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A Review on Freshwater Fish Diversity of India and Concept of DNA Barcoding in Fish Identification

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ABSTRACT

Fish contribute faintly more than one half of the total of vertebrates and India contributes to about 7.7% of global fish diversity. Fish and their diverse progressive phase sometimes find it difficult to recognize by morphological feature alone because of the high variety and phenotypic plasticity. Even within the species of fish, individual genetic distances may be found depending on the environmental stress and water quality which play an important role in its minor morphometric variations. There remains a need for taxonomic experts for single specimen identification. For less experienced user or non-specialist, it is difficult to identify the species which they encounter. Notably, DNA barcoding seems to be a more reliable approach for species identification, which is rapid and cost-effective. DNA barcode is based on the sequence divergence pattern of the cytochrome c oxidase I (COI) gene. DNA barcoding focuses on developing reference libraries of barcode sequence. FISH-BOL (Fish barcode of life) and international barcoding of life (iBOL.org) project is established as a reference DNA barcode library for all fishes. Recently, morphological analysis and DNA barcoding are conjointly used as a taxonomical approach for fish species identification. In this essence, the current review aimed to investigate the fish diversity and status of DNA barcoding of fish in India.

INTRODUCTION

Biodiversity is a term that refers to the variety and variability of life on earth. It includes variation among genes, species and functional traits. Among life forms, it is commonly measured in terms of richness, evenness and heterogeneity **(Cardinale *et al.*, 2012)**. **Noss (1990)** recognized composition, structure and function as main attributes of biodiversity and bolstered those attributes hierarchically into nested form by including other organization levels: regional landscape, community-ecosystem, population species and genetic. Biodiversity can be measured as to genetic diversity, species characteristics (individuality, number and accumulation), biotic communities, their processes and structure **(Press & Delong, 2002).** An ecosystem that has rich biodiversity provides more alternatives for transferring energy and also has a better ability for resisting shocks like fires, flood etc. compared to a system with low biodiversity **(Folke *et al.*, 2002)**. **Cardinale *et al.* (2012)** concluded that biodiversity damage changes the ecosystem functioning and also their ability to deliver better goods and services needed to flourish a society. India, a biologically diverse country hosts 7.6% of all mammalian, 12.6% of all avian, 6.2% of all reptilian, 4.4% of all amphibian, 11.7% of all piscine, and 6.0% of all flowering plant species **(Stephen *et al.*, 2015)**. Sampling, identifying and arranging a species systematically are the pioneer work toward protection of biodiversity. Thus, it is a duty of researcher to precisely identify a species for the purpose of conservation and sustainable use.

Fish are cold-blooded aquatic chordates resides in seas, river, lakes, canals, reservoirs, estuaries etc., and have a pharyngeal gill for respiration. Fish contributes faintly more than one half of the total vertebrates with 34300 species. India contributes to about 7.7% of global fish diversity, of which 1,673 are marine and 994 are freshwater **(Froese & Pauly, 2020)** and also in various ways to the diversity of the aquatic ecosystem. **Coad and Murray (2006)** estimated more than 32,000 valid species of fish on earth included in 85 orders and 536 families and 43% of fishes are freshwater fishes. Earth's surface freshwater encompasses only a small share but involves a large number of fish species. Fishbase **(www.Fishbase.org)** classifies fish species of fresh and brackish water into the following categories (a) exclusively freshwater, (b) occurring in fresh and brackish waters (c) or in fresh, brackish and marine waters. An extensive study on the taxonomy and biology of the freshwater fishes in India has been achieved. Scientific study on Indian freshwater fishes started with **Hamilton (1822)**. Moreover, several magnificent contributions to Indian fish fauna were made by **Talwar and Jhingran (1992), Menon (1999)** and **Jayaram (2010).**

Review on Fish diversity In India

India is one of the megadiverse countries in the world enriched with varied taxonomic, genetic and ecosystem diversity. It is endowed with different riverine system; with tributaries, streams, canals, lakes, ponds, and reservoirs etc. that are rich in fish. In the 21st century, noteworthy Ichthyofaunal studies were carried out in various rivers, lakes, ponds reservoir and stream of India **(Table 1**).

Table 1: A concise view of freshwater fish diversity in India.

Sr. No.	Study Area	Number of Species present	Recorded species belonging to			References
			Genera	Families	Orders	
1	Gomti river	56	42	20		**Sarkar *et al.* (2010)**
2	Betwa river	63	45	20		**Lakra *et al.* (2010)**
3	Ganga river	143	72	32	11	**Sarkar *et al.* (2012)**

4	Ganges river	143	92	40		**Das *et al.* (2013)**
5	Ken river	57	42	20		**Sarkar *et al.* (2014)**
6	Yamuna river	112	73	29	10	**Joshi *et al.* (2016**
7	Ghaggar, Yamuna and Ganga	26			4	**Johal and Rawal (2005)**
8	Jamner river,	27	16	9	4	**Vyas and Vishwakarma (2013)**
9	Tawa river middle stretch	57	35	13	6	**Bose *et al.* (2013)**
10	Narmada river in Western Zone	51		15	7	**Bakawale and Kanhere (2013)**
11	Dudhi river	19	18	5	4	Vishwakarma and Vyas (2017)
12	wan river	21		8	5	**Khade *et al.* (2017)**
13	Barna river	33	21	9	5	**Vishwakarma *et al.* (2014)**
14	Koyna river	58	35	16		**Jadhav *et al*. (2011)**
15	Indrayani river	57	39	18		**Dahanukar *et al*. (2012)**
16	Pavana river	59				**Chandanshive *et al.* (2007)**
17	Mutha and Mula river	62				**Wagh and Ghate (2003)**
18	Mullameri river	14			5	**Vijaylaxmi *et al*. (2010)**
19	River fishes in Western Ghat Mountains of Penisular India	60	27	12	4	**Arunachalam (2000)**
20	Bhadra river	56	31	15		**Shahnawaz *et al.* (2010)**
21	Adan and Kathani river of Godavari basin	47				**Heda (2009)**
23	Godavari river	26	15	7	5	**Rankhamb (2011)**
24	Cauvery river	37				**Arunkumar *et al.* (2016)**

25	Brahmaputra river	141	84	29		**Bhattacharjya *et al.* (2017)**
26	Dihing river	50		18		**Deori *et al*. (2015)**
27	Upper reaches of river Brahmaputra	52		15		**Baishya *et al.* (2016)**
28	Ranganadi river	61			6	**Kaushik and Bordoloi (2016)**
29	Siang river	82	53	24	8	**Das *et al.* (2017)**
30	Harike wetlands	37	25	14		**Kaur *et al.* (2017)**
31	Pong Dam lake	57		12	5	**Sharma and Mehta (2009)**
32	Basantar river	32				**Sharma and Dutta (2012)**
33	Ujh river	42			5	**Rathore and Dutta (2015)**
34	Chenab in J.K.	5		3	3	**Bhutyal and Langer (2015)**
35	Beas river in H.P	6		3	3	**Kumar (2010)**
36	Ornamental fish fauna of Himachal Pradesh	58	36	13		**Sharma and Dhanze (2018)**
37	Beas, Ravi, Sutlej in H.P.	61		13		**Jagtap (2013**
38	Similipal Biosphere Reserve, Odisha	66	42	19	6	**Baliarsingh *et al.* (2013)**
39	Kangsabati reservoir	39	26	15	7	**Bera *et al.* (2014)**
40	Tighra reservoir, Gwalior	40	22	10	6	**Bhat and Rao (2018)**
41	Manjeera reservoir, Telangana	57	42	20	11	**Prasad *et al.* (2020)**
42	Gobind Sagar Dam	46	27	08	05	Sharma (2018)
43	Thotapalli and Gottabarrage reservoir	31	20	11	6	Ramaneswari and Sridhar (2015)

44	Sone Lake, Assam	69	49	24	11	**Kar *et al.* (2006)**
45	Kamalpur Lake, Telangana	25	18	11	7	**Thirupathiah *et al.* (2014)**
46	Highland of Himalaya, Central Highland and the Western Ghats	367 (266 in Himalayan, 155 in Western ghat & 95 in central Highland)				**Nautial (2005)**
47	Kumaon Himalaya	10	9	4	3	**Negi and Negi (2010)**
48	Western Ghats of India	288	109	41	12	**Dahanukar *et al.* (2004)**
49	North East India along with Himalayan and Indo Burma Biodiversity hotspots	422	133	38		**Goswami *et al.* (2012)**
50	Fish Fauna of Himachal Pradesh	104	48	14	8	**Mehta and Uniyal 2005**
51	Fish fauna of Himachal Pradesh	81	49	18	6	**Sharma and Sidhu (2016)**
52	Rakchham – chhitkul wildlife sanctuary H.P.	2	2	1	1	**Negi and Banyal (2017)**

Fish diversity of some Riverine system-

The Ganga River system- The Ganga river system comprises Ganga and Yamuna as its major rivers. **Sarkar *et al.* (2010)** surveyed the fish diversity, their distribution, abundance and threats of fish in river Gomti which is a tributary of river Ganga and collected 56 fish species coming under 42 genera and 20 families. **Lakra *et al.* (2010)** conducted a study to investigate the fish diversity in the Ganga basin (Betwa River). They also studied habitat ecology of fish and correlate species richness with hydrological attributes. Their result revealed that physical habitat variables show a crucial role in the fish distribution in Betwa River. They detected 63 fish species coming under the 20 families and 45 genera and the threat status of River Betwa and recommended that 34.92 % of fish fauna was under threat. **Sarkar *et al.* (2012)** carried out studies in the River Ganga, and addressed the freshwater fish diversity, distribution and abundance and also reviewed the threats to diversity. During their study, they recorded 143 species from which 133 species were native and reaming 10 species were exotic. These species belong to 11 orders, 72 genera and 32 families. The result showed that those fish species were 20 % of freshwater fish reported in India. According to the previous authors, hydrological alteration, dam construction, and overfishing etc. were responsible for alteration in fish diversity and community structure.

Das *et al.* (2013) also observed the ichthyodiversity, distribution and community structure of the Ganges River and also evaluated the ecological integrity of the riverine stretch. Their study found the presence of 143 species pertains to 40 families and 92 genera and the most prevailing family was Cyprinidae (38%). They revealed that with increasing river width and depth, the abundance and distribution of fish species increase. To illustrate, **Sarkar *et al.* (2014)** surveyed the fish diversity, pattern of abundance and distribution in the River Ken, an important tributary of River Yamuna and recorded the presence of 57 species representing 42 genera and 20 families. Assessment of the conservation status showed that 7 fish species were endangered, 13 were vulnerable and 20 at a lower risk, the previous researchers concluded that destructive fishing method, siltation and dams deplete the ichthyofauna of the river to a large extent and an urgent need of conservation strategy is required in River Ken. Furthermore, **Joshi *et al.* (2016)** examined the ichthyodiversity, distribution pattern and invasion of exotic fish species of Yamuna River under the influence of altered hydro-ecological conditions and revealed the presence of 112 species concerning 73 genera, 29 families and 10 orders. By the whole of 112 species, 6 were exotics. Evaluation of the threat status showed that 15 species were threatened and 10 were near threatened. The water quality parameters indicated the polluted condition of the river stretches due to the discharge of untreated domestic and industrial effluents.

Management of Western Himalayan hill stream concerning fish species richness and diversity were examined by **Johal and Rawal (2005)**. They selected 10 study sites coming from Rivers Ghaggar, Yamuna and Ganga and recorded the presence of 26 species. Those species were related to 4 orders; namely, Cypriniformes, Siluriformes, Synbranchiformes and Perciformes, with the Cyprinidae, a leading group with 21 species. Their study revealed that high altitude stream, having steep gradient along with boulder, dominated substrate with rapid habitat type, had lower fish species richness (FSR=3-4) and Shannon and Weaver diversity index of *H`=0.55-0.99*, whereas lower altitude hill stream having gentle gradient along with cobble dominated substrate with pools, riffles, runs, rapid and cascade habitat had high fish richness index (FSR=6-14) and Shannon and Weaver diversity index of *H`=1.67-2.35.*

The West Coast River system

Narmada and Tapti are the two main rivers of west coast river systems. **Vyas and Vishwakarma (2013)** studied the Jamner River, a tributary of the Narmada River and noted 27 fish species from 4 orders, 9 families and 16 genera. Cypriniformes (21) was foremost order followed by Ophiocephaliformes (3), Perciformes (2) and then Mastacembeliformes (1). Tawa River middle stretches, a left-bank tributary of Narmada was investigated by **Bose *et al.* (2013)**. They attempted to evaluate the fish diversity and documented 57 species specify 6 orders, 13 families and 35 genera and Cypriniformes was the dominant order constitute (59.64%) followed by order Perciformes (15.78%), Siluriformes (15.78%), Synbrnchiformes (3.50%), Beloniformes (1.75%), Osteglossiformes (1.75%). They also revealed that the river supports 25 ornamental fish and 17 aquaculture fish. Their study observed that 4 fish species were under threatened category and also indicated that the water of the river was not favourable for fish health. **Bakawale and Kanhere (2013)** surveyed to explore the fish diversity of the Narmada River in the western zone and found the presence of 51 fish species belonging to 7 orders and 15 families. They correlated the fish diversity with biological and Physico-chemical parameters. Their study also determined the declining and disappearances of some species from the river due to the interference of anthropogenic activity and habitat destruction. They suggested studying the life history character such as age, growth, and reproduction etc. of threatened fishes along with their demography.

In addition, **Vishwakarma and Vyas (2017**) conducted a study on the Dudhi River which is a tributary of Narmada basin and generated a primary database of Ichthyofaunal diversity. They recorded 19 species under 4 orders and 5 families. They also revealed that the diversity assessment of Ichthyofauna plays an important role to determine the water quality of any river because fishes respond instantly even to minute change in physicochemical parameters of their habitat. It is worthy to mention that, **Khade *et al.* (2017)** explored the fish diversity of Wan River, a tributary of the Tapti River and reported 21 species from 8 families and 5 orders. They found the dominancy of the Cyprinidae family (55%). **Vishwakarma *et al.* (2014)** explored the fish fauna of Barna River, Madhya Pradesh. Simpson dominance index, Simpson index of diversity; Shannon –Weiner index; Evenness index and Margalef index with the help of software PAST (2.15) were used to quantify the diversity of the assemblage and also for statistical comparison of the diversity. During their study, they identified 33 species of fish belonging to 5 orders, 9 families and 21 genera. Cyprinidae was the most abundant family having 250 (75%) individuals followed by Cobitidae with 32 (10%) individuals.

The East Coast River system

The East coast river system in India consists of Krishna, Cauvery and Godavari rivers. Koyna, a tributary of the Krishna river system in western Maharastra that was documented for fish fauna by **Jadhav *et al*. (2011).** They recorded 58 species from 16 families and 35 genera and revealed that 22 fish species in the Koyna River were endemic to the Western Ghats. They concluded even though there was a modest fishing pressure from tourism and pollution in some stretches of the river, there was a rich diversity of fishes in the Koyna River that means fishes were less threatened by anthropogenic stressors. **Dahanukar *et al.* (2012)** studied the Indrayani River, a northern tributary of the Krishna River system and noted a total of 57 fish species that regard to 18 families and 39 genera. Out of the 57 species, 12 were endemic to the Western Ghats while 6 were endemic to the Krishna River system. Their study also revealed that that river is threatened due to alien species and human-induced activities. Furthermore, **Chandanshive *et al.* (2007)** studied the fish fauna of the Pavana River, a major tributary of Mula and Mutha Rivers, Pune (Maharastra). They collected 59 fish species from the Pavana River and also found that fish fauna was threatened due to industrial and domestic waste. **Wagh and Ghate (2003)** evaluated the status of fish species from Mutha and Mula Rivers, tributaries of Bhima River and found the presence of 62 fish species. They concluded that 30% of the fish species reported earlier were not found during their study. According to the previous authors, the main reason for running down of fish species in the river was industrial and sewage pollution and the pervasiveness of exotic fishes. Additionally, **Vijaylaxmi *et al.* (2010)** documented the Ichthyofaunal diversity of Mullameri River, which is a trivial waterway of river Bheema in Gulbarga district of Karnataka. Fish diversity was assessed by using Shannon- Weiner biodiversity index, Simpson’s Dominance index, Simpson’s index of diversity, Pielous Evenness and Margalef index of species richness. Their result revealed the occurrence of 14 species coming under 5 orders with Cypriniformes as a dominant order followed by Siluriformes and followed by order Channiformes, Mastacembeliformes and Osteoglossiformes.

Fish assemblage structure of river fishes in the Western Ghat Mountains of Penisular India was characterized by **Arunachalam (2000)**. After analyses; macro and micro habitat for characterization of fishes in the stream, they recorded altogether 60 fish species, representing the 27 genera, 12 families and 4 orders and cyprinids were the most dominant group. Their finding also revealed that tremendous habitat diversification was related to immense species diversity. Notably, **Shahnawaz *et al.* (2010)** examined the fish diversity of the Bhadra River of Western Ghat and also correlated fish diversity with physicochemical variables. They recorded a total of 56 species signifying 31 genera and 15 families, with the Cyprinids as a leading group. Their study showed an encouraging relationship between fish species and physicochemical parameters. They found that effluents discharged by industries affect the species richness.

Heda (2009) explored the fish diversity in Adan and Kathani Rivers of Godavari basin which are tributaries of Painganga and Wainganga, respectively. The Jackknife-1 measure was used to calculate species richness estimate, and Biodiversity Pro software was used for calculation of richness estimates, diversity indices and similarity measure. Her finding revealed the presence of 47 species. **Rankhamb (2011)** investigated the Fish fauna of Godavari River at Mudgal which is a sacred place located on the bank of a river and found the presence of 26 species from 15 genera, 7 families and 5 orders. In their study, Cypriniformes was the dominated order with 15 species. Ichthyofauna of Cauvery River was studied by **Arunkumar *et al*. (2016).** They used geographical information system (GIS) for mapping habitat quality, fish diversity pattern and revealed the presence of 37 fish species from different sites in Cauvery River.

The Brahmaputra River system

The River Brahmaputra is running through Assam and Arunachal Pradesh, India. **Bhattacharjya *et al.* (2017)** studied the Ichthyofauna of Brahmaputra River along with its tributaries in Assam and reported 141 fin fishes from 84 genera and 29 families. **Deori *et al.* (2015)** explored the ichthyodiversity of Dihing River, a tributary of Brahmaputra River and revealed the presence of 50 species belonging to 18 families. Cyprinidae (32%) was the dominant family followed by Bagridae (12%), Siluridae and Osphronemidae (8%), Channidae and Mastacembelidae (6%) etc. They also studied the physico-chemical properties of the river and found that the water parameters were within the permissible range. **Baishya *et al.* (2016)** conducted a study that knew the fish diversity of small indigenous species in the upper reaches of the River Brahmaputra, and listed 52 fish species from 15 families. Their study perceived Cyprinidae as a dominant family with 42.31 % (22 species) followed by Bagridae (9 species) and Cobitidae (4 species). **Kaushik and Bordoloi (2016)** reported the fish fauna of Ranganadi River, a tributary of Brahmaputra and found 61 fish species from 6 orders. Their study also revealed the negative effects of electric Dam on fish fauna. **Das *et al.* (2017)** studied the fish diversity of Siang River, largest river of Brahmaputra River system and revealed the presence of 82 fish species under 8 orders, 24 families and 53 genera. They found Cypriniformes as a dominate order. They also evaluated the conservation status of fish and found most of fishes with least concern status.

The Indus River System

The main tributaries of the Indus River system in India are the Jhelum, the Chenab, the Ravi, the Beas and the Sutlej. **Kaur *et al*. (2017)** explored the fish diversity in Harike wetlands, a confluence of Beas and Sutlej River and recorded 37 fish species belonging to 25 genera and 14 families. Cyprinidae (16) was the dominant family followed by Bagridae (4), Siluridae (3) etc. **Sharma and Mehta (2009)** surveyed to study the ichthyofauna of Pong Dam Lake, built on the Beas River and accounted for 57 species under 5 orders and 12 families from the Dam and its tributaries. Their assessment of conservation status showsed 2 critical endangered and 4 endangered species. **Sharma**

and Dutta (2012) studied the Ichthyofauna of Basantar River, which is a tributary of Ravi in India and found the presence of 32 fish species with the dominance of Cypriniformes (18 spp.). **Rathore and Dutta (2015)** explored the fish diversity of Ujh River, a tributary of Ravi in Jammu and documented 42 fish species belonging to 5 Orders. Their study found Cypriniformes as prevailing order. They also related the fast depletion of fish diversity in the river to anthropogenic activity. **Bhutyal and Langer (2015)** conducted a survey to recognize the current status of Ichthyofauna of Chenab, Jammu and Kashmir and depicted 5 species of fish belonging to 3 orders and 3 families. Additionally, **Kumar (2010)** studied the fish fauna and hydrological condition of the Beas River in Kullu, Himachal Pradesh and reported 6 fish species from 3 orders and 3 families. While, **Sharma and Dhanze (2018)** studied the ornamental fish fauna of the Beas, Satluj, Ravi and their tributaries in Himachal Pradesh and reported 58 ornamental species of fish related to 13 families and 36 genera. Of these; 42 species were native and 16 were imported for the aquarium trade. Cyprinidae was the most dominant family of native Ichthyofaunal. On the other hand, **Jagtap (2013)** carried a case study on the fish fauna of Himachal Pradesh concerning available water resources such as Beas, Ravi, Sutlej and their tributaries. His study reported the presence of 61 fish species belonging to 13 families. The family Cyprinidae (36) was dominant followed by Cobitidae (05), Bagridae (04), Sisoridae (03), Channidae (03), Siluridae (02), and remaining Amblycipitidae, Schilbeidae, Belonidae, Mugilidae, Anabantidae and Mastocembelidae carried only 1 species.

Fish diversity of some lakes and reservoirs

Baliarsingh *et al.* (2013) studied the species diversity of freshwater fishes in the Similipal Biosphere Reserve, Odisha. There were altogether 66 species coming from 42 genera and 19 families and 6 orders. Cypriniformes have been the highest diversity followed by Perciformes, Synbranchiformes, Osteoglossiformes and Belongiformes. Biosphere reserve included 1 endangered, 1 vulnerable, 6 near threatened, 42 least concern and 3 data deficient fish species. **Bera *et al.* (2014)** define the ichthyodiversity in Kangsabati reservoir (West Bengal) concerning physicochemical parameters of water and they also evaluate the appropriateness of water to foster fishery activity, they investigated that physicochemical parameter of the reservoir was compatible for 39 commercially important fish species and revealed that the aquatic environment and water physicochemical parameters affect the Ichthyofaunal diversity and development of fish. **Bhat and Rao (2018)** examined the fish diversity in Tighra reservoir, Gwalior. They also find the conservation status and conservation measure of fishes in Tighra reservoir and recorded the presence of 40 fish species coming from 22 genera, 10 families and 6 orders. The maximum numbers of species belonged to family Cyprinidae (22), followed by Channidae and Begridae (4), and followed by Mastacembelidae and Siluridae (2). The family Ambassidae, Belonidae, Clariidae and Heteropneustidae represented by one species. In the IUCN status, out of 40 species of fishes 1 was endangered (*Tor putitora*), 1 was vulnerable (*Cyprinus carpio*), 3 were near threaten, 1 was data deficient and the rest was the least concern. Ichthyofaunal diversity in Manjeera reservoir, Telangana examine by **Prasad *et al.* (2020).** They find 57 fish species of 42 genera, 20 families and 11 orders and Cyprinidae (33.3%) was the most dominant family followed by Danionidae (14.0%), Bagridae (7.0%), Channidae (5.2%), Cichlidae (5.2%), Ambassidae (5.2%), Cobitidae (3.5%), Siluridae (3.5%) and Mastacembelidae (3.5%). **Sharma (2018)** studied the fish fauna of Gobind Sagar Dam, one of the highest gravity dam constructed on Sutlej river in Himachal Pradseh. They documented 46 species belonging to 27 genera, 08 families and 05 orders. They also reveal about the effect of exotic species on the diversity of indigenous fish fauna. **Ramaneswari and Sridhar (2015)** conducted a study to find fish diversity in Thotapalli and Gottabarrage reservoir in Vizianagaram, Srikakulam districts respectively of Andhra Pradesh state and reveal the presence of totally 31 species, in Thotapalli reservoir (28) and Gottabarrage reservoir (26). **Dua & Parkash (2009)** explore the fish biodiversity of Harike Wetland which is a Ramsar site in Punjab and find the occurrence of 61 fish species belonging to 17 families and 35 genera. Their study reveals Wetland as a rich source of fish diversity as it represents fish fauna of Beas and Sutlej river.

Kar *et al.* (2006) focus on the diversity of the fish population in Sone Lake, Assam. Diversity has been measured by the number of species (species richness) and by using two indices that were Shannon- Weaver and Simpson indices. The study revealed the occurrence of 69 species concerning 49 genera, 24 families and 11 orders. Of all these 84.2% belong to the primary freshwater group. **Thirupathaiah *et al.* (2014)** studied the fish diversity, evaluated fish abundance and also conservation status in Kamalpur Lake, district Karimnagar, Telangana. They revealed the presence of 25 species of 18 different genera, 11 families and 7 orders. Among fishes, leading order was Cypriniformes followed by Siluriformes, Perciformes, Symbranchiformes, Ostoeoglossiformes, Beloiniformes and Anguilliformes. In their finding IUCN status of 13 species are least concerned, 4 are data deficient, 6 are not evaluated, 1 species of fish is vulnerable and one species of fish is near threatened. **Ramaneswari and Sridhar (2015)** conducted a study to find fish diversity in Thotapalli and Gottabarrage reservoir in Vizianagaram, Srikakulam districts respectively of Andhra Pradesh state and reveal the presence of totally 31 species, in Thotapalli reservoir (28) and Gottabarrage reservoir (26).

Fish Diversity in Himalaya and Western Ghat

The distribution of Ichthyofauna in the highland of Himalaya, Central Highland and the Western Ghats was investigated by **Nautiyal (2005).** He consulted the published works for analysis and listed the presence of 367 species. Among the three highlands, the Himalayan region nurtures the largest number of species. He detected 266 species in the Himalayan region, 155 species in Western Ghat and 95 species in Central Highland. **Negi and Negi (2010)** analyzed the stream fish’s assemblage structure in the Kumaon Himalaya. The parameters like water sources, channel material, dominant habitat type and stream were taken into consideration for the Kumaon Himalayan streams. They documented 10 species of orders Cypriniformes, Mastacembelis and Perciformes. Out of 10 species, Cypriniformes comprised the dominant group with 8 species. **Dahanukar *et al.* (2004)** studied the large-scaled scattering pattern of fishes, their endemism and uniqueness and also threat status in the Western Ghats of India. They documented 288 fish species belonging to 12 orders, 41 families and 109 genera. Out of 288 species, 118 were endemic. 22 species were distributed all over the range of Western Ghat. **Goswami *et al.* (2012)** studied the fish diversity of North East India along with Himalayan and Indo Burma Biodiversity hotspots zones and recorded 422 species from 38 families and 133 genera. The maximal diversity was noted in Cyprinidae along with 154 species. They also concluded that the northeastern region of India has more than 62.81% of the total freshwater fishes of the country.

Himachal Pradesh which is situated in North Western Himalaya occupies 1.7 % geographical area of India endows more than 4% of ichthyofauna **(Mehta & Uniyal, 2005)**. They made a checklist of the fish fauna of Himachal Pradesh and found the presence of 104 fish species from 48 genera 14 families and 8 orders. They recorded the maximum number of fish species from Sirmour (57) followed by Kangra (55), Bilaspur (50), Solan (50). The Trans Himalayan district Kinnaur and Lahul Spiti have 5 and 2 species, respectively. They concluded that Himachal Pradesh encourages plentifully and diversified Ichthyofauna and having a significant number of commercially important fishes and also revealed the sharp decline in fish abundance due to anthropogenic activity. They also noticed the drying of hill streams which act as an area for breeding for many fishes and emphasized the need for restoration of those hill streams. **Sharma and Sidhu (2016)** studied the fish fauna of Himachal Pradesh and found 81 fish species comprising of 49 genera, 18 families and 6 orders. **Negi and Banyal (2017)** studied the ichthyofauna in Rakchham – chhitkul wildlife sanctuary, Kinnaur, Himachal Pradesh and detected the presence of 2 fish species related to 2 genera, 1 family and 1 order.

Methods of Species Identification

Every species that plays an important role in the ecosystem and diversity of species is beneficial to preserve a sustainable ecosystem. In the present scenario, some species are being hastily lost due to anthropogenic activity. To plan a conservation strategy for species a proper identification of species is necessary. But without truthful taxonomy, it remains awkward to identify a species. In fish species identification, in term of classical taxonomy fish identification is usually based on morphological characters (Morphometry, colour, fin formula, scale counting etc.). Differentiating fish species based on their morphological feature is the most practical, rapid and low-cost method. Local fishermen and fishmongers learn to identify fishes at a young age. Many researchers interlaced such traditional knowledge into modern ichthyology **(Drew, 2005).** For identification of fish, commonly used characters are morphometric and meristic count. Morphometric characters are those characters which can be measured in fish (Fig. 1); whereas, in case of meristic count the countable characters are calculated like the number of fin rays, fin spines etc. **(**Fig. 2). It is a convenient approach to distinguish one species from another through morphometric and meristic count. Moreover, they are also used to measure intraspecific differences among the species. Generally, the scientist identifies the fish through following standard literature or using the key. A taxonomic key is a systematic sequence of alternate choices provided by the diagnostic (morphological) character of an organism that leads to authentic identification of the organism. The formal or taxonomical scope of a key is usually restricted to printed material or presented in digital format. However, due to high diversity and phenotypic plasticity, in many cases, it seems difficult to accurately identify a fish species only on morphological basis. The morphological character finds trouble in the identification of cryptic species, morpho-species and also for identification of developmental stages. It is problematic for a less skilled person to identify a species reliably.

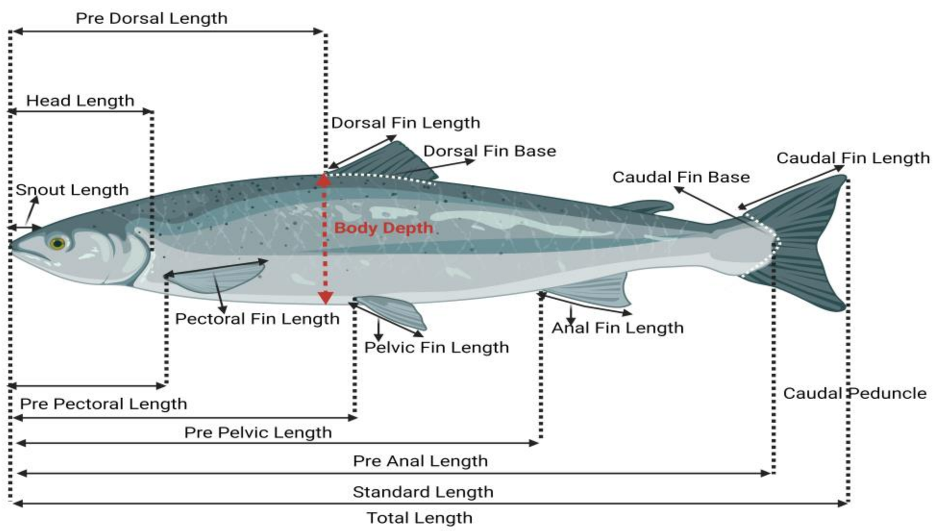

Fig. (1): Hypothetical Common Morphometric Parameters of a Fish.

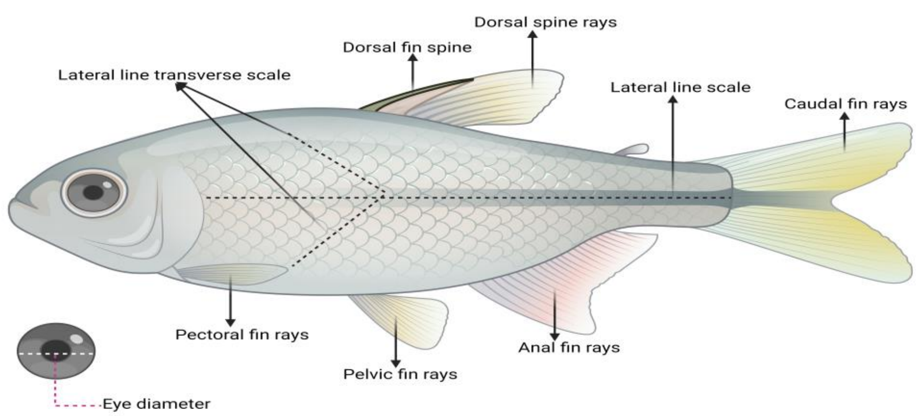

Fig. (2): Hypothetical Meristic Character of a Fish.

Fischer (2013) emphasized on the various methods of fish species identification including expert taxonomist, folk local experts, identification key, image recognition system, use of body parts (Scale, otolith), Acoustic method, genetic identification through single nucleotide polymorphism and DNA barcoding. Some web resources such as Fish Base (www.fishbase.org), the Eschmeyer's Catalog of fishes offer direction to decipher the matter concerning the correct scientific name for a species.

DNA barcoding as a new aspect in the systematics of fish taxonomy

The concept of DNA barcoding as a genomic identification system was developed by Paul Hebert and it is a well-accepted taxonomic method to facilitate species identification even by a non-specialist. In DNA barcoding, a short standardized nucleotide sequence of DNA is used for the identification of fish. Generally, cytochrome oxidase subunit 1 (cox 1), which is a mitochondrial DNA gene used as a global bio-identification system for an animal **(Hebert *et al.* 2003)**. The process of DNA barcoding includes the following steps: DNA extraction i.e. isolation of DNA from the sample, PCR amplification of the target DNA barcode region, DNA sequence of PCR products and analysis. The basic principle behind DNA barcoding is that the interspecies variations are more than intraspecies variation, which helps to differentiate the species using short standardized nucleotide sequences. The main objective of DNA barcoding emphasis on the gathering of reference libraries of barcode sequences for known species so that after assembly a consistent molecular tool is developed for species identification.

DNA barcoding has been effectively used for both marines and freshwater species. **Ward *et al.* (2005)** first of all discriminated Australian marine fish through DNA barcoding. They generated 754 barcodes for 207 species. They sequenced multiple specimens of 3 species of Chimaerids, 61 species of sharks and ray, and 143 species of teleost for the barcode region of cox1. **Moura *et al.* (2008)** attempted to test the competence of the DNA barcode approach to discriminate deepwater shark species: *Centrophorus squamosus*, *Centrophorus granulosus*, *Centroscymnus coelolepis* and *Centroscymnus owstoni* and their study revealed a low level of haplotypic and genetic diversities. **Holmes *et al.* (2009)**) identified shark species from dried fins in Australian water. They examined 211 left pectoral fins, 193 fins out of them provided a chondrichthyan sequence which when matched with reference specimen in a DNA barcode database identified 27 species of shark and rays. Among them, twenty species were sharks while seven were rays. They found that *Carcharahinus dussumieri* was the most abundant fish. Additionally, **Steinke *et al.* (2009)** constructed a DNA barcode reference sequence library for introduced ornamental fish species in North America.

For illustration, Hubert *et al.* (2008) categorized Canadian freshwater fish species through DNA barcoding. They sampled 194 fish species but the primer used amplified the target region of 190 species. A total of 1360 COI barcodes of 652-bp have been obtained for 190 species distributed among 85 genera and 28 families. Furthermore,

Aquilino *et al.* (2011) examined the fish fauna of Taal Lake, Philippines through DNA barcoding using mitochondrial cytochrome c oxidase subunit I (COI) gene. They barcoded 118 individuals of 23 fish species related to 21 genera, 17 families and 9 orders. All COI sequences for each of the 23 species were clearly discriminated among genera. The average genetic divergence increased in ranking, from within species (0.60%), within genus (11.07%), within family (17.67%) and within order (24.08%). The megadiverse neotropical freshwater fish fauna was examined by **Pereira *et al.* (2013)** using DNA barcoding. They analyzed 254 species, out of which 252 were correctly identified by DNA barcode sequences (99.2%). They tested the competence of the COI gene in identifying freshwater fish fauna from the neotropical region. Genetic divergence, measured by using K2P, was 0.3% and 6.8% for intra and inters specific diversity, respectively.

Remarkably, in India, many researchers examined Ichthyofaunal diversity using DNA barcoding as a molecular appliance both for marine as well as freshwater fishes (Table 2). **Khedkar *et al.* (2014)** constructed firstly a DNA barcode library for freshwater fishes of Narmada River. They collected 820 fish specimens from different locations across the river basin. They generated a 314 different COI sequence and revealed that the specimens belonged to 85 species representing 63 genera, 34 families and 10 orders. Their study revealed that, out of 85 species, 5 species were endemic to India whereas 3 were exotic. In addition, **Lakra *et al.* (2011)** barcoded the Indian marine fishes for the first time using mitochondrial COI gene. They characterized 115 marine fish species belonging to 7 orders and 37 families including Carangids, Clupeids, Scombrids, Groupers, Sciaenids, Silverbellies, Mullids, Polynemids and Silurids of Indian marine fishes for the generation of DNA barcodes. The average Kimura two-parameter (K2P) distances increased hierarchically from within species to orders. In this context, **Sachithanandam *et al.* (2011)** examined the barcode sequences for the identification of the *Plectropomus*, results of DNA barcode for *Plectropomus leopardus* revealed the potential ability of mini-barcodes to discriminate among species. Moreover, **Sachithanandam *et al.* (2012)** carried out the identification of *Epinephelus* spp. in the Andaman coastal region by DNA barcoding using the COI gene. They evaluated the mean genetic distances using Kimura 2 parameter (K2P) between the studied *Epinephelus* spp. from Andaman coastal region and same species from the world over. Genetic divergences among *Epinephelus longispinis*, *Epinephelus ongus* and *Epinephelus aerolatus* were 0.0004, 0.0183, and 0.0437, respectively. While, **Sadurudeen *et al.* (2017)** generated DNA barcodes for 32 fish species, delineating 13 families of order Perciformes in the Indian Coast.

Additionally, catfish diversity of North- East India was examined by **Bhattacharjee *et al.* (2012)** using a collective method of morphological feature and DNA barcoding. They collected 75 native catfish specimens and identified 25 species belonging to 17 genera and 9 families through morphological features whereas the DNA barcoding approach described 21 distinct species. The competence of DNA barcoding was revived by successfully identifying 84% of catfish and they also constructed 27 new barcodes for seven species. The high conspecific divergence was shown by *Amblyceps apangi*, *Glyptothorax telchitta*, *Glyptothorax trilineatus* and *Erethistes pusilus* species so their identification through DNA barcoding remained indecisive. **Chakraborty and Ghosh (2014)** evaluated the DNA barcode of freshwater fishes in North-East India. They analyzed 1383 barcode sequences of 175 freshwater species, out of which 172 barcode sequences depicting the 70 fish species. DNA barcoding also helps to resolve the species uncertainty of Northeast Indian mahseer **(Laskar *et al.*, 2013).** They categorized *Neolissochilus hexastichus* to be an accurate species while *Tor progeneius* to be a synonym of *Tor putitora*.

DNA barcodes for freshwater fishes were also generated by **Lakra *et al.* (2016).** They collected the specimen of 72 species from the Ganges and Brahmaputra Rivers system. They generated 284 COI sequences for 72 species signifying 50 genera and 19 families. Fish taxonomy followed the FAO (Food and agriculture organization) fish identification sheets. They reported COI (a mtDNA gene) as an idyllic marker for DNA barcoding. **Sarma and Mankodi (2017)** identify inland fishes of Gujarat using a combined approach of the morphological feature as well as DNA barcoding. Their study reveals the presence of 38 species belonging to Actinopterygii. Species were discriminated using barcode sequences of 655bp region of COI gene.

In the Western Himalaya, **Chandra *et al.* (2012)** utilized Cytochrome Oxidase I (COI) mtDNA barcodes for the recognition of two commercially important Indian coldwater fishes of genus Schizothorax (Snow trout). The average K2P distance of individual species of *Schizothorax richardsonii* was 0.00% and *Schizothorax progastus* was 0.00%, respectively whereas the mean divergence between *Schizothorax richardsonii* and *Schizothorax progastus* was 1.75%. **Barman *et al.* (2018)** studied the DNA barcoding of fishes in the Kaladan River. They obtain 291 COI barcodes from 49 species, signifying 28 genera. Mean genetic divergence (K2P) increased orderly with increasing taxonomic rank from 0.14 % within species, 08.88% within a genus, 12.04 % within families and 19.19% within the order. In this essence, **Barman *et al.* (2018)** conducted the DNA barcoding of freshwater fishes of Indo-Myanmar biodiversity hotspot. They barcoded 363 individuals that represented 109 morphologically were identified species using the COI gene. Total of 363 COI sequences was represented by 109 species. The average Kimura two-parameter (K2P) genetic divergences (%) within-species, genera, families, and orders were 0.42, 10.19, 12.77 and 19.21, respectively. **Pandey *et al.* (2020)** examined the DNA barcoding of freshwater fishes of Ranganadi River in Arunachal Pradesh. They identified 22 fish species and constructed COI barcodes for 114 specimens native to 22 species and found to be 98-100% identical from GenBank and BOLD databases.

Table 2: Inclusive view of DNA barcoding in fish using the COI gene and identified K2P values.

S.No.	Title	% of the identified	Average within species K2P values (%)	Average within genus K2P values (%)	Average within family K2P values (%)	Average within order K2P values (%)	**References**
1	DNA Barcodes for the Fishes of the Narmada, One of India's Longest rivers	98%	0.36%	12.29%	17.87%	22.43%	**Khedkar *et al.* (2009)**
2	DNA barcoding Indian marine fishes	100%	0.30%,	6.60%,	9.91%,	16.00%	**Lakra *et al.* (2011)**
3	DNA barcoding of selected Perciformes (Infra Class: Teleostei) fishes from Indian coast		0.42,	13.91	18.05%		**Sadurudeen *et al.* (2015)**
4.	Identification and Re-Evaluation of Freshwater Catfishes through DNA Barcoding	84%					**Bhattacharjee *et al.* (2012)**
5	An assessment of the DNA barcodes of Indian freshwater fishes	87%	1.6%	9.92%	15.66%	25.32%	**Chakraboty and Ghosh (2013)**
6	DNA barcoding Indian	100%	0.40%,	9.60%,	13.10%,	17.16%,	**Lakra *et al.* (2016)**

	freshwater fishes						
7	DNA barcoding and genetic diversity analyses of fishes of Kaladan river of Indo-Myanmar biodiversity hotspot.	100%	0.14%	08.88%	12.04%	19.19%.	**Barman *et al.* (2017)**
8	DNA Barcoding of Freshwater Fishes of Indo-Myanmar Biodiversity Hotspot	≈ 100%	0.42%	10.19%	12.77%	19.21%	**Barman *et al*. (2018)**
9	DNA Barcoding and Phylogenetics of Freshwater Fish Fauna of Ranganadi river, Arunachal Pradesh	98-100%	0.23%	11.31%	26.25%	27.30%	**Pandey *et al*. (2020)**

Future prospective

To understand an aquatic ecosystem it is necessary to transcript its biodiversity. Over the last few decades, the riverine ecosystem has been facing intense anthropogenic pressure due to the existence of degradation and loss of habitat for the fishes; many riverine fish species have become endangered. They also signified the impact of altered aquatic habits on freshwater fishes and revealed their sensitive nature **(Sarkar *et al*., 2010).** Fishes are generally used as a bioindicator for assessing environmental pollution as well as indicators of the ecological integrity of running waters **(Chovanec *et al*., 2003**). When we compare freshwater biodiversity to marine or terrestrial biodiversity, it may be noted that freshwater biodiversity deteriorated more rapidly than other **(WWF, 2016)** and the reason behind it is the increasing demand of freshwater resources. Other reasons for the loss of fish biodiversity include pollution, overfishing, industrial waste and alien species introduction etc. So, there is a need to enforce robust conservation measures to conserve Ichthyofauna.

The knowledge of fish diversity is essential for adopting proper conservation strategies. Accurate identification of fish is the major task for a taxonomist to develop appropriate conservation management. Every fish species has individual genetic distances depending upon the environmental stress and water quality which play an important role in its minor morphometric variations. Many species have been considered cryptic that mean they are morphologically indistinguishable so that their status remains argumentative **(Darshan *et al.*, 2010)**. Consequently, classification and identification of fishes through classical taxonomy have various barriers and limitations. Nowadays, many researchers use morphological analysis and DNA barcoding work collaboratively for species identification. DNA barcoding as a molecular approach provides an effective tool in fish identification. After the establishment of global barcode database for fishes, even inexpert or less experienced user by accessing DNA sequences will be able to distinguish all fish species and the identification sustained by it could be used to evaluate fish biodiversity, monitor fish conservation and manage fisheries. FISH-BOL (the Fish barcode of life campaign), international research collaboration, collected DNA barcode records for the entire world's fishes with the aim to develop COI gene sequences library **(Ward *et al.*, 2009)**. In India, DNA barcoding is in the infancy period and should be focused on in the sampling of marine and freshwater species. Other than the COI barcode marker (mitochondrial gene), the effort should be targeted to develop a nuclear barcode because sometime COI fails to distinguish fishes formed through introgressive hybridization. Thus, this may help to develop fish DNA barcoding more easily as well as quickly. But the challenge is to find out 600-1000 bp long nuclear coding region undisrupted by introns, with fast rates of evolution **(Dasmahapatra & Mallet, 2006).** However, researches from interdisciplinary sciences are desirable in this direction to fulfill that goal.

CONCLUSION

In conclusion, India contributes about 7.7% of global fish diversity. Fish diversity has been documented from various riverine systems and their tributaries such as Ganga, Yamuna, Narmada, Tapti, Krishna, Cauvery, Godavari, Brahmaputra, Ravi, Beas and Sutlej etc. along with distribution of fish fauna in Himalaya and Western Ghat. Freshwater fishes are also the most threatened group after amphibians because of constant pressure on their habitats by human activities like agricultural pesticides, herbicides, industrial waste, dam construction, exotic species, overfishing etc. Ichthyodiversity and their distribution are convenient for planning and executing conservation strategies. However, in India, freshwater fishes are abysmally recognized and worried due to unsolved cryptic species. Hence, presently there is a need for a molecular approach along with traditional taxonomy for the evaluation of freshwater species. DNA barcoding provides an important molecular tool for identifying unknown fish species, fish diversity and also help the preparation of a suitable approach for sustainable management and conservation.

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Conflicts of Interest

The authors confirm that there is no conflict of interest.

Authors Contribution

Rakesh Kumar, Kushal Thakur: Conception and Design of the work; Kushal Thakur: Drafting of Manuscript; Rakesh Kumar, Bhavna: Critical revision.

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