Gene 527 (2013) 248–258

Contents lists available at SciVerse ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Molecular characterization of major and minor rDNA repeats and genetic variability assessment in different species of mahseer found in North India

Ravindra Kumar *, Mamta Singh [1], Basdeo Kushwaha [1], N.S. Nagpure [1], Indra Mani [1], W.S. Lakra [1]

National Bureau of Fish Genetic Resources, Canal Ring Road, P.O. Dilkusha, Lucknow 226 002, U.P., India

ARTICLE INFO

Article history:
Accepted 4 June 2013
Available online 22 June 2013

Keywords:
Mahseers
Neolissochilus
Phylogeny
rDNA
Tor

ABSTRACT

Relationship among the mahseer species (Family: Cyprinidae) has long been debated in fish systematics. Present study concentrates on the nature of the phylogenetic relationship among the five mahseer species using the sequence of major ribosomal DNA (45S rDNA). We have covered rDNA sequence of approximately 5.2 kb per individual, 26.0 kb per species and 130.0 kb as a whole. We also characterized the 45S and 5S rDNA regions with respect to their nucleotide composition. For phylogenetic analyses, nucleotide sequences were divided into four datasets. First and second datasets contained 18S rDNA and ITS1 sequence, whereas third and fourth datasets consisted of ITS2 and complete 18S-ITS1-5.8S-ITS2-28S, respectively. The NJ tree was constructed for all the datasets. The mahseer species under study formed a monophyletic group well separated from the outgroup species. Similarly, the individuals of *Neolissochilus hexagonolepis* form monophyletic group with *Tor* species, indicating *Neolissochilus* as a sister genus of *Tor*. The findings from the present study provide greater insights into taxonomic status of mahseer, and set the stage for future investigations dealing with phylo-geography, taxonomy, conservation and co-evolution within this interesting and important group of fish.

1. Introduction

Family Cyprinidae is the largest, abundant and globally widespread family of freshwater fishes, which comprises 220 genera and about 2420 species (Nelson, 2006). The cyprinid fishes of the three genera *viz*., *Tor*, *Neolissochilus* and *Naziritor*, constitute an important group referred to as 'mahseer'. They are endemic to Asia with natural distribution encompassing the trans-Himalayan region in the North-West to Sumatra and Borneo islands in the South-East, across a number of Asian countries. Mahseer species are large-scaled barbells (Subfamily: Barbinae), live in upstream, clear, running waters and have high demand as food and attraction for anglers as a sport fish (Ng, 2004; Shrestha, 1990). Some of the species are of great economic value and conservation concern (Nguyen et al., 2006; Sarkar and Srivastava, 2000) as well as of aquaculture potential (Haque et al., 1995; Ingram et al., 2005). At present 46 mahseer species have been recognized; of which 23 species belong to genus *Tor* Gray, 22 to genus *Neolissochilus* Rainboth and one species to genus *Naziritor* Mirza (Eschmeyer et al., 2004). There still exists confusion with regard to taxonomy and systematics, and uniformity in identification of this group. Fishes of the genus *Tor* with the presence of the median lobe are considered to be 'true mahseer', as opposed to *Neolissochilus* and *Naziritor*, where the median lobe is absent. The features such as shape, size and length of the median lobe, often used to distinguish species of *Tor* (Zhou and Cui, 1996), are highly variable (Roberts, 1999) and are also influenced by environmental factors, leading to confusion and as such its reliability as an indicator of species is questionable (Ng, 2004).

DNA sequence data play an indispensable role in reconstruction of evolutionary relationships among the organisms, resulting in insights in genetic affinities that may confirm or conflict with traditional taxonomy. Because of the attractive properties, ribosomal DNA (rDNA) is popular source for examining phylogenetic relationships and for studying genetic variability and divergence within and between species. These properties are secondary structure features, differential

---

Abbreviations: $AgNO_3$, silver nitrate; AIC, Akaike Information Criterion; BCIP, 5-bromo-4-chloro-3′-indolyphosphate p-toluidine salt; CMA_3, Chromomycin A_3; DNA, deoxyribonucleic acid; dNTPs, deoxynucleoside triphosphates; EMBL, European Molecular Biology Laboratory; FISH, fluorescence *in situ* hybridization; IE, intermediate element; ITS1, internal transcribed spacer 1; ITS2, internal transcribed spacer 2; KCl, Potassium chloride; $MgCl_2$, Magnesium chloride; NBT, nitro-blue tetrazolium chloride; NCBI, National Center for Biotechnology Information; NJ, neighbor-joining; NH, *Neolissochilus hexagonolepis*; NTS, Non-transcribed spacer; PCR, polymerase chain reaction; rDNA, ribosomal deoxyribonucleic acid; SM, supplementary materials; TC, *Tor chelynoides*; TP, *Tor putitora*; TPr, *Tor progeneius*; TT, *Tor tor*; Tris–HCl, Tris hydrochloride.

* Corresponding author at: Division of Molecular Biology and Biotechnology, National Bureau of Fish Genetic Resources, Canal Ring Road, P.O. Dilkusha, Lucknow 226 002, U.P., India. Tel.: +91 522 2442440, +91 522 2442441, +91 522 24421735; fax: +91 522 2442403.

E-mail addresses: Ravindra.Scientist@gmail.com, rkumar@nbfgr.res.in (R. Kumar).

[1] Tel.: +91 522 2442440, +91 522 2442441, +91 522 24421735; fax: +91 522 2442403.

0378-1119/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gene.2013.06.032

rates of evolution among divergent regions and tandemly repeated sequences (Arnheim, 1983; Gebri, 1985). As in other eukaryotes, the 45S rDNA of mahseer also contains tandemly repeated transcriptional units, which are separated by intergenic spacers. Within each transcriptional unit, the internal transcribed spacer 1 (ITS1) separates the 18S small subunit (18S rDNA) from the 5.8S large subunit (5.8S rDNA); while the internal transcribed spacer 2 (ITS2) separates the 5.8S from the 28S large subunit (28S rDNA). Each rRNA gene is organized into several divergent domains, also called expansion segments, interspersed between slow evolving, highly conserved cores (Hassouna et al., 1984). Divergent domains are commonly subjected to insertion and deletion events, coupled with substitution rates, that are at least two orders of magnitude higher than that for cores (Olsen and Woese, 1993), and account for the large overall size variation of rRNA genes among eukaryotes. A clear bias toward nucleotide substitutions (especially transitions) rather than insertions or deletions is found in the highly conserved core sequences. These dual modes of evolution in the expansion domains and the cores of nuclear rRNA molecules (particularly 28S) make these genes useful for phylogenetic analyses. The coding regions show little sequence divergence among closely related species, whereas the spacer regions may exhibit higher variability as mutations occur at a relatively rapid rate in internal transcribed spacers. Therefore, these regions may resolve the relationships between closely related species that otherwise show little genetic divergence (Fritz et al., 1994; Porter and Collins, 1991; Tang et al., 1996). Due to above mentioned reasons, several authors used these regions for phylogenetic analyses, *i.e.* in African anguilliform catfishes (Siluriformes: Clariidae), rDNA and spacer regions were used to established the phylogenetic relationship and divergent time estimation (Jansen et al., 2006). Verma and Serajuddin (2012) also used divergent domain 8 of 28S rDNA for phylogenetic analysis of freshwater catfish *Ompok pabda*, *Ompok pabo*, *Ompoc bimaculatus* and *Wallago attu*. There are several reports on phylogenetic studies based on 5S rDNA in fishes belonging to family Anostomidae, Cyprinidae, Merlucciidae, Mugilidae (Campo et al., 2009; Ferreira et al., 2007; Fujiwara et al., 2009; Imsiridou et al., 2007). Till date, no molecular genetic studies based on nuclear DNA or 45S and 5S rDNA sequences, which address questions relating to genetic relationship among mahseer species, were performed. But there are some reports on phylogeny of mahseer using mitochondrial

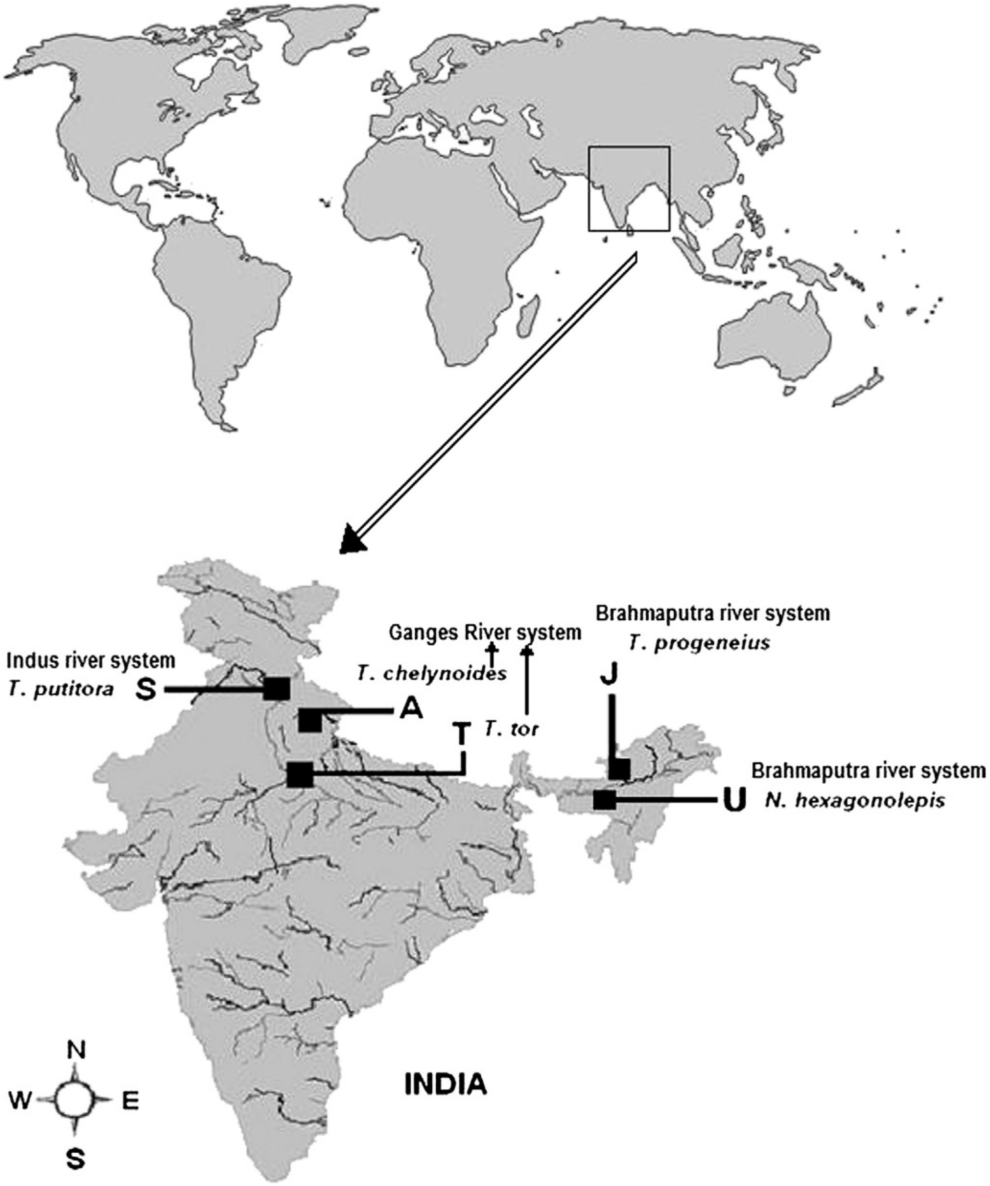

Fig. 1. Collection sites of fish specimens from India.