT---AGTACACACGGCCGGTACAGTGAA
Channa_gachua_18S           CTCAAAGATTAAGCCATGCAAGTCTAAGTACACACGGCCGGTACAGTGAA
Puntius_chelynoides_18S     CTCAAAGATTAAGCCATGCAGGTCTAAGTACACACGGCCGGTACAGTGAA
                            ******************  **   ************************

Channa_punctata_18S         AC-TGCGAATGC--TCAT-AAATCAGTTATGGTTCCTTTGATCGCTCT-C
Channa_striata_18S          AC-TGCGAATGGCATCATTAAATCAGTTATAGTTCCTTTGATCGCTCT-C
Channa_gachua_18S           CTGTGGCAATGGC-TCATTAAATCAGTTATGGTTCCTTTGATCGCTCT-C
Puntius_chelynoides_18S     AC-TGCGAATGGC-TCATTAAATCAGTTATGGTCCCTTTGATCGCTCCAC
                               **  ****   **** *********** ** *************  *

Channa_punctata_18S         ACGTTACTTGGATAACTGTGGCAATTCTAGAGCTAATACATGCCAACGAG
Channa_striata_18S          ACGTTACTTGGATAACTGTGGCAATTCTAGAGCTAATACATGCCAACGAG
Channa_gachua_18S           ACGTTACTTGGATAACTGTGG-AATTCTAGAGCTAATACATGCCAACGAG
Puntius_chelynoides_18S     CCGGTACTTGGATAACTGTGGCAATTCCAGAGCTAATACATGCAAACGGG
                             ** ***************** ***** ************** **** *

Channa_punctata_18S         CGCTGACCTCC--------------GGGGATGCGTGCATTTATCAGACCC
Channa_striata_18S          CGCTGACCTCC--------------GGGGATGCGTGCATTTATCAGACCC
Channa_gachua_18S           CGCTGACTCCC--------------GGGGATGCGTGCATT-ATCAGACCC
Puntius_chelynoides_18S     CGCCGACCCGCCCCTCCCGGGGGGCGGGGACGCGTGCATTTATCAGATCC
                            *** ***   *              ***** ********* ****** **

Channa_punctata_18S         AAAACCCATGCGGGGTGGCCCCCTTCCCGGGGGCGCCCC--GGCCG-CTT
Channa_striata_18S          AAAACCCATGCGGGGTTGCCCCT---CCCGGGGCGCCCC--GGCCG-CTT
Channa_gachua_18S           AAAACCC----GGGGTTGCCCCT---CCCGGGGCGCCCC--GGCCGGCTT
Puntius_chelynoides_18S     AAAACCCATCCGGGGT-GTCGGGGCTCCGGCCTCGCCCCCCGGTCC-CTT
                            *******    ***** * *      ** *   ******  ** *  ***

Channa_punctata_18S         TGGTGACTCTAGATAACCTC
Channa_striata_18S          TGGTGACTCTAGATAACCTC
Channa_gachua_18S           TGGTGACTCTAGATAACCTC
Puntius_chelynoides_18S     TGGTGACTCTAGATAACCTC
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Figure 4. Alignment of 18S rDNA sequences in *C. punctata*, *C. striata*, *C. gachua* and *P. chelynoides.*

mosomes and one pair pericentromericelly located on the q arm of a metacentric chromosome) (Figure 1F). In *C. striata*, CMA_3 bands were present on the telomeric region of three chromosome pairs (Figure 2F). In *C. gachua*, a prominent CMA_3 band was present on a single chromosome pair at the pericentric region (Figure 3F). The presence of a single CMA_3 band on a chromosome of *C. gachua* also indicated its plesiomorphic or primitive condition in comparison to other two species. Further, the position of CMA_3 stained NORs were in agreement with that of Ag-NORs. Such a co-localization has been found in many fish species. Ruma et al. (2006) reported seven CMA_3 positive signals in *C. punctata* and 12 in *C. orientalis*.

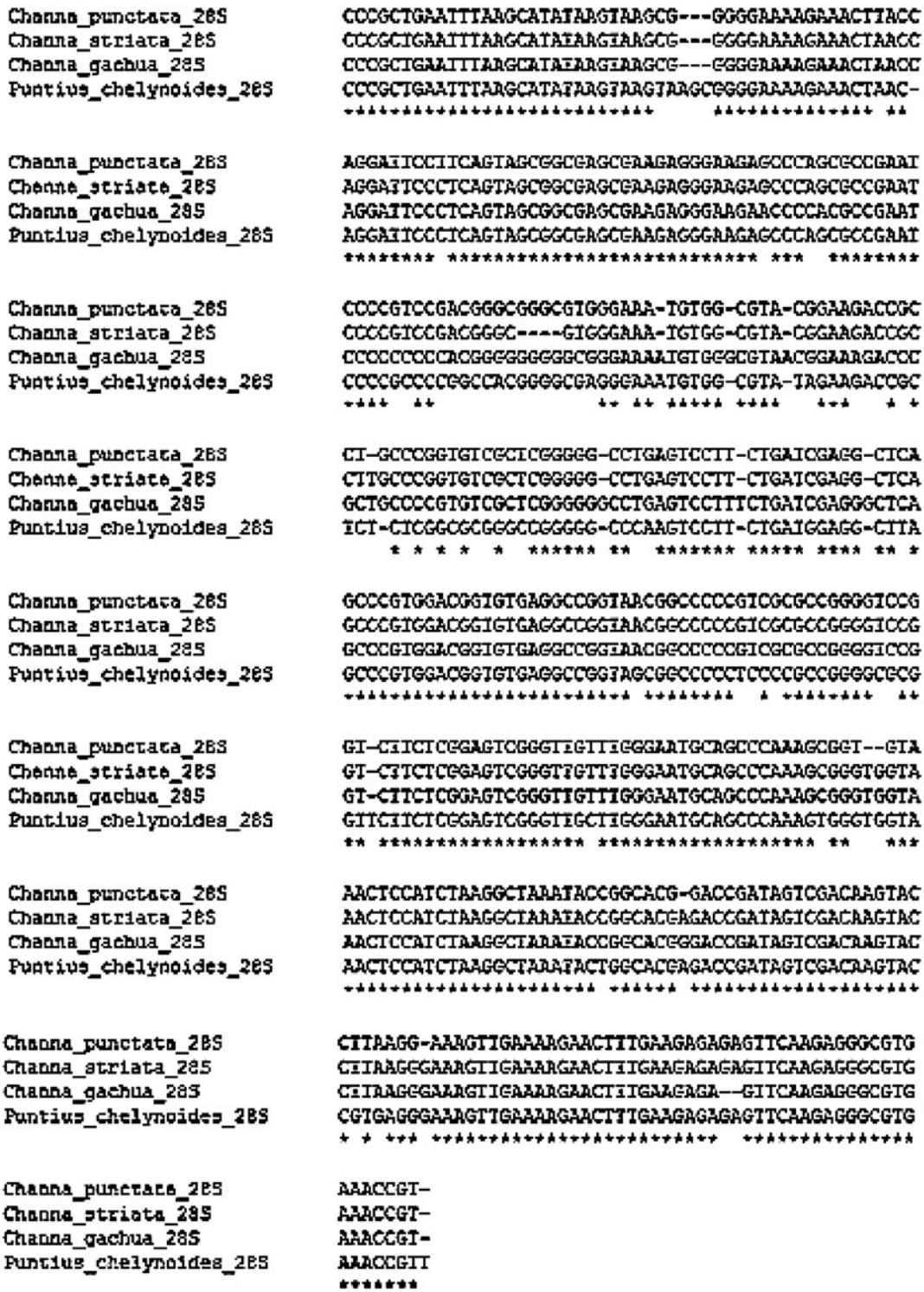

Figure 5. Alignment of divergent domain 1 sequences in *C. punctata*, *C. striata*, *C. gachua* and *P. chelynoides.*

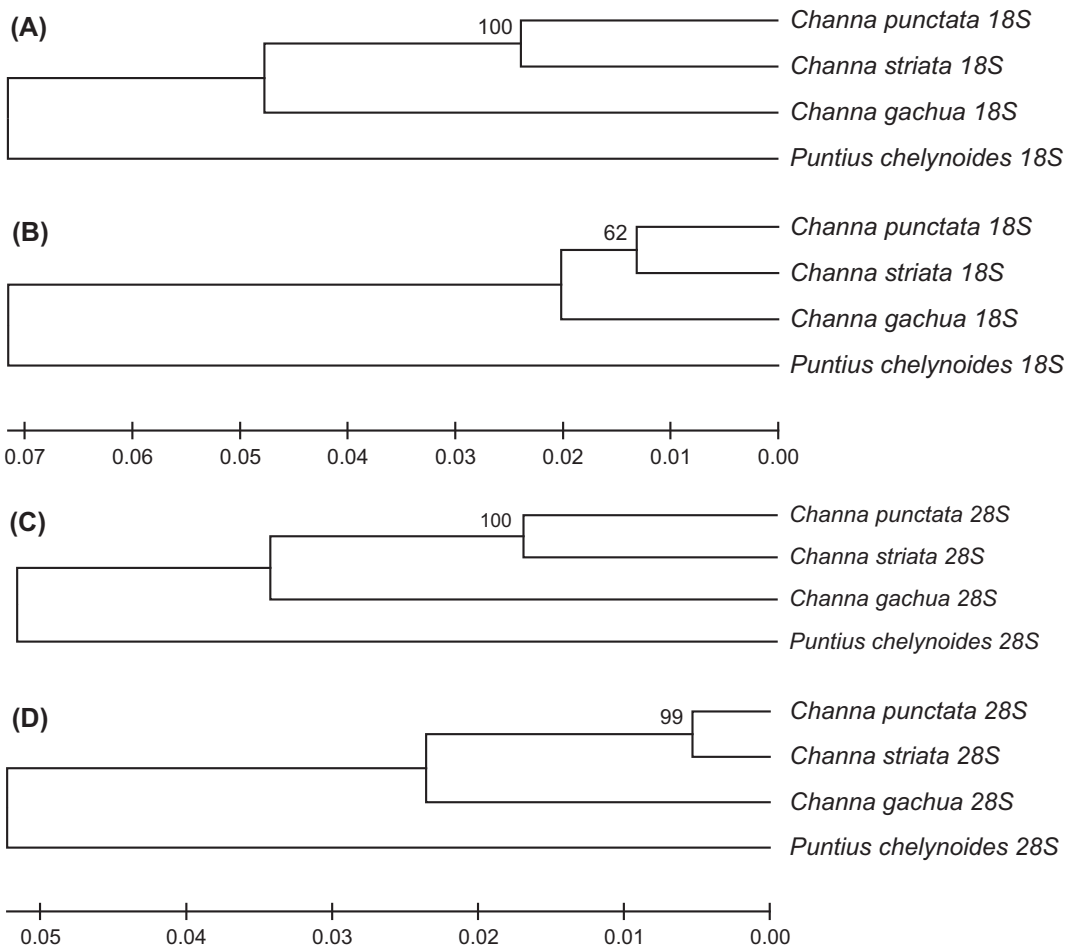

Figure 6. Unrooted phylogenetic tree constructed on the basis of 18S rDNA sequences using maximum parsimony (A) and UPGMA (B) and of divergent domain 1 of 28S rDNA sequences using maximum parsimony (C) and UPGMA (D) methods.

It has been observed that that the C-band region on the metacentric chromosome was flanked with a NOR site in *C. punctata* and *C. gachua*. In a number of fish species, NORs have been found to be associated with C-band regions (Pendas et al. 1993; Martinez et al. 1996; Wasko and Galetti 2000; Kushwaha et al. 2011). The significance of association is not clear but it indicates that small portions of heterochromatin may be genetically active and important for gene expression (Rishi and Rishi 1992).

In eukaryotes, each repeat rDNA unit consists of three rRNA genes (18S, 5.8S and 28S), separated by two internal transcribed spacers (ITS 1 and ITS 2) and surrounded by two external transcribed spacers (5′ ETS and 3′ ETS) (Long and David 1980; Hillis and Dixon 1991). Each repeat of 18S is highly conserved, whereas 28S rDNA is organized into several highly conserved cores interrupted by divergent domains, also called 'D domains' (Hassouna et al. 1984). The divergent domains evolve rapidly with substitution rates that are at least two orders of magnitude higher than those of core regions. These domains show a high rate of insertion and deletion events (Olsen and Woese 1993). Variations in the more rapidly evolving divergent domains make them suitable for phylogenetic comparisons among closely related species.

The sequence lengths of 18S and 28S D1 regions were determined after sequencing as 248, 245 and 243 bp as well as 392, 393 and 401 bp, respectively, in *C. punctata*, *C. striata* and *C. gachua* and were submitted to the National Center for Biotechnology Information (NCBI) database (accession nos. EU836885, EU836886, EU836887, EU836889, EU836890, and EU836891). In 18S and 28S D1 regions, 28 and 35 variable sites were observed after multiple alignments, respectively (Figures 4 and 5). The GC contents of 18S and 28S D1 domain were also calculated and all the species have more than 51% and 58%, respectively. The 28S domains appear closely related to the transcribed spacers of rRNA precursor but a sizable fraction displays a much slower rate of sequence variation. The D1 of vertebrate are characterized by a slight bias to exclude adenine and for high G + C content.

The partial 18S sequence of *C. punctata* showed 95% identity with *C. striata* and 92% identity with *C. gachua*, whereas such identity between *C. striata* and C. *gachua* was 93%. All these sequences showed more than 91% identity with other fish species listed in the

NCBI database. Similarly, the partial 28S sequence of *C. punctata* showed 97% identity with *C. striata* and 93% identity with *C. gachua*, whereas such identity between *C. striata* and *C. gachua* was 94%. These 28S nucleotide sequences showed more than 90% identity with other fish species listed in NCBI. The phylogenetic analyses using two methods, namely MP and UPGMA, showed almost consistent topology, where *C. punctata* and *C. striata* clustered together and showed a closer relationship to each other, while *C. gachua* formed another arm and *P. chelynoides* radiated far away and showed distant relationships with the *Channa* species (Figures 6 A, B, C, D). This is the first report on the nucleotide composition of 18S and D1 regions of 28S nuclear rDNA and the phylogenetic relationship among three *Channa* species. In a study, Bhat et al. (2012) categorized eight *Channa* species in gachua (comprising *C. amphibeus*, *C. aurantimaculata*, *C. barca*, *C. bleheri*, *C. gachua*, *C. punctata* and *C. stewartii*) and marulius (comprising C. *marulius and C. striatus*) groups using RAPD markers.

Application of genetic methods in fish conservation and management is still relatively scarce and awaits the availability of simple, accurate and easily implemented methods. This study will provide key support in effective management and conservation of typically multi-species *Channa*, which shows differential sensitivity to intensive exploitation and requires the collection of biological and fishery information on a species basis.

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