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Comparative and evolutionary analysis of mitochondrial genes in Indian major carps

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ABSTRACT

Direct sequencing of mitochondrial DNA regions such as cytochrome *b*, ATPase 6/8 and control region was performed to study comparative and evolutionary status of the three mitochondrial genes in *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala*. DNA sequence alignment among species using specific software revealed comparative rates of divergence with considerably faster and more heterogeneous substitution rate for control region as compared to cytochrome *b* and ATPase 6/8. Despite the relatively high variability of control region, the overall levels of sequence divergence were low in coding regions. Two protein coding genes and the control region with varying degree of sequence divergence established two distinct groups which are genetically distant from each other exhibiting identical phylogenetic structure in IMCs. Closest relationship was between *Labeo rohita* and *Catla catla* indicating that they might have diverged from a common ancestral stock in genealogical lineage whereas *Cirrhinus mrigala* showed greater divergence with all the three DNA regions studied. Findings of this study will help to understand evolution of mitochondrial DNA genes in carps and facilitate future investigations on phylogeographic structure of Indian carps.

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1. Introduction

Indian major carps, *Labeo rohita* (*L. rohita*), *Catla catla* (*C. catla*) and *Cirrhinus mrigala* (*C. mrigala*) form the mainstay of freshwater aquaculture in the Indian subcontinent. Intensification of carp culture in recent years is attributed to their high commercial value and fast growth rate as well as the popularity. Together they contribute about 87% of inland aquaculture production in India (FAO, 2009). Genetic information on cultivable fish and shellfish species is useful for identification of stocks, stock enhancement, breeding programs, management for sustainable yields and preservation of genetic diversity (Dinesh et al., 1993; Garcia and Benzie, 1995; Tassanakajon et al., 1997). Techniques using mtDNA have been widely employed for aquaculture and fisheries related genetic studies because this marker has several useful characteristics including rapid rate of mutation making it effective for detecting recent population isolation (Ward and Grewe, 1994) and for establishing genealogical relationships among populations within species

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Table 1
Partial mtDNA genes of Indian major carps with Genbank Accession numbers.

Species	Genes	Accession No.
Labeo rohita	Cyt *b*	JN224867–JN224869
		JN253604–JN253629
	ATPase6/8	JN859621–JN859649
	CR	JN315802–JN315818
Catla catla	Cyt *b*	JN859708–JN859736
	ATPase6/8	JN859650–JN859677
	CR	JN859678–JN859707
Cirrhinus mrigala	Cyt *b*	JN859796–JN859825
	ATPase6/8	JN859737–JN859767
	CR	JN859768–JN859795

(Avise, 2000). From a population genetics perspective, mtDNA has been extensively used as a marker for evolution, genetic diversity studies and identification of species (Curole and Kocher, 1999). Mitochondrial genes have proven effective for elucidating phylogenetic and taxonomic relationships in many freshwater fish groups (Briolay et al., 1998; Na-Nakorn et al., 2006) and for investigating intraspecific variation and even for establishing species boundaries (Nguyen et al., 2008). Knowledge on evolutionary and biogeographical history of freshwater fish including carps is limited. In the present investigation, we studied comparative evolution of three mtDNA genes, control region (CR), cytochrome *b* (cyt *b*) and ATPase 6/8 in Indian major carps. Results of this study would be useful for comparative evolution of mtDNA in carps.

2. Materials and methods

2.1. Sample collection

A total of about 90 samples from three species (*C. catla*, *L. rohita* and *C. mrigala*) belonging to family Cyprinidae were collected during 2009–10 from the river Mahanadi (Latitude 20.27°N and Longitude 85.52°E). Morphological identification of species was done based on Talwar and Jhingran (1991).

2.2. DNA isolation

Fin clipping was done from each individual fish, preserved in 95% ethanol and stored at −20 °C until DNA extraction. Total DNA was isolated from fin tissue by proteinase K digestion followed by standard phenol and chloroform extraction (Sambrook et al., 1989). The DNA samples were then resuspended in 1× TE buffer. The concentration and purity of isolated DNA was estimated at wavelength 260/280 nm using a UV spectrophotometer.

2.3. Amplification and sequencing

The partial cyt *b* and CR genes were PCR amplified in a 25 μl reaction volume with 1X PCR buffer (Banglore genei), 0.25 mM of dNTP mix, 10 pmol of each primer, 0.25U of Taq polymerase and 50 ng/μl genomic DNA using a thermal cycler (ABI). The primer pairs used for PCR were L14841 (5′AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA 3′) and H15149 (5′AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA 3′) for cyt *b* gene (Kocher et al., 1989) and CR1 (5′ ACCCCTGGCTCCCAAAGC 3′) and CR2 (5′ GTTTCGGGGTTTGACAAGGATA 3′) for CR gene (Guo et al., 2003), respectively. The PCR temperature profile used was 1 cycle of initial denaturation at 94 °C for 4 min followed by 34 cycles (denaturation: 94 °C for 30 s, annealing: 50 °C

```
Cyt b
[                    111 111 111 111 111 111 222 222 222 222]
[          112 247 889 001 134 455 566 677 888 001 122 244 455]
[       16 051 728 179 284 704 703 628 947 039 890 958 903 928]
#CM1502 TC CCT CTA TTC TCT CTT CTG CCC CCT AAG ATT TTG CGA TAC
#CM1509 .. ... ... ... ... ... ... ... ... G.. ... ... ... ...
#CM1514 A. ... .C. ... ... ... .C. ... ... ... ... ... ... ...
#CM1521 .. ... ... ... ... ... ... ... ... ... ..A ... ... ...
#CC1501 C. T.C TCT ..A .T. T.C TC. .T. .T. .CA ..A CAA TAG .CT
#CC1502 C. T.. TCT ..A .T. T.C TC. .T. .T. .CA ..A CAA TAG CCT
#LR2511 CT .TC TCT CCA CTC ... TCA T.T T.C ... CTA CAA TA. CCT
#LR2513 CT .TC TCT CCA CTC ... TCA T.T T.C ... CTA CAA TA. CCT
#LR2515 CT .TC TCT CCA CTC ... TCA T.T ..C ... CT. CAA TA. CCT
#LR2505 CT .TC TCT CCA CTC .C. TCA T.T T.C ... CT. CAA TA. CCT
```

Fig. 1. Alignment of partial DNA sequences of cyt *b* gene. (Only variable sites are reported).

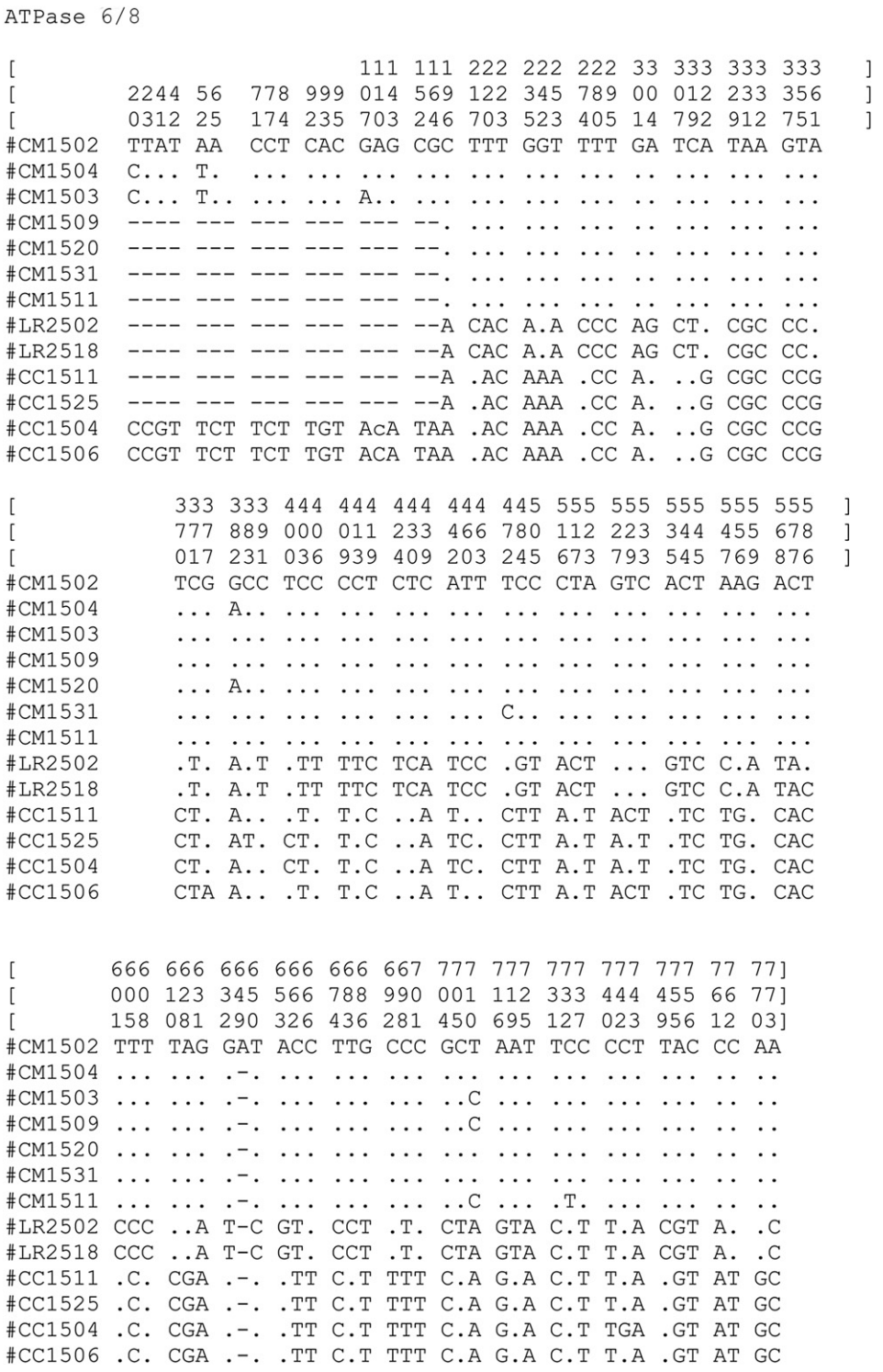

Fig. 2. Alignment of partial DNA sequences of mitochondrial ATPase 6/8 gene. (Only variable sites are reported).

for1 min and extension: 72 °C for 2 min) and a 7 min final extension at 72 °C. In case of ATPase 6/8, annealing temperature was 55 °C with all other parameters being same as above. Primer pair used for ATPase 6/8 was L8331 (5′ AAAGCRTTRGCCTTTTAAGC 3′) and H9236 (5′ GTTAGTGGTCAKGGGCTTGGRTC 3′) (Thai et al., 2004). PCR products were checked on 1% agarose gels and the most specific and intense products were selected for sequencing. Amplified PCR products were purified using the PCR purification kit (Qiagen) and 200 ng of purified PCR product was used for cycle sequencing reaction. The same forward and reverse PCR primers were used for sequencing to get complete sequence of the fragment. Sequencing was done with an automated DNA sequencer (ABI Prism 310 genetic analyzer). Raw sequence data were edited manually and aligned using the software Bioedit version 7.0.9.0 (Hall, 1999) to get a consensus sequence of individual gene fragment.

2.4. Sequence analysis

All the sequences generated in this study have been submitted to GenBank (Table 1). The consensus nucleotide sequence of each gene fragment gathered from about 30 individuals in each species was aligned using CLUSTAL W (Thompson et al., 1994). Pairwise distances of the nucleotide sequences were computed using Kimura's two parameter and pairwise distances of the amino acid sequences of each gene were computed using the MEGA version 4.0 (Molecular Evolutionary Genetics Analysis). The polymorphic sites were computed by using DnaSP version 5.10 (DNA Sequence Polymorphism) (Librado and Rozas, 2009). To assess the degree of saturation, amount of variation and reconstruction of phylogeny, pairwise sequences were compared using MEGA version 4.0 (Tamura et al., 2007).

3. Results

3.1. Cytochrome b

A total of 28 individuals of *L. rohita*, 29 of *C. catla* and 30 individuals of *C. mrigala* were used for sequence analysis of cyt *b*. Simplicity and ambiguity were observed among the sequences as there were no insertions, deletions and stop codons in the sequences. Sequencing of the cyt *b* gene brought out an average of 288 bp sequence. Multiple alignments were done to get consensus sequence. *C. catla* had 2 haplotypes whereas *L. rohita* and *C. mrigala* had 4 haplotypes each for cyt *b* gene. Out of the 288 sites, 234 were conserved, 45 variable, 39 parsimony informative and 6 were singleton. The polymorphic sites are given in Fig. 1. The nucleotide frequencies observed were $T = 28.3\%$, $C = 27.7\%$, $A = 29.1\%$ and $G = 15\%$. Average transitional pairs ($si = 15$) were more frequent than transversional pairs ($sv = 2$) with an average ratio of 6.6. In the cyt *b* gene analysis, the maximum number of amino acids were composed of Leucine (17.16%) & Arginine (14.54%) followed by Aspartate (0.36%) and Glutamate (0.00%).

Pairwise genetic distance values (Kimura 2 parameter) based on cyt *b* gene using MEGA 4.0 are given in Table 2. The mean genetic distance among all the three Indian major carps was estimated as 0.077. Intraspecific distances ranged from 0.004 to 0.007 and the interspecific distances ranged from 0.085 to 0.127. The highest interspecific genetic distance (0.127) was between *L. rohita* and *C. mrigala* and the lowest (0.085) was between *L. rohita* and *C. catla*.

The phylogenetic analysis using MEGA 4.0 included NJ and ME trees for cyt *b* gene for all the three Indian major carps. The branches of phylogenetic tree were constructed by keeping same topology and similar bootstrap probabilities. The reliability of the tree topology was assessed by 1000 bootstrap replications. The NJ and ME trees revealed identical phylogenetic relationship among the species. The tree profile showed that *L. rohita* and *C. catla* form one cluster with *C. mrigala* on other (Fig. 4a and b).

3.2. ATPase 6/8

A total of 29 individuals of *L. rohita*, 29 of *C. catla* and 35 individuals of *C. mrigala* were used for sequence analysis of ATPase 6/8 gene. Sequencing of the ATPase 6/8 gene brought out an average of 785 bp nucleotide sequence. Multiple

Table 2
Pairwise genetic distances (Kimura 2 parameter) LR = *Labeo rohita*, CC = *Catla catla* and CM = *Cirrhinus mrigala* haplotypes based on cytochrome *b* gene sequences.

[	1	2	3	4	5	6	7	8	9	10]
[1] #CM1502										
[2] #CM1509	0.004									
[3] #CM1514	0.011	0.014								
[4] #CM1521	0.004	0.007	0.014							
[5] #CC1501	0.096	0.101	0.088	0.092						
[6] #CC1502	0.096	0.101	0.088	0.092	0.007					
[7] #LR2511	0.123	0.127	0.114	0.118	0.085	0.085				
[8] #LR2513	0.123	0.127	0.114	0.118	0.085	0.085	0.000			
[9] #LR2515	0.114	0.118	0.105	0.118	0.085	0.085	0.007	0.007		
[10] #LR2505	0.123	0.127	0.114	0.127	0.093	0.093	0.007	0.007	0.007	

CR

```
[                                                  111 1111 1111 111 111 111 111]
[          112 333 335 6666 777 77 888 999 000 0011 1112 222 233 333 444]
[       17 125 345 796 4578 345 67 234 129 025 7802 5670 145 812 459 245]
#LR2507 AT TAA CTC TAA C-AT GTA GA AGA GTT CAG CCAA TAAC TAA TAT ATC AAA
#LR2505 .. C.. T.. ... .-.. ... .. ... AC. ... .... .... ... ... ... ...
#LR2503 .. ... ... ... .-.. ... .. ... A.. ... .... .... ... ... ... ...
#LR2510 .. C.. T.. ... .-.. ... .. ... AC. ... .... .... ... ... ... ...
#LR2501 ... .. ... ... .-.. ... .. ... A.. ... .... .... ... ... ... ...
#LR2509 .. C.. T.. ... .-.. ... .. ... AC. ... .... .... ... ... ... ...
#LR2513 .. ... ... ... .-.. ... .. ... A.. ... .... .... ... ... ... ...
#LR2511 .. ... ... ... .-.. ... .. ... A.. ... .... .... ... ... ... ...
#LR2512 .. ... ... ... .-.. ... .. ... A.. ... .... .... ... ... ... ...
#LR2514 .. ... ... ... .-.. ... .. ... A.. ... .... .... ... ... ... ...
#LR2515 .. C.. ... ... .-.. ... .. ... AC. ... .... .... ... ... ... ...
#LR2517 .. .G. ... ... .-.. ... .. ... A.. .T. .... .... ... ... ... ...
#LR2519 .. ... ... ... .-.. ... .. ... A.. ... .... .... ... ... ... ...
#LR2521 .. C.. ... ... .-.. ... .. ... AC. ... .... .... ... ... ... ...
#LR2524 .. ... ... ... .-.. ... .. ... A.. ... .... .... ... ... ... ...
#LR2525 .. ... ... ... .-.. ... .. ... A.. ... .... .... ... ... ... ...
#LR2527 .. ... ... ... .-.. ... .. ... A.. ... .... .... ... ... ... ...
#CM1506 .. ... ... ..G A-T. ... TT .A. T.. ..A ATT. CCGT .GT .G. ... .G.
#CM1505 .. ... ... ..G A-T. ... TT .A. T.. ..A ATT. CCGT .GT .G. ... .G.
#CM1511 .. ... ... ..G A-T. ... TT .A. T.. ..A ATT. CCGT .GT .G. ... .G.
#CM1510 .. ... ... ..G A-T. ... TT .A. T.. ..A ATT. CCGT .GT .G. ... .G.
#CM1509 .. ... ... ..G A-T. ... TT .A. T.. ..A ATT. CCGT .GT .G. ... .G.
#CM1512 .. ... ... ..G A-T. ... TT .A. T.. ..A ATT. CCGT .GT .G. ..T .G.
#CC1505 .C ... .C. C.G TA.C ACG .. ..G A.C T.T .ATG .T.A CG. C.. T.. G.C
#CC1503 .C ... TCT CGG TA.C ACG .G ..G A.C T.T TATG .T.A .G. CG. T.. G.C
#CC1502 .C ... TCT C.G TA.C ACG .G ..G A.. T.T TATG .T.A .G. C.. T.. G.C
#CC1510 .C ..G TCT C.G TA.C ACG .G ..G A.C T.T TATG .T.A .G. C.. T.. G.C
#CC1501 .C ... .C. ..G TA.C ACG .. ..G A.C T.T .ATG .T.A CG. C.. T.. G.C
#CC1509 .C ... TC. C.G TA.C ACG .. ..G A.C T.T .ATG .T.A .G. C.. T.. G.C
#CC1512 .C ... .C. C.G TA.C ACG .. ..G A.C T.T .ATG .T.A CG. C.. T.. G.C
#CC1521 .C ... .C. C.G TA.C ACG .. ..G A.C T.T .ATG .T.A CG. C.. T.. G.C
#CC1522 .C ... TCT C.G TA.C ACG .G ..G A.C T.T TATG .T.A .G. C.. T.. G.C
#CC1523 .C ... TCT CGG TA.C ACG .G ..G A.C T.T TATG .T.A .G. CG. T.. G.C
#CC1524 .C ... TCT C.G TA.C ACG .. ..G A.C T.T TATG .T.A .G. C.. T.. G.C
#CC1525 .C ... TCT C.G TA.C ACG .G ..G A.C T.T TATG .T.A .G. C.. T.. G.C
#CC1528 .C ... TCT C.G TA.C ACG .G ..G A.. T.T TATG .T.A .G. C.. T.. G.C
#CC1529 .C ... TCT C.G TA.C ACG .G ..G A.C T.T TATG .T.A .G. C.. T.. G.C
#CC1530 .C ... ... C.G TA.C ACG .. ..G A.C T.T .ATG .T.A CG. C.. T.. G.C
#CM1513 .. ... ... ..G A-T. ... TT .A. T.. ..A ATT. CCGT .GT .G. ..T ...
#CM1514 G. ... ... ..G A-TC ... TC .AG T.. ..A ATT. .TGT .GT .GC .C. ...
#CM1516 .. ... ... ..G A-T. ... TT .A. T.. ..A ATT. CCGT .GT .G. ... .G.
#CM1518 .. ... ... ..G A-T. ... TT GA. T.. ..A ATT. CCGT .GT .G. ... .G.
#CM1521 .. ... ... ..G A-T. ... TT .A. T.. ..A ATT. CCGT .GT .G. ... .G.
#CM1522 .. ... ... ..G A-T. ... TT .A. T.. ..A ATT. CCGT .GT .G. ... .G.
#CM1529 .. ... ... ..G A-T. ... TT .A. T.. ..A ATT. CCGT .GT .G. ..T ...
#CM1531 G. ... ... ..G A-TC ... TT .AG T.. ..A ATT. .TGT .GT .GC .C. ...
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Fig. 3. Alignment of partial DNA sequences of mitochondrial control region. (Only variable sites are reported).

```
[       111 111 111 111 11 11 111 122 222 22 22 222 22222 222 2222 3333 ]
[       455 566 777 888 88 88 999 911 222 22 23 333 33466 677 7788 2233 ]
[       816 948 014 123 56 79 015 835 345 67 90 367 89003 701 4504 3418 ]
#LR2507 TCA ACA ATC AAA TC CC TAC AGT -AA TA AA TC- AATAA TCT GAAC TCAC
#LR2505 ... ... ... ... .. .. ... ... -.. .. .. ..- ..... ... .G.. ....
#LR2503 ... ... ... ... .. .. ... ... -.. .. .. ..- ..... ... .G.. ....
#LR2510 ... ... ... ... .. .. ... ... -.. .. .. ..- ..... ... .G.. ....
#LR2501 ... ... ... ... .. .. ... ... -.. .. .. ..- ..... ... .G.. ....
#LR2509 ... ... ... ... .. .. ... ... -.. .. .. ..- ...G. ... .G.. ....
#LR2513 ... ... ... ... .. .. ... ... -.. .. .. ..- ..... ... .G.. ....
#LR2511 ... ... ... ... .. .. ... ... -.. .. .. ..- ..... ... .G.. ....
#LR2512 ... ... ... ... .. .. ... ... -.. .. .. ..- ..... ... .G.. ....
#LR2514 ... ... ... ... .. .. ... ... -.. .. .. ..- ..... ... .G.. ....
#LR2515 ... ... ... ... .. .T ... ... -.. .. .. ..- ..... ... .G.. ....
#LR2517 ... ... ... ... .. .. ... ... -.. .. .. ..- ..... ... .G.. ....
#LR2519 ... ... ... ... .. .. ... ... -.. .. .. ..- ..... ... .G.. ....
#LR2521 ... ... ... ... .. .T ... ... -.. .. .. ..- ..... ... .G.. ....
#LR2524 ... ... ... ... .. .. ... ... -.. .. .. ..- ..... ... .G.. ....
#LR2525 ... ... ... ... .. .. ... ... -.. .. .. ..- ..... ... .... ....
#LR2527 ... ... ... ... .. .. ... ... -.. .. .. ..- ..... ... .G.. ....
#CM1506 .TC T.C .AT ..G .T .T CTT .AC T.. .T .T AGG T.A.. ... A.T. GACA
#CM1505 ..C T.C .AT ..G .T .T CTT .AC T.. .T GT AGG T.A.G ... A.T. GACA
#CM1511 ..C T.C .AT ..G .T .T CTT .AC T.. .T .T AGG T.A.. ... A.T. GACA
#CM1510 ..C T.C .AT ..G .T .T CTT .AC T.. .T .T AGG T.A.. .T. A.T. GACA
#CM1509 ..C T.C .AT ..G .T .T CTT .AC T.. .T .T AGG T.A.. ... A.T. GACA
#CM1512 ..C T.C .AT ..G .T .T CTT .AC T.. .T GT AGG T.A.. ... A.T. GACA
#CC1505 C.. T.. G.T GG. CT TT C.. T.C -CT AT .C AGG ...G. CTC .GC. ..CA
#CC1503 C.. T.. G.T GG. CT TT ... T.C -CT AT .C AGG ...G. CTC .GC. ..CA
#CC1502 C.. T.. G.T GG. CT TT ... T.C -CT AT .C AGG ...G. CTC .GC. ..CA
#CC1510 C.. T.. G.T GG. CT TT ... T.C -CT AT .C AGG ...G. CTC .GC. ..CA
#CC1501 C.. T.. G.T GG. CT TT C.. T.C -CT AT .C AGG ...G. CTC .GC. ..CA
#CC1509 C.. T.. G.T GG. CT TT C.. T.C -CT AT .C AGG ...G. CTC .GC. ..CA
#CC1512 C.. T.. G.T GG. CT TT C.. T.C -CT AT .C AGG ...G. CTC .GC. ..CA
#CC1521 C.. T.. G.T GG. CT TT C.. T.C -CT AT .C AGG ...G. CTC .GC. ..CA
#CC1522 C.. T.. G.T GG. CT TT ... T.C -CT AT .C AGG ...G. CTC .GC. ..CA
#CC1523 C.. T.. G.T GG. CT TT ... T.C -CT AT .C AGG ...G. CTC .GC. ..CA
#CC1524 C.. T.. G.T GG. CT TT ... T.C -CT AT .C AGG ...G. CTC .GC. ..CA
#CC1525 C.. T.. G.T GG. CT TT ... T.C -CT AT .C AGG ...G. CTC .GC. ..CA
#CC1528 C.. T.. G.T GG. CT TT ... T.C -CT AT .C AGG ...G. CTC .GC. ..CA
#CC1529 C.. T.. G.T GG. CT TT ... T.C -CT AT .C AGG ...G. CTC .GC. ..CA
#CC1530 C.. T.. G.T GG. CT TT C.. T.C -CT AT .C AGG ...G. CTC .GC. ..CA
#CM1513 ..C T.C .AT ..G .T .T CTT .AC T.. .T GT AGG T.A.. ... A.TT GACA
#CM1514 ..C T.C ..T ... .T .T CTT .AC T.. .T .T AGG T.A . ... A.T. GACA
#CM1516 ..C T.C .AT ..G .T .T CTT .AC T.. .T .T AGG T.A . .T. A.T. GACA
#CM1518 ..C T.C .AT ..G .T .T CTT .AC T.. .T GT AGG T.A . .T. A.T. GACA
#CM1521 ..C T.C .AT ..G .T .T CTT .AC T.. .T .T AGG T.A . ... A.T. GACA
#CM1522 ..C T.C .AT ..G .T .T CTT .AC T.. .T GT AGG T.A . .T. A.T. GACA
#CM1529 ..C T.C .AT ..G .T .T CTT .AC T.. .T GT AGG T.A . ... A.TT GACA
#CM1531 ..C TTC ..T  ... .T .T CTT .AC T.. .T .T AGG T.A . ... A.T.GACA
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Fig. 3. (*continued*).

```
[        333 333 333 33 344 444 4444 444 44 444 444 555 55 555 555 555  ]
[        444 455 889 99 900 113 3444 455 67 778 899 000 11 222 334 455  ]
[        478 905 190 12 502 144 9015 689 77 890 823 689 18 129 180 801  ]
#LR2507  CCC AAT ACG TC GCC AAT -TTT GTC TA GCT TC- TTC TT TAA GTG GAT
#LR2505  ... ... ... .. ... ... -C.. ... .. ... ..- ... .. ..G ... T..
#LR2503  ..A ... ... .. ... ... -... ... .. ... ..- ... .. ... ... ...
#LR2510  ... ... ... .. ... ... -C.. ... .. ... ..- ... .. ..G ... T..
#LR2501  ... ... ... .. ... ... -... ... .. ... ..- ... .. ... ... ...
#LR2509  ... ... ... .. ... ... -C.. ... .. ... ..- ... .. ..G ... T..
#LR2513  ... ... ... .. ... ... -... ... .. ... ..- ... .. ..T ... ...
#LR2511  ... ... ... .. ... ... -... ... .. ... ..- ... .. ..T ... ...
#LR2512  ..A ... ... .. ... ... -... ... .. ... ..- ... .. ... ... ...
#LR2514  ... ... ... .. ... ... -... ... .. ... ..- ... .. ... ... ...
#LR2515  ... ..C ... .. ... ... -C.. ... .. ... ..- ... .. ..G ..A T..
#LR2517  ... ... ... .. ... ... -... ... .. ... ..- ... .. ... ... ...
#LR2519  ... C.. ... .. ... ... -... ... .. ... ..- ... .. ... ... ...
#LR2521  ... ..C ... .. ... ... -C.. ... .. ... ..- ... .. ..G ... T..
#LR2524  ... ... ... .. .A. ... -... ... .. ... ..- ... .. ... ... ...
#LR2525  ... ... ... .. ... ... -... ... .. ... ..- ... .. ... ... ...
#LR2527  ..A ... ... .. ... ... -... ... .. ... ..- ... .. ... ... ...
#CM1506  ... ..C ..A .T A.A ... C... ..T C. ATA .TT ACT C. ..G TCT A.C
#CM1505  ..- ..C .TA .T A.A ... C... ..T C. ATA .TT ACT C. ... TCT A.C
#CM1511  ... ..C ..A .T A.A ... -... ..T C. ATA .TT ACT C. ..G TCT A.C
#CM1510  ... ..C .TA .T A.A ... -C.. ..T C. ATA .TT ACT C. ... TCT A.C
#CM1509  ... ..C .TA .T A.A ... C... ..T C. ATA .TT ACT C. ..G TCT A.C
#CM1512  ... ..C .TA .T A.A ... C... ..T C. ATA .TT ACT C. ... TCT A.C
#CC1505  TT. .G. ... C. ..A GG. -C.. ..T .G ..A ..- ..T .. CT. T.. AG.
#CC1503  T.. .G. ... C. ..A G.. -C.. .CT .G ..A ..- ..T .. CT. T.A AG.
#CC1502  ... .G. ... C. ..A ... -C.. .CT .G ..A ..- ..T .. CT. T.A AG.
#CC1510  T.. .G. ... C. ..A GG. -C.. .CT .G ..A ..- ..T .. CT. T.A AG.
#CC1501  TT. .G. ... C. ..A GG. -C.. ..T .G ..A ..- ..T .. CT. T.. AG.
#CC1509  T.. .G. ... C. ..A G.. -C.. .CT .G ..A ..- ..T .. CT. T.A AG.
#CC1512  TT. .G. ... C. ..A GG. -C.. ..T .G ..A ..- ..T .. CT. T.. AG.
#CC1521  TT. .G. G.. C. ..A GG. -C.. ..T .G ..A ..- ..T .C CT. T.. AG.
#CC1522  ... .G. ... C. ..A ... -C.. .CT .G ..A ..- ..T .. CT. -.A AG.
#CC1523  T.. .G. ... C. ..A G.. -C.. .CT .G ..A ..- ..T .. CT. T.A AG.
#CC1524  T.. .G. ... C. ..A G.. -C.. .CT .G ..A ..- ..T .. CT. T.A AG.
#CC1525  ... .G. ... C. ..A G.. -C.. .CT .G ..A C.- ..T .. CT. T.A AG.
#CC1528  ... .G. ... C. ..A ... -C.. .CT .G ..A ..- ..T .. CT. T.A AG.
#CC1529  ... .G. ... C. ..A G.. -C.. .CT .G ..A C.- ..T .. CT. T.A AG.
#CC1530  TT. .G. ... C. ..A GG. -C.. ..T .G ..A ..- ..T .. CT. T.. AG.
#CM1513  ..- ..C .TA .T A.A ..G C.T. T.T C. ATA .TT ACT C. ... TCT A.C
#CM1514  ..A ... ..A .T A.A ... -CT. ..T C. ATA .TT ACT C. ... TCT A..
#CM1516  ... ..C .TA .T A.A ... -CT. ..T C. ATA .TT ACT C. ... TCT A.C
#CM1518  ... ..C .TA .T A.A ... C.TG -.T C. ATA .TT ACT C. ... TCT A.C
#CM1521  .-- ..C ..A .T A.A ... C.T. ..T C. ATA .TT ACT C. ..G TCT A.C
#CM1522  ... ..C .TA .T A.A ... C.TG -.T C. ATA .TT ACT C. ... TCT A.C
#CM1529  ... ..C .TA .T A.A ... C.T. ..T C. ATA .TT ACT C. ... TCT A.C
#CM1531  ..A ... ..A .T A.A ... -CT. ..T C. ATA .TT ACT C. ... TCT A..
```

Fig. 3. (*continued*).

```
[       555 5 555 555 555 66 666 6 ]
[       555 5 566 777 999 11 122 2 ]
[       345 8 907 126 345 14 901 2 ]
#LR2507 AAG T TAT ATA TTC TC ATG C
#LR2505 ... . ... ... ... .. ... .
#LR2503 ... . C.. ... ... .. ... .
#LR2510 ... . ... ... ... .. ... .
#LR2501 ... . ... ... ... .. ... .
#LR2509 ... . ... ... ... .. ... .
#LR2513 ... . ... ... ... .. ... .
#LR2511 ... . ... ... ... .. ... .
#LR2512 ... . C.. ... ... .. ... .
#LR2514 ... . C.. ... ... .. ... .
#LR2515 ... . ... ..G ... .. ... .
#LR2517 ... . ... ... ... .. ... .
#LR2519 ... . ... ... ... .. ... .
#LR2521 ... . ... ... ... .. ... .
#LR2524 ... . ... ... ... .. ... .
#LR2525 ... . ... ... ... .. ... .
#LR2527 ... . C.. ... ... .. ... .
#CM1506 GGA C ... G.G .CT .T TAT G
#CM1505 GGA C ... GCG .CT .T TAT G
#CM1511 GGA C ... G.G .CT .T TAT G
#CM1510 GGA C ... GCG .CT .T TAT G
#CM1509 GGA C ... G.G .CT .T TAT G
#CM1512 GGA C ... GCG .CT .T TAT G
#CC1505 ... . .G. G.G C.. C. ... .
#CC1503 ... . .GC G.G C.. C. ... .
#CC1502 ... . .GC G.. C.. C. ... .
#CC1510 ... . .GC G.G C.. C. ... .
#CC1501 ... . .G. G.G C.. C. ... .
#CC1509 ... . .GC G.G C.. C. ... .
#CC1512 ... . .G. G.G C.. C. ... .
#CC1521 ... . .G. G.G C.. C. ... .
#CC1522 ... . .GC G.G C.. C. ... .
#CC1523 ... . .GC G.G C.. C. ... .
#CC1524 ... . ... G.G C.. C. ... .
#CC1525 ... . .GC G.G C.. C. ... .
#CC1528 ... . .GC G.. C.. C. ... .
#CC1529 ... . .GC G.G C.. C. ... .
#CC1530 ... . .G. G.G C.. C. ... .
#CM1513 GGA C ... GCG .CT .T TAT G
#CM1514 GGA C ... ..G .CT .T TAT G
#CM1516 GGA C ... GCG .CT .T TAT G
#CM1518 GGA C ... GCG .CT .T TAT G
#CM1521 GGA C ... G.G .CT .T TAT G
#CM1522 GGA C ... GCG .CT .T TAT G
#CM1529 GGA C ... GCG .CT .T TAT G
#CM1531 GGA C ... ..G .CT .T TAT G
```

Fig. 3. (*continued*).

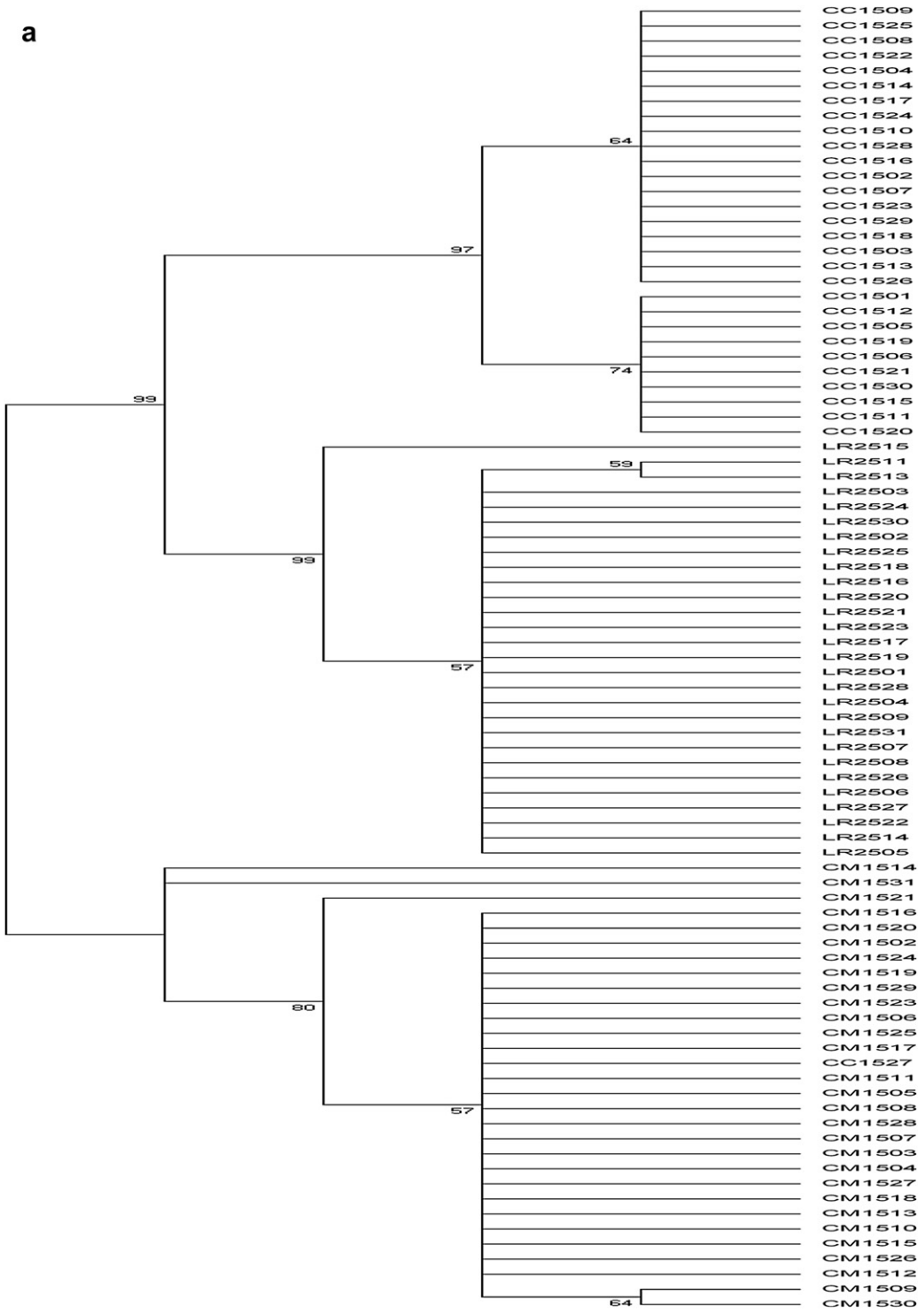

Fig. 4. a & b NJ and ME phylogenetic tree of Indian major carps inferred from DNA sequences of mitochondrial gene cytochrome *b*.

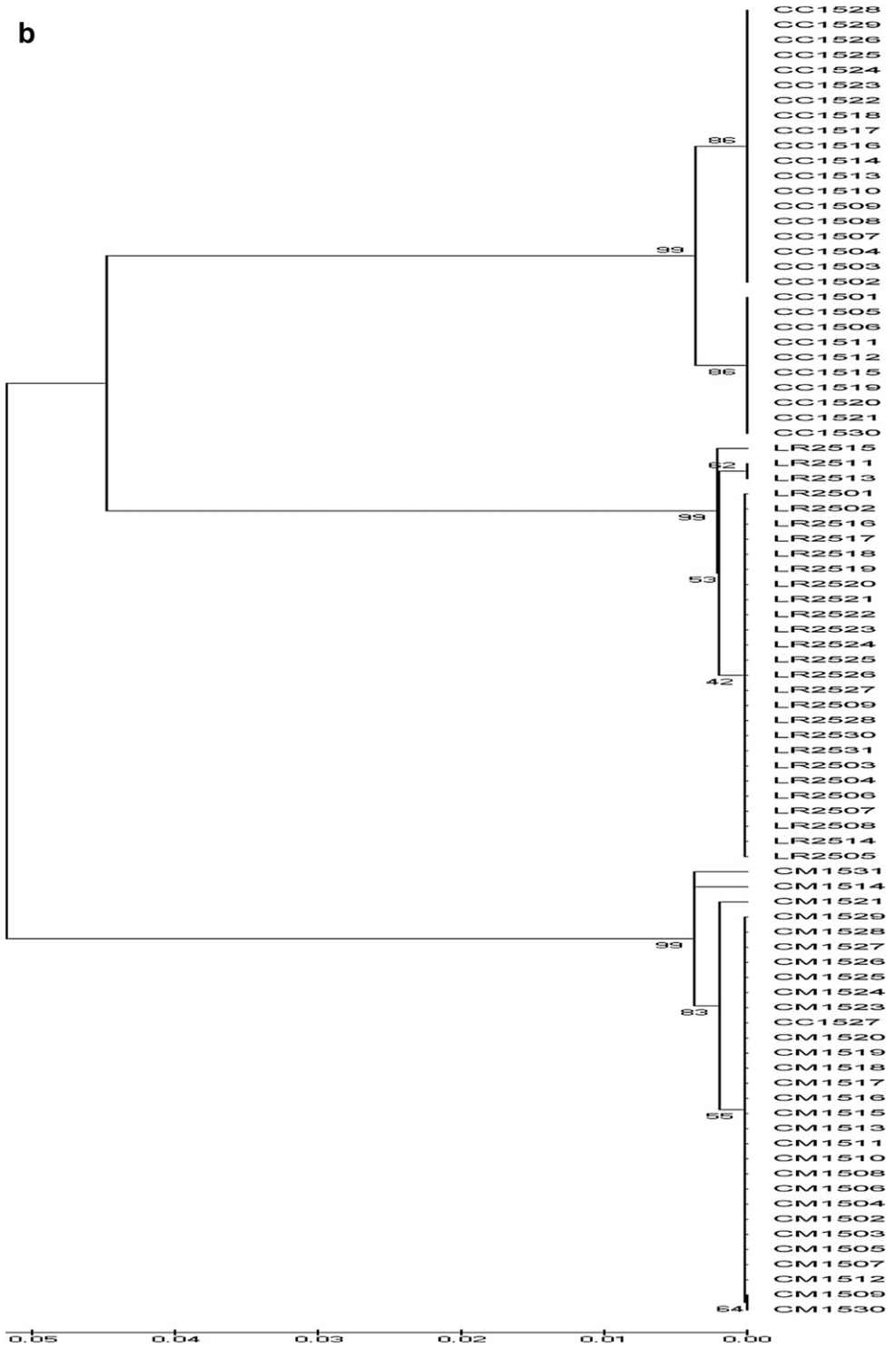

Fig. 4. (*continued*).

Table 3
Pairwise genetic distances (Kimura 2 parameter) of LR = *Labeo rohita*, CC = *Catla catla* and CM = *Cirrhinus mrigala* haplotypes based on ATPase 6/8 gene sequences.

[	1	2	3	4	5	6	7	8	9	10	11	12	13	]
[1] #CM1502														
[2] #CM1504	0.002													
[3] #CM1503	0.002	0.003												
[4] #CM1509	0.002	0.003	0.000											
[5] #CM1520	0.002	0.000	0.003	0.003										
[6] #CM1531	0.002	0.003	0.003	0.003	0.003									
[7] #CM1511	0.003	0.005	0.002	0.002	0.005	0.005								
[8] #LR2502	0.129	0.127	0.129	0.129	0.127	0.131	0.131							
[9] #LR2518	0.131	0.129	0.131	0.131	0.129	0.133	0.133	0.002						
[10] #CC1511	0.115	0.113	0.115	0.115	0.113	0.113	0.117	0.078	0.076					
[11] #CC1525	0.119	0.117	0.119	0.119	0.117	0.117	0.121	0.078	0.076	0.007				
[12] #CC1504	0.119	0.117	0.119	0.119	0.117	0.117	0.121	0.078	0.076	0.007	0.003			
[13] #CC1506	0.117	0.115	0.117	0.117	0.115	0.115	0.119	0.080	0.078	0.002	0.008	0.008		

alignments were done to get consensus sequence. Two haplotypes of catla, four of rohu and seven haplotypes of mrigal were found for ATPase 6/8 gene. From 785 bp sequence, 476 sites were conserved, 112 variable, 14 singletons and 98 sites were parsimony informative. The polymorphic sites are shown in Fig. 2. The nucleotide frequencies observed were; $T = 26.9\%$, $C = 29.7\%$, $A = 30.7\%$ and $G = 12.8\%$. Average transitional pairs (si = 33) were more frequent than transversional pairs (sv = 11) with an average ratio of 2.9. In ATPase gene the most frequently occurring amino acids were Proline (12.38%) and Threonine (11.44%) whereas least frequently occurring amino acids were Glutamate (0.59%) and Tryptophan (0.20%).

Pairwise genetic distance values (Kimura 2 parameter) based on ATPase 6/8 gene using MEGA 4.0 are given in Table 3. The average genetic distance among all the three Indian major carps was estimated as 0.077. Intraspecific distances ranged from 0.002 to 0.008 and the interspecific distances ranged from 0.076 to 0.133. The highest interspecific genetic distance (0.133) was between *L. rohita* and *C. mrigala* and the lowest (0.076) was between *L. rohita* and *C. catla*. Phylogenetic analysis was done using MEGA 4.0 to produce Neighbor joining and Minimum evolution trees (Fig. 5a and b).

3.3. Control region (CR)

A total of 28 individuals of *L. rohita*, 29 of *C. catla* and 31 individuals of *C. mrigala* were used for sequence analysis of mitochondrial CR. The sequences of the control region had no insertions, deletions and stop codons in the sequences. Sequencing of the partial gene brought out an average of 710 bp sequence. Forward and reverse sequences were aligned to get the consensus sequence. Fifteen haplotypes of *C. catla*, 17 of *L. rohita* and 14 haplotypes of *C. mrigala* were found for CR. In 710 bp fragments, 513 sites were conserved, 158 variable, 8 singleton and 150 sites were parsimony informative. The polymorphic sites are given in Fig. 3. The nucleotide frequencies observed were: $T = 32.3\%$, $C = 21.1\%$, $A = 33.2\%$ and $G = 13.5\%$. Average transitional pairs (si = 43) were more frequent than transversional pairs (sv = 21) with an average ratio of 2.1.

Pairwise genetic distance values (Kimura 2 parameter) based on mitochondrial CR using MEGA 4.0 are given in Table 4. The average genetic distance among all the three Indian major carps was estimated as 0.103. Intraspecific distances ranged from 0.000 to 0.036 and the interspecific distances ranged from 0.129 to 0.211. The highest interspecific genetic distance (0.211) was between *C. catla* and *C. mrigala* and the lowest (0.129) was between *L. rohita* and *C. catla*. Phylogenetic analysis was done using MEGA 4.0 to produce Neighbor joining and Minimum evolution trees (Fig. 6a and b).

4. Discussion

Analysis of mtDNA variation has indicated that the most variable segments of the mitochondrial genome are located in the noncoding CR (Cann et al., 1984). Consequently, it has been shown that sequencing of these "hypervariable" segments reveals higher variation, relative to RFLP analysis (Desmarais, 1989; Vigilant et al., 1989). There is little information available to confirm whether the higher variability of the CR also holds true in other vertebrates namely, in fish. There are reports that CR may not be as variable in some fish species as it is in mammals (Meyer et al., 1990; Bernatchez et al., 1992). Conversely, Brown et al. (1993) observed that the substitution rate of white sturgeon (*Acipenser transmontanus*) CR was comparable to rates of hypervariable sequences in the human CR. In our study the analysis of CR alone revealed 46 haplotypes, followed by 13 in ATPase 6/8 and 10 haplotypes in cyt *b*. Maximum number of haplotypes found in CR shows its hyper variability. These results suggest that differences in patterns of mutation and constraints may exist between the CR of teleost and chondrostean fish (Bernatchez and Danzmann, 1993). Our results indicated that there is

Table 4

Pairwise genetic distances (Kimura 2 parameter) of LR = *Labeo rohita*, CC = *Catla catla* and CM = *Cirrhinus mrigala* haplotypes based on control region sequences.

[	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	]
[1]																																															
[2]	0.013																																														
[3]	0.005	0.012																																												
[4]	0.013	0.000	0.012																																											
[5]	0.003	0.010	0.002	0.010																																										
[6]	0.015	0.002	0.013	0.002	0.012																																								
[7]	0.005	0.010	0.003	0.010	0.002	0.012																																							
[8]	0.005	0.010	0.003	0.010	0.002	0.012	0.000																																						
[9]	0.005	0.012	0.000	0.012	0.002	0.013	0.003	0.003																																					
[10]	0.005	0.012	0.000	0.012	0.002	0.013	0.003	0.003	0.000																																				
[11]	0.018	0.008	0.017	0.008	0.015	0.010	0.015	0.015	0.017	0.017																																			
[12]	0.007	0.013	0.005	0.013	0.003	0.015	0.005	0.005	0.005	0.005	0.018																																		
[13]	0.005	0.012	0.003	0.012	0.002	0.013	0.003	0.003	0.003	0.003	0.017	0.005																																	
[14]	0.015	0.005	0.013	0.005	0.012	0.007	0.012	0.012	0.013	0.013	0.003	0.015	0.013																																
[15]	0.005	0.012	0.003	0.012	0.002	0.013	0.003	0.003	0.003	0.003	0.017	0.005	0.003	0.013																															
[16]	0.002	0.012	0.003	0.012	0.002	0.013	0.003	0.003	0.003	0.003	0.017	0.005	0.003	0.013	0.003																														
[17]	0.005	0.012	0.000	0.012	0.002	0.013	0.003	0.003	0.000	0.0000	0.017	0.005	0.003	0.013	0.003	0.003																													
[18]	0.144	0.152	0.148	0.152	0.146	0.154	0.146	0.146	0.148	0.148	0.144	0.150	0.148	0.146	0.148	0.144	0.148																												
[19]	0.148	0.161	0.152	0.161	0.150	0.163	0.152	0.152	0.152	0.152	0.152	0.154	0.152	0.154	0.152	0.148	0.152	0.010																											
[20]	0.142	0.150	0.146	0.150	0.144	0.152	0.144	0.144	0.146	0.146	0.142	0.148	0.146	0.144	0.146	0.142	0.146	0.002	0.008																										
[21]	0.148	0.156	0.152	0.156	0.150	0.158	0.152	0.152	0.152	0.152	0.148	0.154	0.152	0.15	0.152	0.148	0.152	0.01	0.007	0.008																									
[22]	0.144	0.152	0.148	0.152	0.146	0.154	0.146	0.146	0.148	0.148	0.144	0.15	0.148	0.146	0.148	0.144	0.148	0.003	0.007	0.002	0.007																							
[23]	0.148	0.161	0.152	0.161	0.150	0.163	0.152	0.152	0.152	0.152	0.152	0.154	0.152	0.154	0.152	0.148	0.152	0.01	0.003	0.008	0.007	0.007																							
[24]	0.133	0.135	0.131	0.135	0.129	0.133	0.131	0.131	0.131	0.131	0.133	0.133	0.131	0.133	0.131	0.131	0.131	0.194	0.199	0.192	0.190	0.194	0.199																						
[25]	0.145	0.143	0.143	0.143	0.141	0.141	0.143	0.143	0.143	0.143	0.141	0.145	0.143	0.145	0.143	0.143	0.143	0.201	0.206	0.199	0.197	0.201	0.206	0.020																						
[26]	0.133	0.131	0.131	0.131	0.129	0.129	0.131	0.131	0.131	0.131	0.133	0.133	0.131	0.133	0.131	0.131	0.131	0.197	0.201	0.194	0.192	0.197	0.201	0.024	0.010																					
[27]	0.145	0.143	0.143	0.143	0.141	0.141	0.143	0.143	0.143	0.143	0.141	0.145	0.143	0.145	0.143	0.143	0.143	0.206	0.211	0.204	0.201	0.206	0.211	0.017	0.007	0.010																				
[28]	0.131	0.133	0.129	0.133	0.127	0.131	0.129	0.129	0.129	0.129	0.131	0.131	0.129	0.131	0.129	0.129	0.129	0.192	0.197	0.19	0.187	0.192	0.197	0.002	0.022	0.025	0.018																			
[29]	0.137	0.135	0.135	0.135	0.133	0.133	0.135	0.135	0.135	0.135	0.133	0.137	0.135	0.137	0.135	0.135	0.135	0.197	0.201	0.194	0.192	0.197	0.201	0.010	0.010	0.013	0.010	0.012																	
[30]	0.133	0.135	0.131	0.135	0.129	0.133	0.131	0.131	0.131	0.131	0.133	0.133	0.131	0.133	0.131	0.131	0.131	0.194	0.199	0.192	0.190	0.194	0.199	0.000	0.020	0.024	0.017	0.002	0.010																
[31]	0.137	0.139	0.135	0.139	0.133	0.137	0.135	0.135	0.135	0.135	0.137	0.137	0.135	0.137	0.135	0.135	0.135	0.199	0.204	0.197	0.194	0.199	0.204	0.003	0.024	0.027	0.020	0.005	0.013	0.003															
[32]	0.137	0.135	0.135	0.135	0.133	0.133	0.135	0.135	0.135	0.135	0.133	0.137	0.135	0.137	0.135	0.135	0.135	0.197	0.201	0.194	0.192	0.197	0.201	0.020	0.007	0.003	0.007	0.022	0.010	0.020	0.024													
[33]	0.145	0.143	0.143	0.143	0.141	0.141	0.143	0.143	0.143	0.143	0.141	0.145	0.143	0.145	0.143	0.143	0.143	0.201	0.206	0.199	0.197	0.201	0.206	0.020	0.000	0.010	0.007	0.022	0.010	0.020	0.024	0.007											
[34]	0.135	0.133	0.133	0.133	0.131	0.131	0.133	0.133	0.133	0.133	0.131	0.135	0.133	0.135	0.133	0.133	0.133	0.197	0.201	0.194	0.192	0.197	0.201	0.015	0.008	0.012	0.008	0.017	0.008	0.015	0.018	0.008	0.008										
[35]	0.141	0.139	0.139	0.139	0.137	0.137	0.139	0.139	0.139	0.139	0.137	0.141	0.139	0.141	0.139	0.139	0.139	0.201	0.206	0.199	0.197	0.201	0.206	0.020	0.007	0.007	0.007	0.022	0.010	0.020	0.024	0.003	0.007	0.008									
[36]	0.133	0.131	0.131	0.131	0.129	0.129	0.131	0.131	0.131	0.131	0.133	0.133	0.131	0.133	0.131	0.131	0.131	0.197	0.201	0.194	0.192	0.197	0.201	0.024	0.010	0.000	0.010	0.025	0.013	0.024	0.027	0.003	0.010	0.012	0.007								
[37]	0.141	0.139	0.139	0.139	0.137	0.137	0.139	0.139	0.139	0.139	0.137	0.141	0.139	0.141	0.139	0.139	0.139	0.201	0.206	0.199	0.197	0.201	0.206	0.020	0.007	0.007	0.007	0.022	0.010	0.020	0.024	0.003	0.007	0.008	0.000	0.007							
[38]	0.131	0.133	0.129	0.133	0.127	0.131	0.129	0.129	0.129	0.129	0.131	0.131	0.129	0.131	0.129	0.129	0.129	0.192	0.197	0.190	0.187	0.192	0.197	0.002	0.022	0.025	0.018	0.003	0.012	0.002	0.005	0.022	0.022	0.017	0.022	0.025	0.022						
[39]	0.150	0.163	0.154	0.163	0.152	0.165	0.154	0.154	0.154	0.154	0.154	0.156	0.154	0.156	0.154	0.15	0.154	0.015	0.008	0.013	0.012	0.012	0.005	0.201	0.208	0.203	0.213	0.199	0.203	0.201	0.206	0.203	0.208	0.203	0.208	0.203	0.208	0.199					
[40]	0.140	0.148	0.144	0.148	0.142	0.150	0.144	0.144	0.144	0.144	0.144	0.146	0.144	0.146	0.144	0.14	0.144	0.03	0.034	0.029	0.030	0.030	0.034	0.179	0.185	0.181	0.190	0.176	0.181	0.179	0.183	0.181	0.185	0.181	0.185	0.181	0.185	0.176	0.036				
[41]	0.148	0.156	0.152	0.156	0.150	0.158	0.152	0.152	0.152	0.152	0.148	0.154	0.152	0.15	0.152	0.148	0.152	0.01	0.007	0.008	0.000	0.007	0.007	0.190	0.197	0.192	0.201	0.187	0.192	0.190	0.194	0.192	0.197	0.192	0.197	0.192	0.197	0.187	0.012	0.030			
[42]	0.152	0.165	0.156	0.165	0.154	0.167	0.156	0.156	0.156	0.156	0.156	0.158	0.156	0.158	0.156	0.152	0.156	0.013	0.007	0.012	0.007	0.010	0.007	0.199	0.206	0.201	0.210	0.196	0.201	0.199	0.203	0.201	0.206	0.201	0.206	0.201	0.206	0.196	0.012	0.037	0.007		
[43]	0.142	0.150	0.146	0.150	0.144	0.152	0.144	0.144	0.146	0.146	0.142	0.148	0.146	0.144	0.146	0.142	0.146	0.002	0.008	0.000	0.008	0.002	0.008	0.192	0.199	0.194	0.204	0.190	0.194	0.192	0.197	0.194	0.199	0.194	0.199	0.194	0.199	0.190	0.013	0.029	0.008	0.012	
[44]	0.150	0.163	0.154	0.163	0.152	0.165	0.154	0.154	0.154	0.154	0.154	0.156	0.154	0.156	0.154	0.150	0.154	0.012	0.005	0.010	0.005	0.008	0.005	0.196	0.203	0.199	0.208	0.194	0.199	0.196	0.201	0.199	0.203	0.199	0.203	0.199	0.203	0.194	0.010	0.036	0.005	0.002	0.010	
[45]	0.148	0.161	0.152	0.161	0.150	0.163	0.152	0.152	0.152	0.152	0.152	0.154	0.152	0.154	0.152	0.148	0.152	0.013	0.007	0.012	0.010	0.010	0.003	0.199	0.206	0.201	0.211	0.197	0.201	0.199	0.204	0.201	0.206	0.201	0.206	0.197	0.002	0.034	0.010	0.010	0.012	0.008	
[46]	0.140	0.148	0.144	0.148	0.142	0.150	0.144	0.144	0.144	0.144	0.144	0.146	0.144	0.146	0.144	0.140	0.144	0.029	0.032	0.027	0.029	0.029	0.032	0.179	0.185	0.181	0.190	0.176	0.181	0.179	0.183	0.181	0.185	0.181	0.185	0.181	0.185	0.176	0.034	0.002	0.029	0.036	0.027	0.034	0.032

[1] #LR2507; [2] #LR2505; [3] #LR2503; [4] #LR2510; [5] #LR2501; [6] #LR2509; [7] #LR2513; [8] #LR2511; [9] #LR2512; [10] #LR2514; [11] #LR2515; [12] #LR2517; [13] #LR2519; [14] #LR2521; [15] #LR2524; [16] #LR2525; [17] #LR2527; [18] #CM1506; [19] #CM1505; [20] #CM1511; [21] #CM1510l; [22] #CM1509; [23] #CM1512; [24] #CC1505; [25] #CC1503; [26] #CC1502; [27] #CC1510; [28] #CC1501; [29] #CC1509; [30] #CC1512; [31] #CC1521; [32] #CC1522; [33] #CC1523; [34] #CC1524; [35] #CC1525; [36] #CC1528; [37] #CC1529; [38] #CC1530; [39] #CM1513; [40] #CM1514; [41] #CM1516; [42] #CM1518; [43] #CM1521; [44] #CM1522; [45] #CM1529; [46] #CM1531.

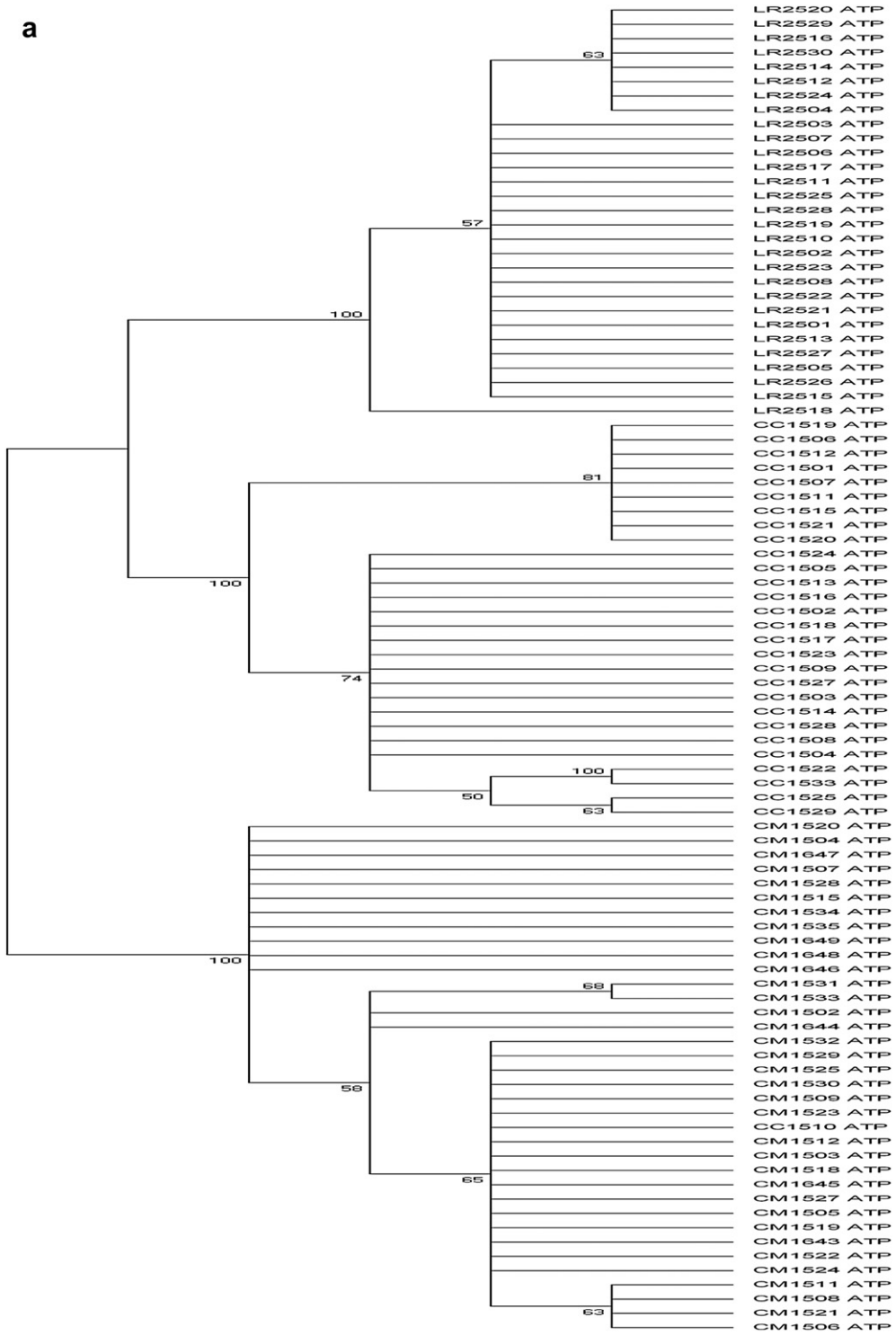

Fig. 5. a & b NJ and ME phylogenetic tree of Indian major carps inferred from DNA sequences of mitochondrial ATPase 6/8 gene.

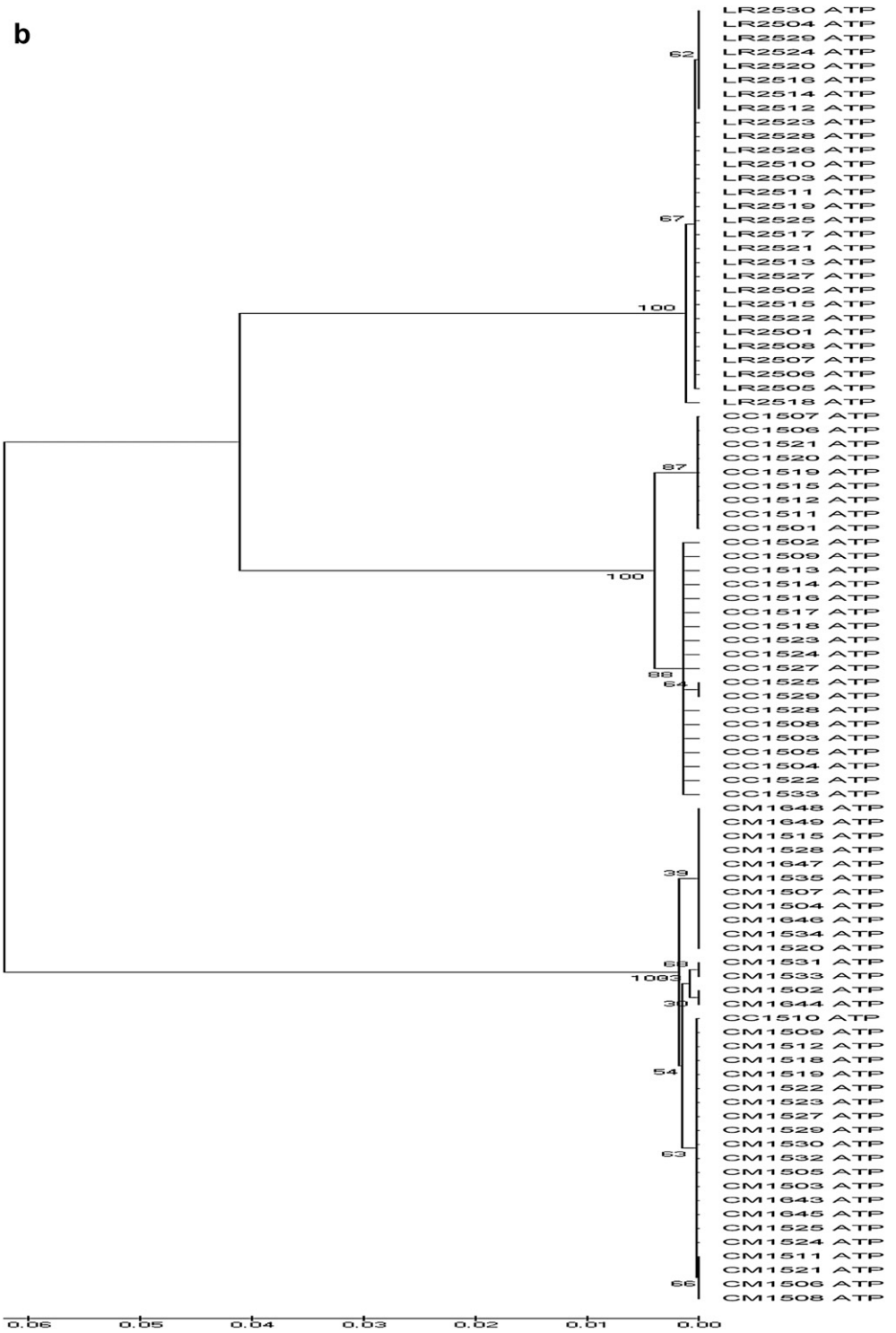

Fig. 5. (*continued*).

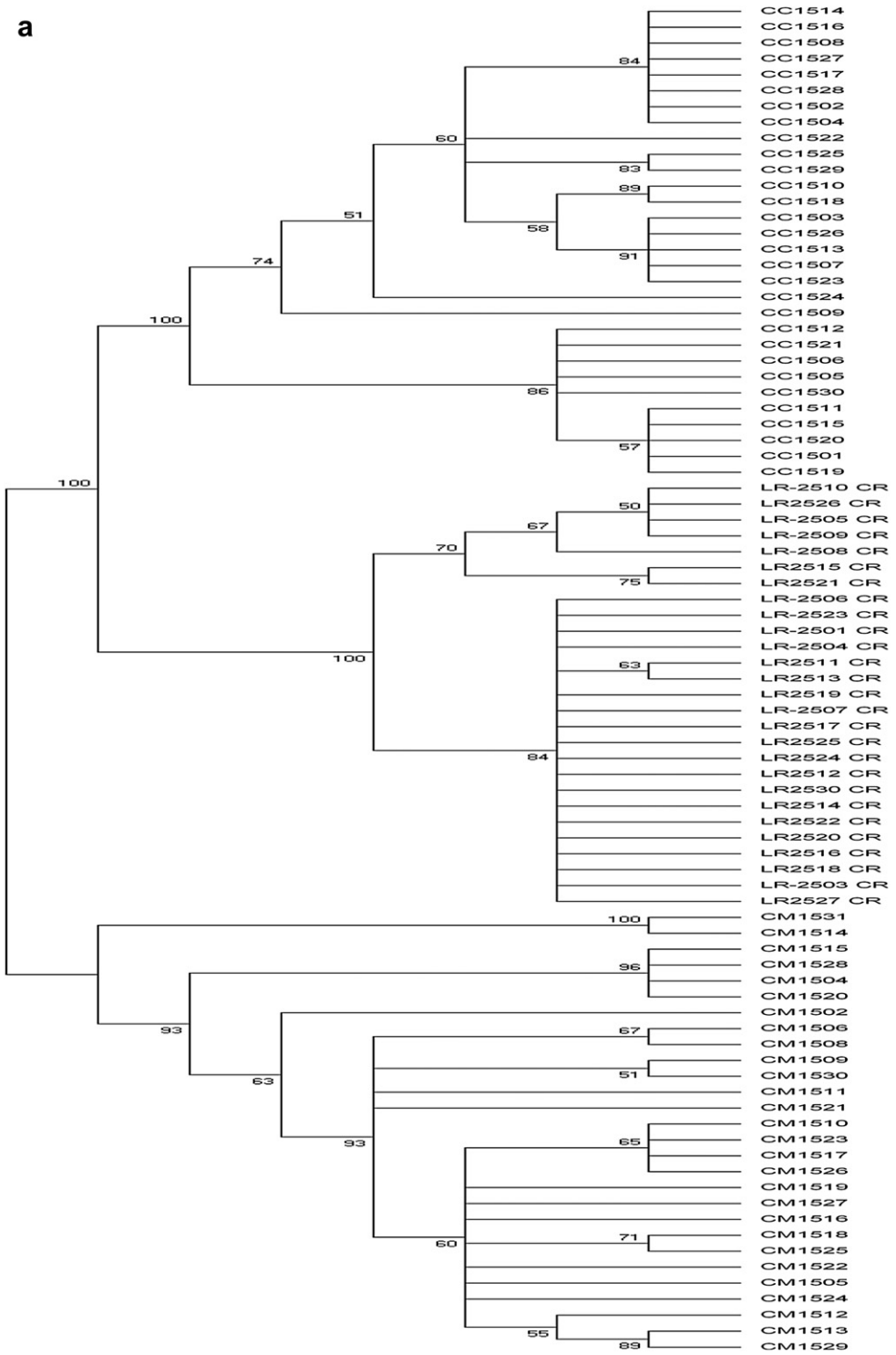

Fig. 6. a & b NJ and ME phylogenetic tree of Indian major carps inferred from DNA sequences of control region.

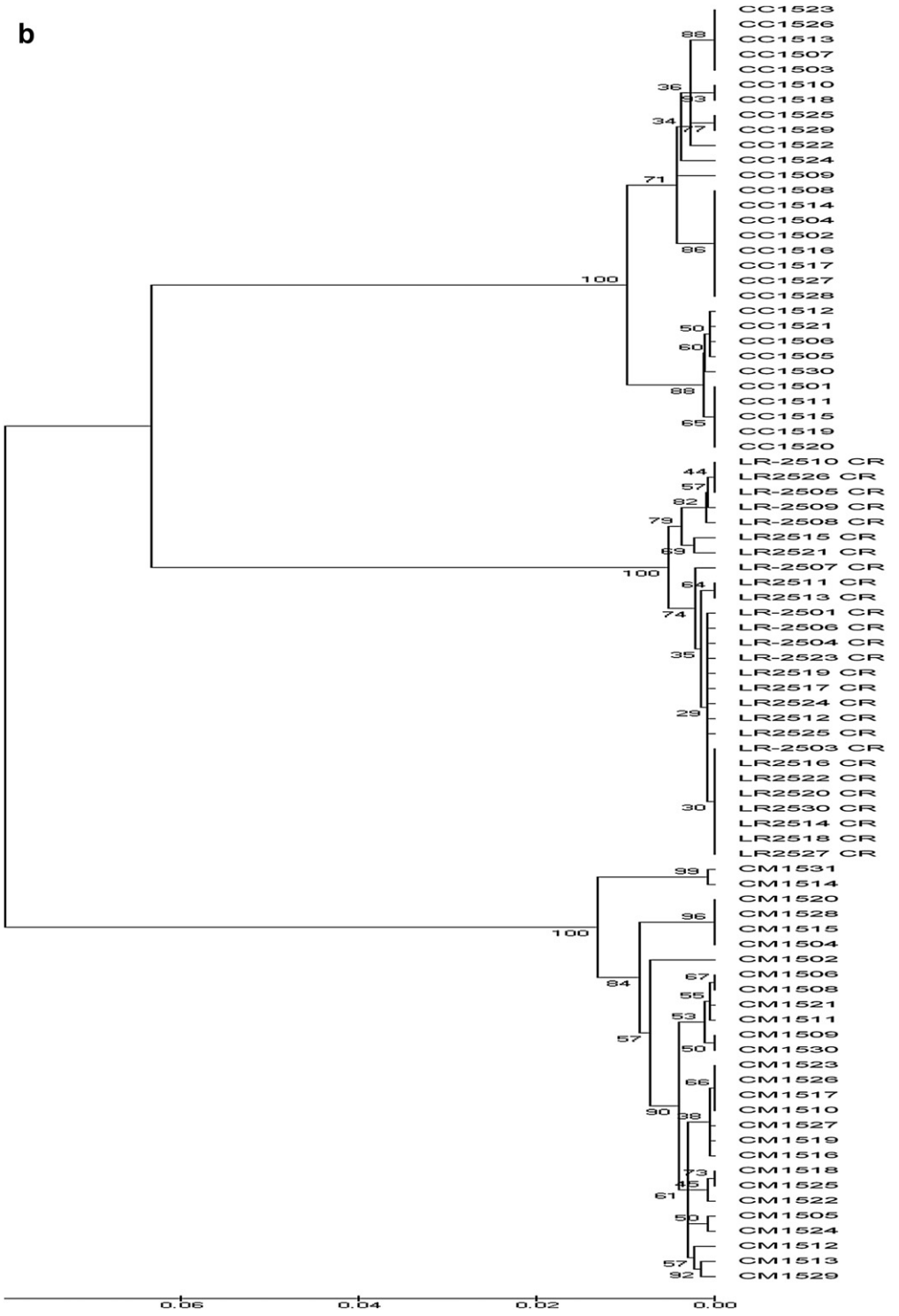

Fig. 6. (*continued*).

higher substitution rate in CR, which is in contradiction to the findings of Meyer et al. (1990). Intraspecific variations exhibited by cyt *b* and ATPase 6/8 genes are less than CR. The cyt *b* gene has minimum variations as compared to ATPase 6/8 gene and CR. However, the mtDNA CR appears to have mutation rate that parallels to that of the evolution of a species (Page and Holmes, 1998) and in this case, most polymorphic sites could have emerged during a demographic expansion that probably would have reached moderate population size, which might explain the presence of 46 haplotypes.

The phylogenetic analysis was done by taking protein coding genes: ATPase 6/8 and non coding CR in Indian major carps revealed that CR performs well for resolving species level splits. The CR is usually described as hypervariable (Simon, 1991), but this may result in part because only the more variable portions are usually targeted for analysis. This result indicated the importance of evaluating rates and patterns of mtDNA evolution prior to employing it as a phylogenetic marker.

The phylogenetic trees exhibiting 3 clusters by all the three genes in these carps suggest that they appear to have diverged almost at the same time. Phylogenetic trees (NJ, ME) in the present study revealed two lineages among three Indian major carps and it illustrates clearly that *L. rohita* always makes a sister clade with *C. catla* than *C. mrigala*. These data indicated the existence of significant differences among the three Indian major carps and this also favored the results of genetic distance (cyt *b*, ATPase 6/8 and CR) which was found to be more evident between *L. rohita* and *C. mrigala*.

It has been frequently reported in mammals that segments of the CR evolve 5–15 times faster than the rest of the mtDNA genome (Aquadro and Greenberg, 1983; Cann et al., 1984; Desmarais, 1989; Vigilant et al., 1989). This is evident from our result that in CR variable sites and singleton sites are more in numbers followed by ATPase 6/8 and cyt *b* showing its faster rate of evolution in CR, whereas parsimony informative sites are less in case of CR and more in cyt *b* indicating high levels of nucleotide dissimilarity in CR than cyt *b*. It can be inferred from the present study that CR shows faster rate of evolution followed by the two protein coding genes, ATPase 6/8 and cyt *b*, respectively. Again, the genetic distance data between all the three Indian major carps inferred from three mitochondrial genes reveals that cyt *b* and ATPase 6/8 might be evolving slowly than CR as evident by low levels of genetic distance between cyt *b* and ATPase 6/8 in all the three Indian major carps. But in case of CR, a relatively high level of genetic distance was observed. Because mitochondrial DNA evolves very rapidly compared to nuclear DNA (Avise, 1994), it was traditionally used as a genealogical tool mainly to examine closely related species. A high level of divergence is usually associated either with a long evolutionary history in a large stable population or with secondary contacts between previously differentiated allopatric lineages (Grant and Bowen, 1998). In cyt *b* gene the nucleotide variance has been found to be very less in *L. rohita* and *C. catla* although there may exists interspecific substitution/deletion. Same result was also obtained from other two genes. *C. mrigala* always formed a different clade that gives a sign of divergence from *L. rohita* and *C. catla* in genealogical lineage.

Here the attempt was to study evolutionary rates in three mtDNA genes by reconstructing a robust phylogeny of the Indian major carps. While comparing methods of phylogenetic reconstruction using neighbor joining and minimum evolution, individual mtDNA gene regions produced identical results with a single model applicable to the entire dataset. The findings of the present study provide useful insights into rate of evolution of these three genes along with the taxonomic status of Indian major caps and facilitate future investigations dealing with phylogeography, taxonomy, conservation and co-evolution of this important group of fish.

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