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DNA barcoding of freshwater fish from different drainage systems of Telangana in Southern India

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Abstract

The diversity of freshwater fish in the state of Telangana is currently recorded as 143 species under 34 families and 14 orders. The current study principally deals with the identification of fish fauna based on morphology and the generation of DNA barcode data. DNA sequence data on a partial segment of the mitochondrial cytochrome oxidase C subunit I (mtCOI) gene were generated from freshwater fishes sampled from various localities within the state of Telangana. The study identified 57 species under 41 genera, 24 families, and 11 orders. Morphological identification was concordant with the molecular basis of taxon assignment, where 57 OTU (Operational Taxonomic Units) correspond to 57 species, and no BIN discordance was found in the dataset. The present phylogenetic tree discriminates all the studied species based on the partial mtCOI gene. The study contributed DNA sequence data for many of the important species for the first time to the global database.

Keywords: Deccan Peninsula, Genetic Diversity, Ichthyofauna, Morphology, Molecular Marker

Introduction

The Convention on Biodiversity (CBD) has laid major emphasis on the inventory and conservation of biodiversity. Species-level identification of biota is vital for inventory from an ecosystem to a broader landscape. Surveys and inventories provide the essential baseline data for monitoring changes caused by factors such as habitat conversion, climate change and for determining conservation priorities (Kerr, *et al.*, 2000).

Efforts to generate molecular information on the native and indigenous fish of India are progressing well, but potentially lagging behind the global pace. A pilot search in the National Center for Biotechnology Information (NCBI) nucleotide database with criteria selected 'Nucleotide' and search keywords "COI fish India" revealed 13,517 results as on September 9, 2021. However, the results hardly discriminated against the habitat specificity of the specimens whether abode from freshwater or marine. The Animal Discoveries 2019 (Chandra, *et al.*, 2020) recorded the occurrence of 3,439 species of fish inhabiting the Indian Territory, including both freshwater and marine inhabitants with around 206 endemic species and around 18 introduced species.

Freshwater fishes are one of the most important groups because they are more or less confined to drainage systems which can be thought of as dendritic islands of water surrounded by land, which in turn are bordered by a saltwater barrier (Berra, 2007). A search in the global fish database (www.fishbase.org) revealed a total of 1,035 freshwater fish species reported from India and its islands together. However, the information on fish species diversity is updating faster with the discovery of several new species and new records, particularly from the western ghats and northeast India.

The knowledge of the fish diversity of the Deccan Peninsular Biogeographic Zone is largely sporadic. The species composition in the region as a whole is facing a shortage of annotated checklists. Previously reported

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freshwater fish diversity in the erstwhile Andhra Pradesh is known to be comprised of 158 species under 68 genera, 27 families, and 10 orders (Barman, 1993). The available checklist of freshwater fish from the Eastern Ghats of the Deccan Peninsula recorded 127 species (Rema Devi and Indra, 2003). The freshwater fish of the state of Telangana were recorded to be 165 species under 11 orders and 29 families (Laxmappa & Bakshi, 2016). A recent report suggests the occurrence of 143 species of freshwater fish in 14 orders and 34 families (Prasad & Srinivasulu, 2021). However, the fish species inventory of the broader biogeographic region is baffling due to the inclusion of several uncertain and poorly described species. In this milieu, there is an urgent need for an integrative effort to develop an annotated checklist for the region. The checklist would reflect the share of endemic fish species of greater academic importance as well as draw the attention of the biodiversity authority and conservation agencies. Telangana State occupies a potential part of the Deccan Peninsular biogeographic region in terms of water resources and major river systems. This study focuses on generating DNA barcodes for the freshwater fish of Telangana.

Materials and Methods

Survey and Sampling

The study principally deals with the identification of fauna based on morphology and the generation of DNA barcode data for each species. Specimens were collected in a sequential manner in order to record the diversity. Specimens were collected through participatory sampling methods in order to maintain regulatory sampling and avoid damage to the habitat. The specimens were sampled randomly for taxonomic purposes using the recommended permissible tools. The density and abundance of each of the species in any sampling area are beyond the scope of this study.

Specimens were preserved in 90% ethyl alcohol with proper field ID for downstream work. A little amount of muscle tissue was dug out with care from each voucher specimen which was separately preserved in 90% ethanol with proper tagging. The specimens, after collecting the required amount of tissue for DNA study, were fixed in 10% formalin and permanently preserved in 90% ethanol thereafter. The specimens were vouchered and catalogued in the National Zoological Collection of the Zoological Survey of India, Freshwater Biology Regional Centre, Hyderabad. The specimens were examined thoroughly for taxonomic identification at the species level following the original descriptions, the subsequent taxonomic revisions, and the available identification keys. Quantitative data were recorded from the studied specimens following standard procedures and related literature (Talwar & Jhingran, 1991, Jayaram, 1999). Nomenclature follows Eschmeyer's Catalog of Fish (Fricke, 2020).

DNA Isolation, PCR and Sequencing

DNA isolation, PCR, and sequencing of the targeted mitochondrial marker (mtCOI) of each of the specimens followed the standardized protocols (Sambrook & Russell, 2001). Genomic DNA was extracted from muscle tissue through the commercialized Kit based method. The extracted DNA was checked in 1% agarose gel electrophoresis using a standard protocol (Sambrook & Russell, 2001). Approximately 648 bp were amplified from the 5′ regions of the mtCOI gene using various combinations of the primers described in Ward, *et al* (2005). The PCR Fast Cycling kit was used for amplification and the thermal profile was set as per the primer specifications. Approximately, 15 ng of purified PCR product was used for cycle sequencing for both reactions from the outsourced services.

The generated sequences were truncated at both ends after alignment in MEGA6.0 to get a consensus length in the dataset. The evolutionary divergences were estimated between sequences of closely related species or sister species among the congeners using the Kimura 2 Parameter (K2P) in MEGA 6.0 (Tamura, *et al*., 2013). Further, the reciprocal monophyly among the targeted congeners was tested through the Neighbour Joining (NJ) tree method in MEGA 6.0 for species-level differentiation. The species-level delineation was based on different methods of DNA barcoding analysis. The conclusion on species taxonomy was based on the congruent results from different methods of DNA barcoding analysis over the morphologically identified species.

Results and Discussion

A total of 542 examples of freshwater fish were collected from various locations in Telangana (Figure 1). Among them, a total of 96 representative samples were studied through the DNA barcoding technique. These specimens

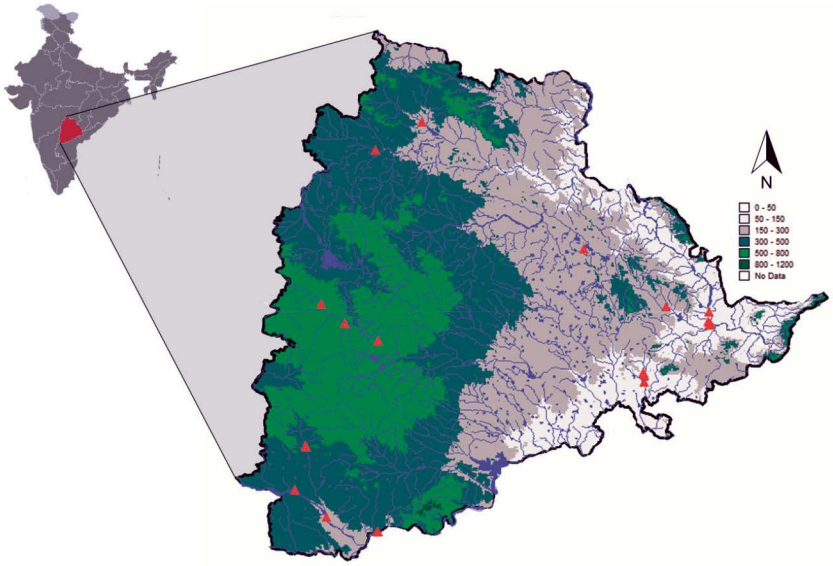

Figure 1. Map of the study area depicting the drainage systems marked by blue line, and the sampling locations marked by orange triangle shape.

were morphologically identified as belonging to 57 species under 41 genera, 24 families and 11 orders. The generated barcode sequences were submitted to both NCBI GenBank and BOLD Systems. The BOLD Systems provide a sequence analysis platform where the selected 96 sequences were analyzed to understand the Taxon ID tree, Distribution Map, Sequence clusters, and BIN discordance. Morphological identification was concordant with the molecular basis of taxon assignment, where 57 OTU (Operational Taxonomic Units) correspond to 57 species, and no BIN discordance was found in the dataset. The neighbour-joining phylogenetic tree of the studied taxa is given in Figure 2. The list of species identified through morphology as well as the DNA barcoding approach is given in Table 1.

The Telangana state was reportedly harbouring nearly 143 species of freshwater fish belonging to 34 families under 14 orders (Prasad & Srinivasulu, 2021). However, this study recorded a few species which supplement the previous studies as discussed below. The study added a few species, like *Awaous ocellaris* (Broussonet, 1782), *Clupisoma bastari* Datta & Karmakar, 1980, *Garra bicornuta* Narayan Rao, 1920, and *Botia striata* Narayan Rao, 1920 as a new record for the state, Telangana. Further, the previously reported *Notopterus notopterus* (Pallas, 1769) from Telangana is revised herein as *Notopterus synurus* (Bloch & Schneider 1801) after Lavoue, *et al* (2020).

A lesser-known catfish, *Clupisoma bastari*, was identified from the Godavari River basin, based on the morphological descriptions, and contributed novel DNA barcode data to the GenBank. The Kimura 2 parameter for genetic divergence between species, and the maximum-likelihood phylogeny clearly depicted a distinct clade of *C. bastari* in the studied dataset. *Clupisoma bastari* maintained sufficient genetic divergence (8.3% to 11.2%) with other congeners, and branched as a sister-species of *C. garua*. Specimens of *C. bastari* were collected from Sriram Sagar Reservoir, Telangana, and from the Godavari-Sabri confluence, near Konavaram bridge, Telangana. Considering the distinct species status of *C. bastari* occurring in the Godavari River basin, the reports of the presence of *C. garua* from Godavari as well as from more southwards in the River Krishna in previous studies (Devi & Indra, 2003) are appearing to be an incorrect identification of *C. bastari*. In the meantime, this article was undergoing review, the phylogeny and DNA barcoding of the species, *C. bastari*, was published elsewhere (Laskar, *et al*., 2022). The study also noted an interesting genetic diversity in the genus *Osteobrama* which is beyond the scope of this communication and is dealt elsewhere, the unidentified species is referred here as *Osteobrama* sp. This study provides evidence of how the integrative approach helps to easily distinguish the morphologically related species. Such molecular approach

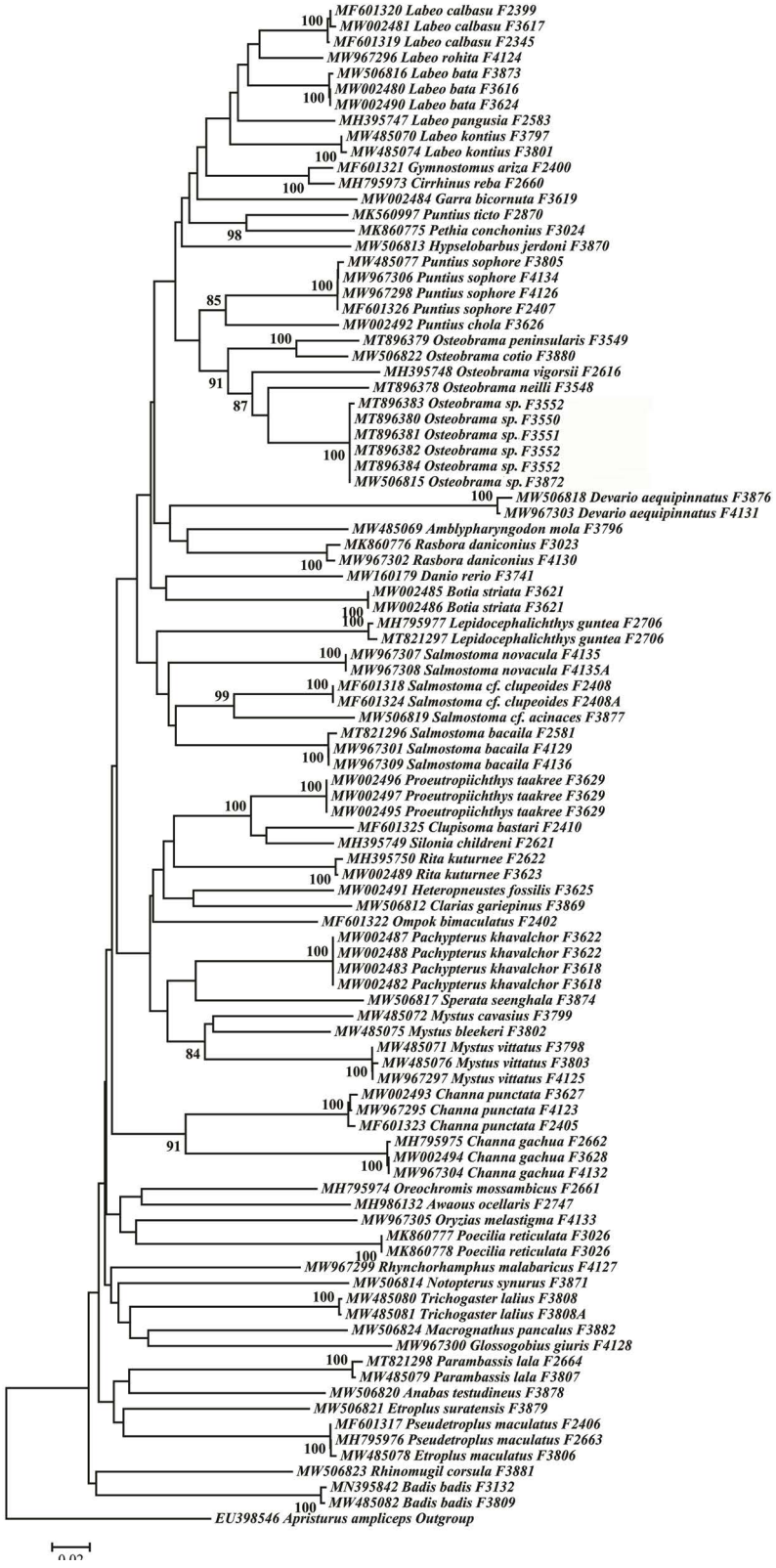

Figure 2. Neighbour-joining phylogeny of the studied fish species depicting distinctive species clades corresponding to the morphospecies. Scale bar corresponds to the length of clade from each node.

Table 1. List of fish species collected from the Telangana state, India with complete information on DNA barcoding available with the NCBI accession numbers. The museum registration numbers prefix by "FBRC/ZSI/"

Order	Family	Organism	Museum Regn. No.	BIN URI	NCBI Accession Number	Latitude	Longitude
Anabantiformes	Anabantidae	1. *Anabas testudineus* (Bloch, 1792)	F3878	BOLD: ACC0743	MW506820	16.37 N	77.694 E
	Badidae	2. *Badis badis* (Hamilton, 1822)	F3132	BOLD: AEG0671	MN395842	18.225 N	79.917 E
			F3809	BOLD: AEG0671	MW485082	17.201 N	80.383 E
	Channidae	3. *Channa gachua* (Hamilton, 1822)	F2662	MH795975	MH795975	17.654 N	78.077 E
			F3628	BOLD: ABA8489	MW002494	17.273 N	80.377 E
			F4132	-	MW967304	16.702 N	77.778 E
		4. *Channa punctata* (Bloch, 1793)	F2405	BOLD: AAE8814	MF601323	18.99 N	78.310 E
			F3627	BOLD: AAE8814	MW002493	17.273 N	80.377 E
			F4123	-	MW967295	16.709 N	77.774 E
	Osphronemidae	5. *Trichogaster lalius* (Hamilton, 1822) valid as *T. fasciatus* Bloch & Schneider, 1801	F3808	BOLD: AAY7699	MW485080	17.201 N	80.383 E
			F3808A	BOLD: AAY7699	MW485081	17.201 N	80.383 E
Beloniformes	Adrianichthyidae	6. *Oryzias melastigma* (McClelland, 1839) valid as *Oryzias dancena* (Hamilton, 1822)	F4133	-	MW967305	16.702 N	77.778 E
	Hemiramphidae	7. *Rhynchorhamphus malabaricus* Collette, 1976	F4127	-	MW967299	16.709 N	77.774 E
Cichliformes	Cichlidae	8. *Etroplus suratensis* (Bloch, 1790)	F3879	BOLD: AAF3969	MW506821	16.37 N	77.694 E
		9. *Oreochromis mossambicus* (Peters, 1852)	F2661	BOLD:AAA8511	MH795974	17.654 N	78.077 E
		10. *Pseudetroplus maculatus* (Bloch, 1795)	F2406	BOLD:AAA8511	MF601317	19.201 N	78.670 E
			F2663	BOLD: ACC0078	MH795976	17.654 N	78.077 E

(Continues)

Table 1 to be continued...

			F3806	BOLD: AAE4466	MW485078	16.046 N	78.326 E
Cypriniformes	Botiidae	11. *Botia striata* Narayan Rao, 1920	F3621	BOLD: AAK1517	MW002486	16.048 N	78.334 E
			F3621	BOLD: AAK1517	MW002485	16.048 N	78.334 E
	Cobitidae	12. *Lepidocephalichthys guntea* (Hamilton, 1822)	F2706	BOLD: AAE4466	MH795977	17.654 N	78.077 E
			F2706	BOLD: ACC0078	MT821297	17.654 N	78.077 E
	Cyprinidae	13. *Amblypharyngodon mola* (Hamilton, 1822)	F3796	BOLD: AAZ2536	MW485069	16.048 N	78.334 E
		14. *Gymnostomus ariza* (Hamilton, 1807)	F2400	BOLD: AAE5780	MF601321	18.99 N	78.310 E
		15. *Cirrhinus reba* (Hamilton, 1822)	F2660	BOLD: AAJ3231	MH795973	17.654 N	78.077 E
		16. *Garra deccanensis* Jadhav, Karuthapandi, Shangningam, Jaiswal & Shankar, 2022	F3619	BOLD: AEF1448	MW002484	16.048 N	78.334 E
		17. *Hypselobarbus jerdoni* (Day, 1870)	F3870	BOLD: AAV2898	MW506813	16.37 N	77.694 E
		18. *Labeo bata* (Hamilton, 1822)	F3616	BOLD: AAV6679	MW002480	16.048 N	78.334 E
			F3624	BOLD: AAV6679	MW002490	16.046 N	78.326 E
			F3873	BOLD: AAV6679	MW506816	16.37 N	77.694 E
		19. *Labeo calbasu* (Hamilton, 1822)	F2345	BOLD: AAD7996	MF601319	17.802 N	77.893 E
			F2399	BOLD: AAD7996	MF601320	18.99 N	78.310 E
			F3617	BOLD: AAD7996	MW002481	16.048 N	78.334 E
		20. *Labeo kontius* (Jerdon, 1849)	F3797	BOLD: ACX2575	MW485070	16.048 N	78.334 E
			F3801	BOLD: ACX2575	MW485074	16.046 N	78.326 E
		21. *Labeo pangusia* (Hamilton, 1822)	F2583	BOLD: AAZ8282	MH395747	17.640 N	80.884 E

Table 1 to be continued...

		22. *Labeo rohita* (Hamilton, 1822)	F4124	-	MW967296	16.709 N	77.774 E
		23. *Osteobrama* sp.	F3550	BOLD: ACM5411	MT896380	16.046 N	78.326 E
			F3551	BOLD: ACM5411	MT896381	16.048 N	78.334 E
			F3552	BOLD: ACM5411	MT896382	16.046 N	78.326 E
			F3552	BOLD: ACM5411	MT896383	16.046 N	78.326 E
			F3552	BOLD: ACM5411	MT896384	16.046 N	78.326 E
			F3872	BOLD: ACM5411	MW506815	16.37 N	77.694 E
		24. *Osteobrama cotio* (Hamilton, 1822)	F3880	BOLD: AAE6868	MW506822	16.37 N	77.694 E
		25. *Osteobrama neilli* (Day, 1873)	F3548	BOLD: ACR7173	MT896378	16.046 N	78.326 E
		26. *Osteobrama peninsularis* Silas, 1952	F3549	BOLD: ACJ3278	MT896379	17.252 N	80.384 E
		27. *Osteobrama vigorsii* (Sykes, 1839)	F2616	BOLD: ABY3071	MH395748	17.743 N	80.880 E
		28. *Pethia conchonius* (Hamilton, 1822)	F3024	BOLD: AAO8787	MK860775	17.520 N	78.332 E
		29. *Puntius chola* (Hamilton, 1822)	F3626	BOLD: AAX7390	MW002492	16.046 N	78.326 E
		30. *Puntius sophore* (Hamilton, 1822)	F2407	BOLD: ACW5482	MF601326	18.99 N	78.310 E
			F4126	-	MW967298	16.709 N	77.774 E
			F4134	-	MW967306	16.702 N	77.778 E
			F3805	BOLD: ACW5482	MW485077	16.046 N	78.326 E
		31. *Pethia ticto* (Hamilton, 1822)	F2870	BOLD: AAU2026	MK560997	16.16 N	77.934 E
	Danionidae	32. *Rasbora daniconius* (Hamilton, 1822)	F3023	BOLD: ABX6594	MK860776	17.520 N	78.332 E
			F4130	-	MW967302	16.709 N	77.774 E
		33. *Salmostoma bacaila* (Hamilton, 1822)	F4129	-	MW967301	16.709 N	77.774 E

(Continues)

Table 1 to be continued...

			F4136	-	MW967309	16.046 N	78.326 E
			F2581	BOLD: ABA0106	MT821296	17.665 N	80.876 E
		34. *Salmostoma novacula* (Valenciennes, 1838)	F4135	-	MW967307	16.046 N	78.326 E
			F4135A	BOLD: ABW5244	MW967308	16.046 N	78.326 E
		35. *Salmostoma* cf. *clupeoides* (Bloch, 1795) *valid as Salmostoma balookee* (Sykes, 1839)	F2408A	BOLD: ADI2223	MF601324	19.201 N	78.670 E
			F2408	BOLD: ADI2223	MF601318	19.201 N	78.670 E
		36. *Salmostoma* cf. *acinaces* (Valenciennes, 1844)	F3877	BOLD: ABA9683	MW506819	16.37 N	77.694 E
		37. *Danio rerio* (Hamilton, 1822)	F3741	BOLD: AAE3739	MW160179	17.778 N	80.552 E
		38. *Devario aequipinnatus* (McClelland, 1839)	F3876	BOLD: AAU1278	MW506818	16.37 N	77.694 E
			F4131	-	MW967303	16.709 N	77.774 E
Cyprinodontiformes	Poeciliidae	39. *Poecilia reticulata* Peters, 1859	F3026	BOLD: ACC0443	MK860777	17.520 N	78.332 E
			F3026	BOLD: ACC0443	MK860778	17.520 N	78.332 E
Gobiiformes	Gobiidae	40. *Awaous ocellaris* (Broussonet, 1782)	F2747	BOLD: AEB5275	MH986132	17.645 N	80.897 E
		41. *Glossogobius giuris* (Hamilton, 1822)	F4128	-	MW967300	16.709 N	77.774 E
Mugiliformes	Mugilidae	42. *Rhinomugil corsula* (Hamilton, 1822)	F3881	BOLD: ABV4537	MW506823	16.37 N	77.694 E
Osteoglossiformes	Notopteridae	43. *Notopterus synurus* (Bloch & Schneider, 1801)	F3871	BOLD: AAF2803	MW506814	16.37 N	77.694 E
Perciformes	Ambassidae	44. *Parambassis lala* (Hamilton, 1822)	F2664	BOLD: ABA7419	MT821298	17.654 N	78.077 E
			F3807	BOLD: ABA7419	MW485079	16.046 N	78.326 E
Siluriformes	Ailiidae	45. *Proeutropiichthys taakree* (Sykes, 1839)	F3629	BOLD: ADA4883	MW002495	16.046 N	78.326 E
			F3629	BOLD: ADA4883	MW002496	16.046 N	78.326 E

Table 1 to be continued...

			F3629	BOLD: ADA4883	MW002497	16.046 N	78.326 E
	Bagridae	46. *Mystus bleekeri* (Day, 1877)	F3802	BOLD: AAZ9696	MW485075	16.046 N	78.326 E
		47. *Mystus cavasius* (Hamilton, 1822)	F3799	BOLD: ADX0539	MW485072	16.048 N	78.334 E
		48. *Mystus vittatus* (Bloch, 1794)	F3798	BOLD: AAX3611	MW485071	16.048 N	78.334 E
			F3803	BOLD: AAX3611	MW485076	16.046 N	78.326 E
			F4125	-	MW967297	16.709 N	77.774 E
		49. *Rita kuturnee* (Sykes, 1839)	F2622	BOLD: ABA2575	MH395750	17.743 N	80.880 E
			F3623	BOLD: ABA2575	MW002489	16.046 N	78.326 E
		50. *Sperata seenghala* (Sykes, 1839)	F3874	BOLD: AAZ3699	MW506817	16.37 N	77.694 E
	Clariidae	51. *Clarias gariepinus* (Burchell, 1822)	F3869	BOLD: AAB2256	MW506812	16.37 N	77.694 E
	Heteropneustidae	52. *Heteropneustes fossilis* (Bloch, 1794)	F3625	BOLD: ACR4875	MW002491	16.046 N	78.326 E
	Horabagridae	53. *Pachypterus khavalchor* (Kulkarni, 1952)	F3618	BOLD: ADB5824	MW002483	16.048 N	78.334 E
			F3618	BOLD: ADB5824	MW002482	16.048 N	78.334 E
			F3622	BOLD: ADB5824	MW002487	16.046 N	78.326 E
			F3622	BOLD: ADB5824	MW002488	16.046 N	78.326 E
	Schilbeidae	54. *Clupisoma bastari* Datta & Karmakar, 1980	F2410	BOLD: ABY1142	MF601325	19.201 N	78.670 E
		55. *Silonia childreni* (Sykes, 1839)	F2621	BOLD: ADM4540	MH395749	17.743 N	80.880 E
	Siluridae	56. *Ompok bimaculatus* (Bloch, 1794)	F2402	BOLD: AAA9421	MF601322	18.99 N	78.310 E
Synbranchiformes	Mastacembelidae	57. *Macrognathus pancalus* Hamilton, 1822	F3882	BOLD: AAF5455	MW506824	16.37 N	77.694 E

already evidenced to be more successful and efficient in discriminating the freshwater fish species from different geographical area in India and abroad (Hubert, *et al*., 2008; Ward, *et al*., 2009; April, *et al*., 2011; Lakra, *et al*., 2011; Mabragana., *et al*. 2011; Bhattacharjee, *et al*., 2012; Khedkar, *et al*., 2014; Chen, *et al*., 2015; Lakra, *et al*., 2016; Barman, *et al*., 2018; Laskar, *et al*., 2018; Kundu, *et al*., 2019a). The integrated approach is not only elucidates the genetic diversity of freshwater fish, but also hinted to the existence of possible cryptic species and evolutionary relationship (Wong, *et al*., 2011; Yang, *et al*., 2012; Yang, *et al*., 2015; Kundu, *et al*., 2019b), resolved taxonomic dilemmas (Laskar, *et al*., 2013; Conte-Grand, *et al*., 2017; Laskar, *et al*., 2018), detection of invasive species and aquarium trade (Steinke, *et al*., 2009; Collins, *et al*., 2012), and food adulteration and human health (Stern, *et al*., 2017; Willette, *et al*., 2017). The DNA barcoding data generated in this study would provide a ready reference in academics as well as in policy-making issues. With the inclusion of the above-mentioned four confirmed species and an unidentified species, the number of freshwater fish in Telangana would be 148 species.

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