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Distributional records of Tor mahseer *Tor tor* (Hamilton, 1822) from Southern India

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Summary

During exploratory surveys in the tributaries (Penganga and Satnala) of Godavari and (Bheema) Krishna basins, specimens of mahseer were collected. The morpho-meristic characteristics of these specimens conformed to the taxonomic keys for *Tor tor*. The mitochondrial COI sequences of these specimens clustered with the *T. tor* specimens from the River Narmada and were distinct from the other mahseer such as *T. khudree* and *T. mussullah*, which are known to exist in the rivers of the region. This confirmed the distribution of *T. tor* in the rivers of peninsular India and indicated an extended distribution of the known range. The major predominating habitat characteristics of collection areas were cobbles mixed with gravel, and a riparian cover of shrubs and trees. The occurrence of fingerling size specimens in the river suggests that the species has adapted and is likely to have established self-recruiting populations in these rivers.

Introduction

Tor mahseer is a well known game and food fish and inhabits mountainous streams and rivers as well as fast flowing rivers in the plains, often preferring clear, swift flowing waters with stony, pebbly or rocky bottoms (Shrestha, 1997). The species is reported to reach 150 cm TL (Mishra, 1959), and gain a maximum weight of 68 kg (Talwar and Jhingran, 1991). Although in view of its population decline due to over exploitation and habitat loss the conservation status of the species is evaluated as 'near threatened' (IUCN, 2011). A fish germplasm exploratory survey in the Godavari River system (Penganga sub-basin) and Krishna River system (Krishna River) in the Peninsular Indian region revealed the distributional records of the Indian mahseer species, *Tor tor*. Wide distribution of only two species of mahseer, i.e. *Tor khudree* and *Tor mussullah* in the rivers was recorded so far in Peninsular India (Eschmeyer and Fricke, 2010; Jayaram, 2010; Froese and Pauly, 2011), and a captive stock of *Tor putitora* is also known at a farm in the region (Daniels and Gadgil, 2002). The peninsula region comprises a geologically stable area with an average elevation between 300 and 1800 m in the south of India. The region is drained by five major river systems: Godavari, Krishna, Penner, Mahanadi and Cauvery. The Penganga River is one of the tributaries of the Godavari (the largest river basin in the northern half of the Indian peninsula) and originates in the Ajantha ranges in Maharashtra, and after flowing through the state border between Maharashtra and Andhra Pradesh finally converges into the Wardha River (another tributary of the Godavari). While the Krishna River originates in the hills of Mahbaleshwar and after merging with several tributaries, forms the Krishna River system that flows through the state of Andhra Pradesh before flowing into the Bay of Bengal. Menon (1992, 1999) described five valid species of the genus *Tor* (*T. tor*, *T. putitora*, *T. khudree*, *T. progenius* and *T. kulkarni*) that occur in different parts of India. However, nine valid species are described in the recent taxonomic descriptions (Jayaram, 2010). The genus is mainly distinguishable by its big head and scales, fleshy lips continuous at the angles of the mouth with an interrupted fold or groove across the lower jaw, two pairs of big barbels, lateral-line scales ranging from 22 to 28, and length of head equal to or greater or less than the body depth.

Tor tor is so far known from the Indus, Ganga (including sub-Himalayan range), Brahmaputra and Narmada river systems in India (Shrestha, 1994; Desai, 2003; Jayaram, 2010).

As there are no previous records, reported here is the first distributional record of *T. tor* from the tributaries of the Godavari and Krishna river systems and its expansion into the southern part of India.

Materials and methods

Sampling

The study was conducted in the two tributaries, the Penganga and Satnala of the Godavari basin and from the Bheema River (a tributary of Krishna basin) in the peninsular region of India (Fig. 1). Samples were caught with the help of local fishermen using 15 mm mesh size gillnets at 1.0 –2.5 m river depths. Altogether 48 specimens of various size groups of *T. tor* were collected in four separate fish germplasm exploratory surveys conducted between 2005 and 2011 (Table 1). High resolution digital photographs were taken of all specimens. These individuals were then fixed in 10% formaldehyde for further studies. Identification of the collected specimens was done according to standard taxonomic keys (Talwar and Jhingran, 1991; Jayaram, 2010). For COI sequence data analysis, eight samples in total were taken (Penganga, n = 3, Satnala, n = 2 and Bheema, n = 3).

DNA isolation

The genomic DNA was extracted from 95% ethanol fixed blood/tissue samples, following the procedure of Ruzzante et al. (1996) with minor modifications. Approximately 50 μl of ethanol fixed blood cells were washed twice with High TE buffer (100 mM Tris. HCl, 40 mM EDTA, pH 8.0) and incubated overnight in 0.5 ml lysis buffer (10 mM Tris. HCl, 1 mM EDTA, 400 mM NaCl, pH 8.0), containing 1%

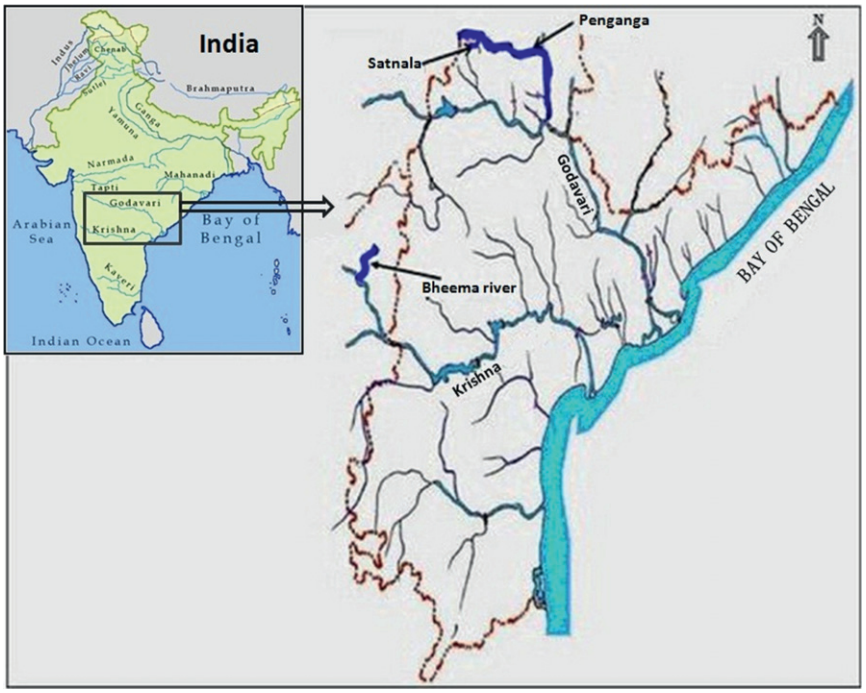

Fig. 1. Drainage map of Godavari and Krishna rivers with sampling locations

Table 1
Details of collection sites, number of specimens, total length and NCBI (National Centre for Biotechnology Information) accessions numbers of *Tor tor*

River	Geographical position (Lat./Long./Alt.)	Number of specimens	Total length range (in cm)	NCBI accessions (COI region)
Penganga	N 19°45.987, E 078° 43.058, 640ft	12	11.5–47.0	JN603184–JN603186
Satnala	N 19° 45.99, E 078° 43.40, 705ft	11	10.0–17.0	JN603183, JN603187
Krishna	N 17° 57.13, E 073° 52.4, 2337ft	25	15.0–20.0	EU714111, EU714114–EU714115

Cm, centimeter; COI, cytochrome c oxidase I.

sodium dodecyl sulphate and 0.2 mg ml^{-1} proteinase K, at 37°C, followed by the phenol extraction protocol.

mtDNA amplification and sequencing

The partial mitochondrial COI region was amplified using primers Fish F1 (5′ TCAACCAACCACAAAGACATTGGCAC 3′) and Fish R1 (5′TAGACTTCTGGGTGGCCAAAGAATCA 3′) (Ward et al., 2005). Polymerase chain reactions (PCR) were performed in 25 μl reaction volume, with each reaction containing 1× PCR buffer (10 mM Tris–HCl, pH 9.0; 50 mM KCl; 0.01% gelatin), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 5 pmol of each primer and 1.5 units Taq DNA polymerase (Genei, Bangalore) and 50 ng of genomic DNA template. PCR amplifications were performed in a thermal cycler (MJ Research, PTC 200) with the parameters: one cycle of initial denaturation at 94°C for 5 min; 30 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min with a final elongation at 72°C for 10 min. After amplification, 2 μl of PCR products were visualized on 1.2% agarose gels and bi-directionally sequenced, to check the validity of the sequence data. All sequences were aligned using ClustalW (Thompson et al., 1997) using default parameters and then checked manually. Sequences of *T. khudree* (GQ469787–GQ469791) and *T. mussallah* (GQ469797–GQ469801), both known as deccan mahseers, were downloaded from the NCBI Genbank to confirm identity of the specimens. Estimates of nucleotide diversity (Librado and Rozas, 2009) and evolutionary divergence between groups of sequences (Tamura et al., 2007) were conducted using the Kimura 2-parameter model. The rate variation among sites was modeled with a gamma distribution (shape parameter = 0.3). Genetic relatedness analysis was performed using Neighbor-joining with maximum likelihood distance (NJ), and significance of nodes determined through 1000 bootstrap as per options available in the software MEGA version 4 (Tamura et al., 2007). *Labeo rohita* accession #GU195120 was taken as the out group.

Results

The *T. tor*, the most widely spread and abundant among the mahseer species is reported herein as new to the Godavari and Krishna river basins. The specimens were stored in the repository of the National Bureau of Fish Genetic Resources, Indian Council of Agricultural Research, India, for future reference. Among the major habitat characteristics are substrate such as cobbles and gravel, and a riparian cover of shrubs and trees predominating the study area. Other fish species recorded in the same study areas were the genera *Garra*, *Puntius, Nemacheilus*, *Notopterus*, *Ompok* and *Mystus* as the dominant species.

Analyses of COI sequence data from samples under study from the Bheema (EU714111, EU714114–EU714115), Penganga (JN603184–JN603186) and Satnala (JN603183, JN603187) rivers with that of *T. tor* (Narmada, EU714116, EU714119) revealed that of 655 sites, conserved sites were 630, variable 17, three parsimoniously informative and 14 singleton. The average compositions in four groups of *Tor* were found to be comparable to each other (A 26.1%, T 29.2%, G 17.8% and C 26.9%) and transition transversion ratio was found to be 4.44. Further analyses of 609 bp sequences with two other species, *T. mussallah* and *T. khudree*, showed that the sequence divergence ranged from 0.003 to 0.038, and that the genetic distance between the two groups ranged from 0.0025 to 0.01890 (Table 2). The COI sequences of the collected specimens clustered together (NJ tree) with accessions obtained from the Narmada River with high bootstrap support, thereby confirming identity of the specimens under study as *T. tor* in peninsular India (Fig. 2).

Discussion

The *T. tor* reported as new from the peninsular river systems was primarily confined to the Ganga, Bramaputra and Narmada drainages covering the uplands of the trans-Himalayan region to the central highlands of India (Table 3). Comparison of morphological descriptions of the recorded specimens with standard taxonomic keys indicated the identification validity of *T. tor*. Identification conformed with results of molecular markers such as the COI region of mtDNA 655 bp fragment (Ward et al., 2005) and was in concordance with the sequences of the same mtDNA region reported for *T. tor* from the Narmada River. The fragment has been recommended for use as a DNA Barcode for species identification. The species was originally described by Hamilton (1822) as *Cyprinus tor* from the Mahananda River (north-eastern part of Bengal), but is now known to be widely distributed. Apart from India, the species is native to Bangladesh, Bhutan, Myanmar, Nepal and Pakistan (Desai, 2003). According to Sehgal (1971), in India this species occurs from Jammu in the west to the Brahmaputra Valley in the east all along the Himalayan range. Others (McDonald, 1948; Hora, 1949; Motwni and David, 1957; Mishra, 1959; Lal and Chaterjee, 1962; Karamchandin et al., 1967; Rajbanshi and Csavas, 1982) noted that besides the snow-fed Himalayan rivers, the rivers having their sources in the highlands of central India also had *T. tor*. The occurrence of *T. tor* in tributaries of the Godavari basin supports the significance of the 'Satpura hypothesis' in mahseer migration, according to which during glacial periods the Garo-Rajmahal gap must have been a few hundred meters higher than the sea level with the colder climate favoring increased precipitation with less evaporation and greater runoff in streams like the Narmada, the Tapti along the Satpura, and the Vindhya. These conditions favoured the spread of torrential fishes from the region of the Assam Himalayas to Peninsular India across the Garo-Rajmahal Bridge (Hora, 1951). Due to habitat loss and heavy utilization of mahseer

Table 2
Evolutionary (K2-P distance, below diagonal) and sequence divergence (above diagonal) between different studied groups of genus *Tor*

	P & S	Narmada	Bheema	*Tor mussullah*	*Tor khudree*
P & S	–	0.0025	0.0029	0.01525	0.1890
Narmada	0.003	–	0.00263	0.01251	0.01564
Bheema	0.003	0.003	–	0.01449	0.01800
Tor mussullah	0.030	0.029	0.030	–	0.01368
Tor khudree	0.038	0.038	0.038	0.027	–

P & S, Penganga and Satnala rivers.

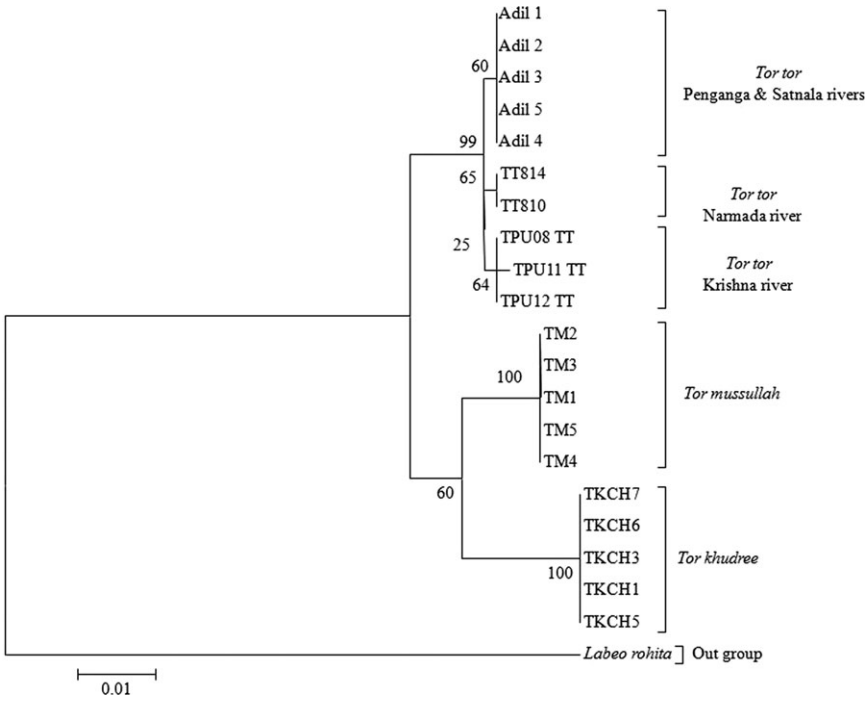

Fig. 2. Neighbour joining tree of *Tor tor* specimens from rivers Penganga, Satnala (Godavari basin), Bheema (Krishna basin) and Narmada along with two Deccan mahseers, *T. khudree* and *T. mussullah* based on COI mtDNA gene. Bootstrap values given at nodes (TT/TPU = *Tor tor*; TM, *Tor mussullah*; TKH, *Tor khudree*)

Table 3
Distribution records of *Tor tor* in various river systems, India

River system	Rivers	References
Indus	Beas, Chenab, Jhelum, Ravi, and Sutlej	Mishra (1959), Raina and Petr (1999)
Ganga	Alaknanda, Bhagirathi, Bhilangna, Ganga, Ghaghra, Gomti, Kosi, Ramganga, Rapti, and Sarda	Badola (1975), Das and Pathani (1978), Pathani (1978), Bisht and Das (1981), Malhotra (1982), Sharma (1986), Pandey (1996), Sarkar et al. (2011)
Brahmaputra	Barak, Brahmaputra, Gumti, and Simsang	Sehgal (1971), Lipton (1983), Dasgupta (1990), Karmakar (2000).
Yamuna	Betwa, Chambal, Hindon, Ken, Rihand, Sind, and Sone	Motwni and David (1957), Dubey and Mehra (1959), Karamchandani and Pisolkar (1967), Chaudhay (1978), Lakra et al. (2010).
Narmada	Mahi and Narmada	Malviya (1961), Desai (1973)
Tapti	Aner, Bori, Girna, Panzara, Purna, and Waghur	Karamchandin et al. (1967)
Mahanadi	Mahanadi	Desai (2003)

as food, sport, and traditional medicine, a steady decline has been reported from several parts of its range (CAMP, 1998; Sharma et al., 2004; Rayamajhi et al., 2009**)**. As the present authors have collected various life stages of *T. tor* including adults and juveniles, it is believed that a self-recruiting population has already been established in the region. Records of this fish species from the Himalayan glacier-fed streams to the present study area in tropical Peninsular India corroborates earlier findings (Desai, 2003), which indicates that *T. tor* is a eurythermal fish species inhabiting both cold and warm waters. However, presence of very similar hill-stream habitat characteristics and the coexistence of species like *Garra* and loaches in the region signifies that the mahseer prefer to stay in a similar habitat throughout their range of distribution. As *T. tor* is a local migratory fish, there is also a need to study in detail the biology and conservation genetics of this fish across its extended distribution ranges.

The present record of *T. tor* from the Penganga and its tributary Satnala of the Godavari basin and River Krishna basin augments information about its extended distribution to the known distributional range of the species. This report is based on the exploration of two peninsular India rivers; however, it raises the possibility that distribution of *T. tor* could be much larger than what is currently known. Additional catches from other river basins and tributaries could further enhance knowledge of the actual distributional range of this species.

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