

APPENDIX

Publications

Published abstracts

- Ghosh SK, Ghosh PR, Trivedi S, Das PJ, Chetry AJ, Mahadani P, Bhattacharjee MJ, Ahenthem M, **Khomdram B**, Kshetrimayum M, Das KC, Tiwary BK, Choudhary A, and Charkraborty CS (2009); DNA Barcode of Life : Species specific DNA Sequence from East and Northeast Indian Biodiversity. 3rd International Conference of Barcode of Life Conference, Mexico City. P.p-193.
- Ghosh SK, Bhattacharjee MJ, Dhar B, **Khomdram, B** and Laskar BA (2011); Molecular characterization of Catfishes of Northeast India: A DNA barcode and mini-barcode Approach. Fourth International barcode of life conference, Adelaide, Australia.

Published papers

- **Bijoya Khomdram**, Bishal Dhar and Sankar K. Ghosh (2014), Jiribam, the ornamental fishes' hot spot zone of Manipur, India, *Journal of agriculture and veterinary science (IOSR-JAVS)*. 7(1): 85-91.
- **Bijoya Khomdram Devi**, Bishal Dhar, Boni Amin Laskar and Sankar Kumar Ghosh (2013), Exploring ornamental Fishes of Manipur through DNA barcoding, *J. Environ & Sociobiol.* : 10 (1): 83-88.
- Mohua Chakraborty, S. K. Ghosh, Bishal Dhar and **Bijoya Khomdram Devi** (2013), Revealing genetic diversity of *Clarias batracus* using DNA barcode, *J. Environ & Sociobiol.* : 10 (1): 25-32.

Others

Book Chapter contribution:

- **Chapter 3** (Sampling and Vouchering) in the book, "A Text Book on DNA Barcoding" by Prof. (Dr.) Sankar Kumar Ghosh. Publisher: Books Space, ISBN: 81-922989-4-8.

Conferences attended and presented

- **Khomdram B**, Bhattacharjee MJ, Miranda Ksh., Ahanthem M and Ghosh SK (2011); Development of DNA based passport of ornamental fishes from North east India, **98th Indian science congress**, Chennai. p.p- 199 (Animal, veterinary and Fishery science).
- Maloyjo Joyraj Bhattacharjee, **Bijoya Khomdram** and Sankar Kumar Ghosh (2011) DNA barcoding of Ornamental Fishes of Northeast India, **22nd Pacific Science Congress, Kuala Lumpur**, Malaysia. p.p-37.

Workshops/Training Programmes Attended

- “National workshop on DNA Barcode of Life” organized by Department of Biotechnology, Assam University, Silchar (07th April, 2009)
- “Workshop on Techniques in Biodiversity Researches” organized by Department of Ecology and Environmental Science, Assam University, Silchar, Assam, India (March, 27, 2010).

Jiribam, the Ornamental Fishes' Hot Spot Zone Of Manipur, India

Bijoya Khomdram, Bishal Dhar and Sankar Kumar Ghosh*

Department of Biotechnology, Assam University, Silchar-788011, Assam, India

Abstract: An investigation on the ornamental fish species availability was carried out in the Jiribam sub division, Imphal east district, Manipur, India. All the possible areas were surveyed and many experts were interacted. Out of the total 139 ornamental fishes found in the state of Manipur, 61 were recorded from Jiribam alone which comprise ~44 %. Therefore, we can categorize Jiribam as one of the hot spots of ornamental fishes in Manipur. The total 61 species belonged to 22 families and 7 orders. 42 species were recorded as threatened species and 3 species are endemic.

Keywords: Jiribam, ornamental fish, hot-spot, endemic, threatened

I. Introduction

Due to the diversity of topographic and climatic features of NE India, this region is rich in endemic fish. Most of the small food fish which are treated as unwanted for conventional farming have good potency as ornamental fishes and are popularly known as Aquarium fishes. These species are attracting hobbyists both locally and globally.

North East India is considered as one of the hot spots of freshwater fish biodiversity in the world (Kottelat and Whitten, 1996). It is a well-recognized fact that there has been drastic reduction in abundance of the fresh water fishes in this region due to destruction of the habitat, overexploitation and other anthropogenic effects. Review of literature indicates that only limited information is available on fish germplasm resources of north east India with special reference to its potential as cultivable, sport and ornamental fishes. There has been a wide variation in the number of fishes reported from this region ranging from 172 (Ghosh and Lipton, 1982) to 267 (Sen, 2000). Also detailed drainage wise distribution, seasonal abundance, endemism and preferred microhabitats have not been critically examined.

The up to date inventory of the fish species of North East India showed 250 potential ornamental fish species. Out of this, the highest no. recorded from Assam (187), followed by Arunachal Pradesh (165), Meghalaya (159), Manipur (139), Tripura(103), Nagaland(71), Mizoram(46), and Sikkim(29). Conservation status of native ornamental fishes have shown that out of 250 sp., 10 are critically endangered, 28 are endangered, 49 are vulnerable, 45 are lower risk near threatened, 8 are lower risk least concern, 3 are data deficient and 107 are not evaluated (Ponniiah, A. G. *et al.*, 2006). NE harbors diversified native ornamental fish species. These include both classified and non-classified types of aquarium fishes (Mahapatra *et al.*, 2004). Small fishes like *Botia derio*, *Danio dangila*, *Puntius shalynius* etc. are classified types on the other hand larger food fishes like *Labeo gonius*, *Rita rita* etc. are termed as non classified ones. There are two major river basins within the state of Manipur, viz. the Barak River Basin and the Manipur River Basin. After the world famous Shiroi Lily and the Sangai, matter has now come to light that Manipur is also home to a number of fish species which are highly prized for their ornamental values in Europe and the United States.

Diversity of fish fauna in Jiribam, Manipur

The north east region shares its fish fauna predominantly with that of the Indo Gangetic fauna and to a little extent with the Burmese and South China fish fauna (Yadav and Chandra, 1994). Exploring the literature shows that 172 fish species with reference to their economic importance were recorded by Ghosh and Lipton (1982) while Sen (1985) and Mahanta *et al.* (1998) recorded altogether 187 fish species from Assam and the neighboring north eastern states of India. Compilation of Yadav and Chandra (1994) listed a total of 129 species. Sinha (1996) in his comprehensive review gave a list of 230 species of fishes as available from north eastern region. Sen (2000) has indicated that more number of species (267) has been reported from north east India. The various reports show a wide variation in the total number of fishes reported. Since Manipur is located in the extreme east zone of India therefore there is a greater chance in the available fish fauna being influenced by Burmese fish fauna. According to recent reports, a total of 139 ornamental fishes are found in Manipur. Jiribam is a small sub division in the westernmost part of Manipur where it borders with the state of Assam. It is drained by a single river, Jiri River and many small canals. The Jiri River joins the Barak River in Tipaimukh and hence becomes the main reason of harboring a large number and variety of fishes. The literature reveals that no efforts have been made to explore the rich ichthyodiversity of this region and also no work have been done to

assess the rich fish resources available in this region with respect to commercial utilization. With the growing demand for consumption and aquarium, it is necessary to evaluate potential species on the basis of different criteria.

In the present paper, an attempt has been made to prioritize among the fishes of this region the potential ornamental fishes along with their endemic status and status of threat on the basis of available literature as well as enquiry and interaction with the local fishery experts. Potential aquarium fishes have been identified based on actual present demand, bright coloration, uncommon look and uniqueness and following the records as mentioned in the literature. Recent estimates suggested that worldwide 20% of all freshwater fish species are extinct, endangered or vulnerable (Maclean and Jones, 1995). As a result fish stocks particularly those dwelling in inland open water areas, have gradually become endangered.

Extensive field survey conducted from September 2009 to December 2010 in Jiribam sub division of Manipur revealed the occurrence of bewildering diversity of ornamental fishes. Study about the species availability helps to know the present status of species variety and their relative abundance in the respective water bodies.

II. Materials And Methods

Study area

The survey work was carried out in Jiribam sub division (Imphal east district) of Manipur, India. Data were collected from all the major fish landing centers and interaction with the fishermen and local people.

Data collection and analysis

In order to collect data field visit was made every month and sometimes daily during the study period according to information and preference in the respective areas. In addition relevant information was also collected from various sources. The data were assembled through field survey using appropriate questionnaire. The questionnaire form was filled in by interviewing the fishermen directly from the field and local fish experts and also the local people. All the collected data were analyzed and the species observed were grouped in different categories.

III. Results And Discussion

Species composition

A total of 61 species were found from the surveyed area out of the total 139 species of ornamental fishes found in the state of Manipur. All the species were Freshwater fishes. They were belonged to 22 families and 7 orders. They are serially depicted in the tables 1, 2 and 3. Out of the 61 species recorded 21 species belonged to the family, Cyprinidae, only 1 sp. belonged to Anabantidae, 2 species belonged to Anguillidae, 1 species belonged to Badidae, 2 species from Balitoridae, 3 species from Ambassidae, 3 species from Channidae, 1 species from Clariidae, 3 species from Osphronemidae, 1 species from Erethistidae, 1 species from Schilbeidae, 3 species from Sisoridae, 4 species from Cobitidae, 5 species from Bagiridae, 1 species from Nandidae, 2 species from Notopteridae, 1 species from Mastacembelidae, 1 species from Chachidae, 1 species from Synbranchidae and 2 species from siluridae, 1 species from Heteropneustidae and 1 from the family Belonidae. Among the 7 orders of fishes found, the order Cypriniformes dominated others with a total number of 27 species then comes Siluriformes (15) and Perciformes ranked third with a total number of 12 species.

Species variation in different season

During the survey it was observed that not all species were available in all season. A total of 14 species were more available in winter season, 12 in summer and 35 fish species were available throughout the year. There were some species which were more available in summer but not in winter. And some species were available only in winter season. The species which have high ornamental value are mostly available during the months of October to December.

Status of the fishes

According to the IUCN (2008) Red List of all life forms, 16,928 species are threatened globally, and of these 1275 species are fishes. There are 9 categories in the IUCN Red List namely, Ex- Extinct, EW- Extinct in the Wild, CR- Critically Endangered, EN- Endangered, VU- Vulnerable, LR/cd- Lower risk/ conservation dependent, NT- Near Threatened (includes LR/nt- Lower Risk/ near threatened), DD- Data deficient, LC-Least Concern (includes LC/lc- Lower Risk/ least concern). Species may move between categories for a variety of reasons, including genuine improvement or deterioration in status, new information being available about the species that was not known at the time of previous assessment, taxonomic changes, or mistakes being made in previous assessment (eg., incorrect information used previously, misapplication of the IUCN Red List criteria, etc.). Out of the 61 species of ornamental fishes recorded from Jiribam, 42 species are found to be in the list of

threats according to the report of CAMP workshop on freshwater fishes of India organized by NBFGR, 1997 and also following the records in the recent NBFGR publication (Lakra, W. S. *et al.*, 2010), after exercising all the related records and publications. These fishes are shown in Table 4 with their category of threat. And we observed 3 endemic fishes in jiribam namely, *Devario acuticephala*, *Schistura manipurensis* and *Garra manipurensis*. Except these 3 species, others are native.

In the present study, the percentage contribution of Cyprinids are found to be 33.3% being the dominant family. Some fishes are found to be surprisingly in the verge of extinction. The noted ornamental fish *Chaca chaca* is reported to be found in some restricted area of Jiribam but due to some reasons we are facing a great problem in getting this fish. The major reason behind the threatening status of this particular fish is the construction of Tipaimukh dam in the water body which is the sole and native home for this very species of ornamental fish. *Bagarius bagarius* is almost extinct in Jiribam nowadays, during the whole survey period it was recorded to catch only once in the month of December. During the last few years many exotic fishes are also introduced by the fishermen so that they could get a greater profit in their business without a second thought of affecting the local and indigenous fish diversity of the area. Freshwater fish are not only the most diverse group of vertebrates but they also represent and feature the greatest proportion of threatened species (Bruton, 1995; Leidy and Moyle, 1998; Duncan and Lockwood, 2001). The principal threats to freshwater fish are the deterioration or destruction of habitats, both by pollution and intense modifications (like damming, channelization and so on.) and introduction of exotic species (Moyle, 1986; Allan and Flecker, 1993). Though most of the fish resources of Manipur are already explored by Vishwanath and his team (Vishwanath and Sarojnalini, 1986, Vishwanath *et al.*, 2007, Vishwanath and Dishma, 2012), the fish species of Jiribam region remain untouched. The present study will help future researchers and others for easy access of the ornamental fishes of this region and will be of great help to conservationist and aquarists.

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Table 1:- List of the ornamental fishes being collected from Jiribam. (A-abundant,LA- less abundant, MA-moderately abundant, R-rare, VR-very rare)

SI no	Local name	Scientific name	Ornamental value	Abundance
1	Muka nga	<i>Amblypharyngodon mola</i>	Medium	A
2	Ngawa	<i>Barillius ngawa</i>	High	R
3	Ngawa phuri thungbi	<i>Barillius bendelisis</i>	High	R
4	Muka nga macha	<i>Devario acuticephala</i>	High	A
5	Ngasang	<i>Esomus dbrnicus</i>	High	A
6	Ngasang macha	<i>Rasbora rasbora</i>	High	MA
7	Ngathi	<i>Labeo calbasu</i>	Medium	R
8	Khabak	<i>Labeo gonius</i>	Medium	MA
9	Phabou	<i>Puntius manipurensis</i>	High	R
10	Phabou	<i>Puntius punctata</i>	Medium	MA
11	Phabou	<i>Puntius sarana</i>	High	MA
12	Phabou	<i>Puntius conchonius</i>	Medium	MA
13	Phabou	<i>Puntius vittatus</i>	High	MA
14	Mirga	<i>Cirhinus mrigala</i>	Medium	A
15	Ngara	<i>Tor tor</i>	High	VR
16	Rou	<i>Labeo rohita</i>	Low	A
17	Ukabi	<i>Anabus testudineus</i>	Medium	MA
18	Ngaril	<i>Anguilla bengalensis</i>	High	R
19	Ngaril leisna	<i>Anguilla bengalensis Gray</i>	High	VR
20	Napet nga	<i>Badis badis</i>	High	MA
21	Ngatup	<i>Schistura sp.</i>	Medium	R
22	Ngatup	<i>Schistura manipurensis</i>	High	VR
23	Ngamhai akoiba	<i>Chanda nama</i>	High	MA
24	Ngamhai asangba	<i>Chanda nama</i>	High	MA
25	Ngamhai anganba	<i>Chanda nama</i>	High	MA
26	Porom	<i>Channa marulia</i>	High	MA
27	Ngamu	<i>Channa punctata</i>	Medium	A
28	Meitei ngamu	<i>Channa orientalis</i>	High	MA
29	Ngakra	<i>Clarias batrachus</i>	High	A
30	Ngabemma	<i>Colisa chuna</i>	High	MA
31	Ngabemma	<i>Colisa fasciata</i>	High	MA
32	Ngabemma	<i>Colisa lalia</i>	High	MA
33	Samu khongpak	<i>Erithistes hara</i>	High	VR
34	Basa	<i>Eutropichthys vacha</i>	Medium	MA
35	Leingoi chabi	<i>Gogangra viridescens</i>	High	R
36	Hangoi nga	<i>Sisor raddophorus</i>	High	VR
37	Ngakijou angangba	<i>Lepidocephalichthys anandalei</i>	High	MA
38	Ngakijou amuba	<i>Lepidocephalichthys guntea</i>	High	MA
39	Ngakijou awaoba	<i>Lepidocephalichthys berdmorei</i>	Medium	MA
40	Sarengkhoibi	<i>Botia derio</i>	High	MA
41	Ngasep	<i>Mystus tengara</i>	Medium	A
42	Ngasep	<i>Mystus vittatus</i>	Medium	MA
43	Ngachou	<i>Sperata singhala</i>	Medium	R
44	Ngarel	<i>Bagarius bagarius</i>	High	VR
45	Litha	<i>Rita rita</i>	Medium	MA
46	Kharaobi	<i>Nandus nandus</i>	High	MA
47	Kandla	<i>Notopterus notopterus</i>	Medium	MA
48	Ngapai	<i>Notopterus chitala</i>	High	MA
49	Ngamoi	<i>Mastacembalus armatus</i>	High	MA
50	Ngaprum	<i>Monopterus cuchia</i>	Medium	MA
51	Ngaseksha	<i>Ompok pabda</i>	High	R
52	Gajeb bakau	<i>Chaca chaca</i>	High	VR
53	Nunga amuba	<i>Garra manipurensis</i>	High	VR
54	Nunga awaoba	<i>Schizothorax richardsoni</i>	High	R
55	Ngaching	<i>Gagata sp.</i>	High	R
56	Nga cheklaobi	<i>Xenentodon cancila</i>	High	MA
57	Bata	<i>Labeo bata</i>	Low	MA
58	Mitlangbi	<i>garra sp.</i>	Medium	R
59	Ngamu sengum	<i>Garra gotyla</i>	Medium	A
60	Ngachik	<i>Heteropneustes fossilis</i>	Medium	MA
61	Sareng	<i>Wallago attu</i>	Medium	MA

Table 2:- Families of the ornamental fishes found in Jiribam along with the number of species

Sl no.	Family	No. of species
1	Cyprinidae	21
2	Anabantidae	1
3	Anguillidae	2
4	Badidae	1
5	balitoridae	2
6	Ambassidae	3
7	Channidae	3
8	Clariidae	1
9	Osphronemidae	3
10	Erethistidae	1
11	Schilbeidae	1
12	Sisoridae	3
13	Cobtidae	4
14	Bagaridae	5
15	Nandidae	1
16	Notopteridae	2
17	Mastacembelidae	1
18	Chachidae	1
19	Synbranchidae	1
20	Siluridae	2
21	Heteropneustidae	1
22	Belonidae	1

Table 3:- Orders of the ornamental fishes found in Jiribam along with the number of species

Sl no.	Order	No. of species
1	Cypriniformes	27
2	Perciformes	12
3	Anguilliformes	2
4	Siluriformes	15
5	Osteoglossiformes	2
6	beloniformes	1
7	Synbranchiformes	2

Table 4:- Threatened species being detected in Jiribam sub division of Manipur based on NBFGR (National Bureau of Fish genetic Resources) data and report of CAMP (Conservation Assessment and Management Plan) workshop organized by NBFGR (EN- Endangered, VU- Vulnerable, CR-Critically Endangered, LRnt- Lower Risk near threatened, LRlc- Lower Risk least concern).

Sl no.	Species name	category
1	<i>Puntius manipurensis</i>	EN
2	<i>Sisor rabdophorus</i>	EN
3	<i>Tor tor</i>	EN
4	<i>Badis badis</i>	VU
5	<i>bagarius bagarius</i>	VU
6	<i>Botia derio</i>	VU
7	<i>Eutropiichthys vacha</i>	VU
8	<i>garra gotyla</i>	VU
9	<i>Heteropneustes fossilis</i>	VU
10	<i>Ompok pabda</i>	VU
11	<i>Puntius sarana</i>	VU
12	<i>Puntius vittatus</i>	VU

13	<i>Schizothorax richardsoni</i>	VU
14	<i>Notopterus chitala</i>	EN
15	<i>Amblypharyngodon mola</i>	LRlc
16	<i>Anabus testudineus</i>	VU
17	<i>Anguilla bengalensis Gray</i>	EN
18	<i>Channa marulia</i>	LRnt
19	<i>Channa orientalis</i>	VU
20	<i>Channa punctata</i>	LRnt
21	<i>Cirrhinus mrigala</i>	LRnt
22	<i>Clarias batrachus</i>	VU
23	<i>Colisa fasciata</i>	LRnt
24	<i>Esomus danricus</i>	LRlc
25	<i>Garra manipurensis</i>	CR
26	<i>Rita rita</i>	LRnt
27	<i>Schistura manipurensis</i>	VU
28	<i>Wallago attu</i>	LRnt
29	<i>Xenentodon cancila</i>	LRnt
30	<i>Labeo bata</i>	LRnt
31	<i>Labeo calbasu</i>	LRnt
32	<i>Labeo rohita</i>	LRnt
33	<i>Lepidocephalus anandalei</i>	LRnt
34	<i>Lepidocephalus berdmorei</i>	EN
35	<i>Monopterusuchia</i>	LRnt
36	<i>Mystus vittatus</i>	VU
37	<i>Nandus nandus</i>	LRnt
38	<i>Gogangra viridescens</i>	LRnt
39	<i>Notopterus notopterus</i>	LRnt
40	<i>Puntius conchoni</i>	VU
41	<i>Puntius vittatus</i>	VU
42	<i>Barilius bendelisis</i>	LRnt

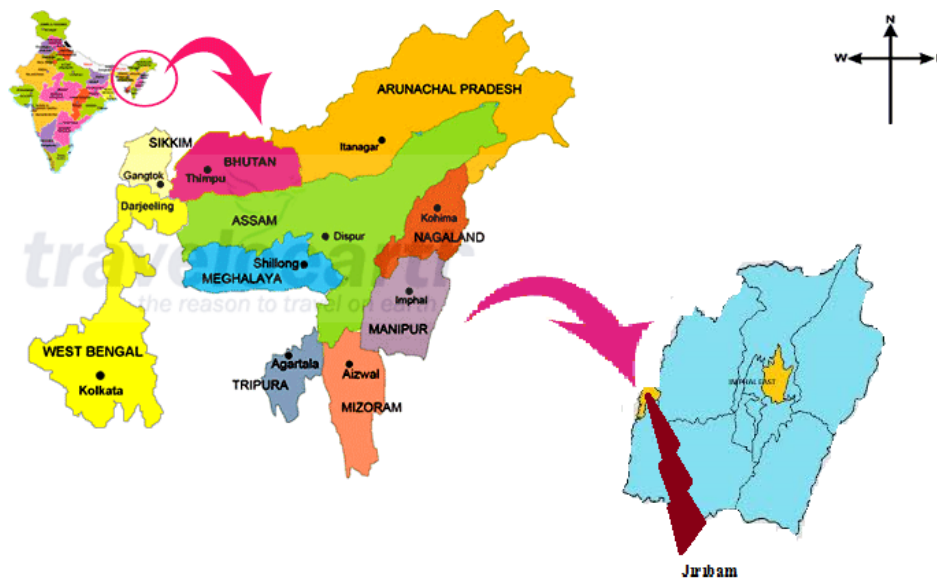
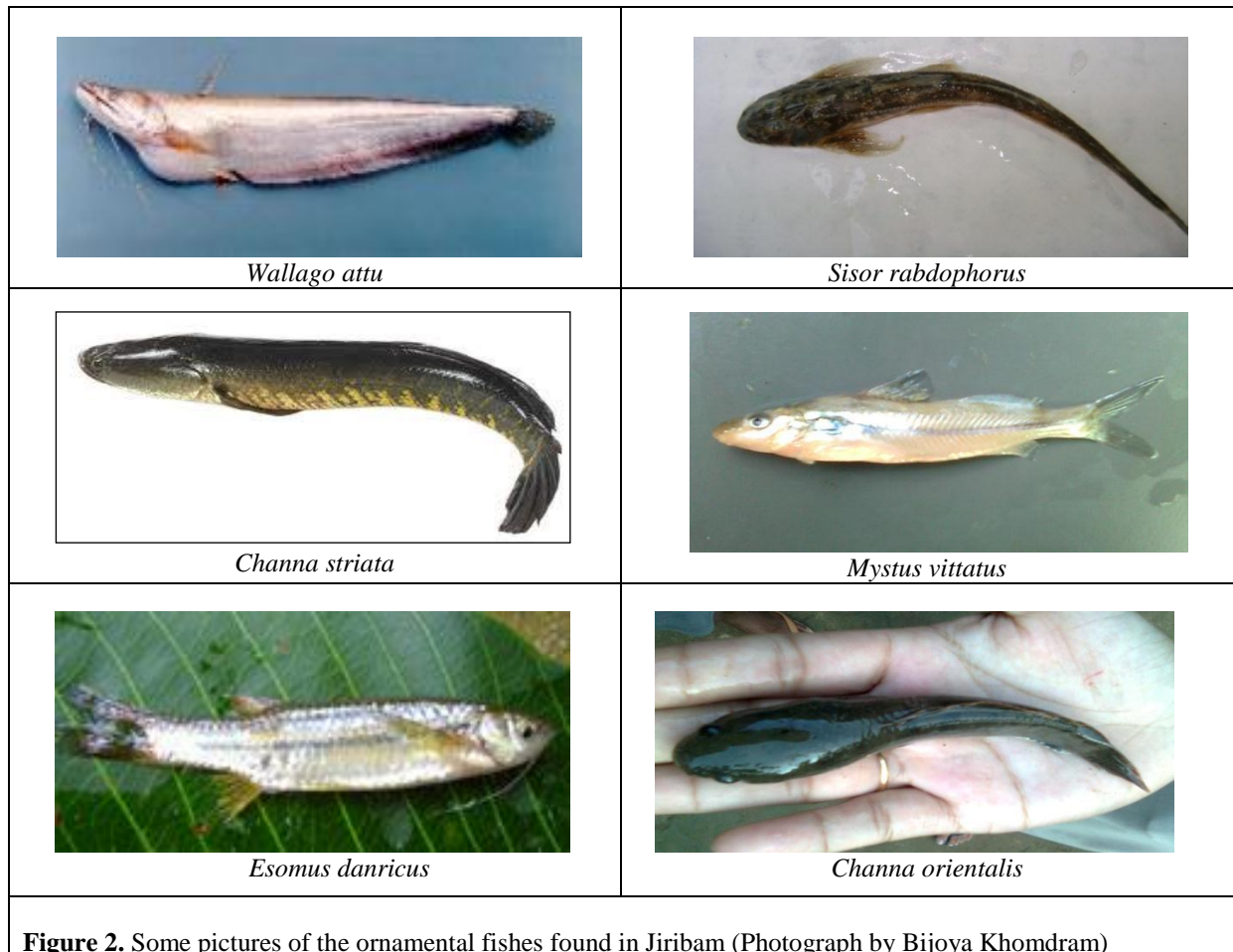


Figure 1. Map of India, Manipur, showing Jiribam, the study area (www.mapsofindia.com).



EXPLORING ORNAMENTAL FISHES OF MANIPUR THROUGH DNA BARCODING

Bijoya Khomdram Devi, Bishal Dhar, Boni Amin Laskar and Sankar Kumar Ghosh*

DNA barcode and Genomics Laboratory, Department of Biotechnology, Assam
University, Silchar-788011, Assam, India

ABSTRACT

DNA barcoding is an effective technique of species identification. The large reference library of DNA barcode should be enriched from every region and may be assessed together with morphological taxonomy for species authentication. In this study, a total of 27 fish samples belonging to 21 different species having ornamental relevance were collected and morphologically identified from Manipur, Northeast India. DNA was isolated from muscle tissue of samples and then COI barcode region was amplified using universal fish primers, sequenced and submitted to NCBI (Accession nos. JQ713844 - JQ713860). The sample sequences were identified through similarity search in two databases: GenBank and BOLD. Both the databases revealed definitive identity matches in the range of 98.37% - 100% for 44% of the species. This indicated that the database still lacks reference data of ornamental fishes of Manipur and this study contributed new barcode sequences of ornamental fishes.

Key words : *Ornamental fish, Manipur, DNA barcoding, COI, sequence homology*

INTRODUCTION

Ornamental fishes usually signify attractive colorful fishes culturable in an aquarium or in a garden pool for fun and fancy. The increasing demand for aquarium fishes gradually paved the avenue towards global trade of ornamental fishes. According to the international trade in tropical aquarium fish, global market of ornamental is about US \$600 million at wholesale level. Indian waters possess a rich diversity of attractive fish, with over 100 indigenous varieties and about 80% of ornamental fishes are from fresh waters (Ponniah and Sarkar, 2006). World trade of the fish is estimated to be about Rs. 2000 crores, but India's share is only Rs. 15 crores, which is very insignificant. The major shares of the country's ornamental fish export are captive collection from Northeastern states comprising about 85% of the total aquarium fish trade of India (Ponniah and Sarkar, 2006). Due to the diversity of topographic and climatic features of Manipur of North-east India, this region is rich in endemic fish. Most of the small food fish (139

* Department of Biotechnology, Assam University, Silchar-788011, Assam, India, Telephone No: +919435372338, Fax: +91 3842270802, Email ID: drsankarghosh@gmail.com

species) are though treated as unwanted for conventional farming but they have good potency as ornamental fishes (Mahapatra *et al.*, 2007).

DNA barcoding has been demonstrated as an effective fish identification tool for consumer protection and fishery's management/conservation (Hebert *et al.*, 2003). Steinke *et al.* (2009) have effectively demonstrated application of this technique for the trade in marine ornamental fish species. The goal of DNA barcoding is to distinguish the majority of world species by using DNA barcode sequence and to produce a large scale reference sequence library of life on the earth. Several studies of Fish Barcode of Life initiative have generated a huge number of reference DNA barcode sequences from taxonomically authenticated fish species (Ratnasingham and Hebert, 2007). Therefore, an approach of similarity search with reference database would be of great potential to identify species for preparation of checklist.

MATERIALS AND METHODS

Fishes were collected randomly from different parts of Manipur, preserved in 10% formalin (whole body preservation) and deposited as vouchers in the Molecular Biology Laboratory, Department of Biotechnology, Assam University, Silchar, Assam. Tissue samples from each specimen were collected fresh and preserved either in TES buffer or in absolute ethanol and kept in 4° C. Total DNA of each sample was extracted and amplified for DNA barcode region of mtDNA according to the method described in Bhattacharjee *et al.* (2012). The Open Reading Frame (ORF) for COI was checked, and correct amino acid sequences were determined by online software ORF prediction (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>). There was no evidence of indels or stop codons in all the sample sequences which might signal the amplification of a Numt. The aligned sequences were corrected manually using BioEdit program (Hall, 1999). GenBank and BOLD database were searched using megablast during November-December 2012. In most of the cases, the search corresponded to the sequence with the high BLAST score. In other cases, the closest match was a shorter target with a higher percent identity. Ambiguous bases in target sequence were considered as matching. A similar procedure was followed for BOLD searches (BOLD Identification System).

RESULTS AND DISCUSSION

All the sample sequences were in the range of 569 to 655 bp, and 98% of the read lengths were greater than 600 bp. The similarity search result thereby confirmed definitive identity showing significant species specific match in BOLD of 100% for consensus sequences of five species (*viz.*, *Barbonymus gonionotus*, *Labeo bata*, *Labeo calbasu*, *Puntius sophore* and *Wallago attu*). These sequences also showed 99% - 100% match in Genbank with similar named species. Further, four more sequences showed 99% similarity with four named species in BOLD (*Labeo gonius*, *Channa punctata*, *Mystus vittatus* and *Sisor raddophorus*) and showed 99% - 100% similarity with the same named species in Genbank. Five more sequences showed similarity in the range of 85% - 99% with same named sequences (*Lepidocephalus guntea*, *Channa striata* and *Amblyceps mangois*) of both BOLD and NCBI. Thus, the species identification search using the 2 databases, *i.e.*,

Table 1. List of ornamental fish species collected, with their local names in Manipuri and locations (by GPS)

Species	Common name in Manipuri	IUCN status	Ornamental relevance	Order	Habitat	Location (generated through GPS)
<i>Cirrhinus mrigala</i>	Mirga	LRnt	Classified	Cypriniformes	Jiri river	24° 48' 28.49" N, 93° 06' 38.99" E
<i>Barbonymus gonionotus</i>	Phabou achoolba	LC	Unclassified	Cypriniformes	Jiri river	24° 48' 0" N, 93° 7' 12" E
<i>Labeo bata</i>	Ngaton	LRnt	Classified	Cypriniformes	Jiri river	24° 48' 0" N, 93° 7' 12" E
<i>Glossogobius giuris</i>	Ngamu sengum	VU	Classified	Cypriniformes	Jiri river	24° 48' 28.49" N, 93° 06' 38.99" E
<i>Esomus danricus</i>	Ngasang	LRlc	Classified	Cypriniformes	Sana khong	24° 48' 09.64" N, 93° 06' 39.77" E
<i>Rasbora daniconius</i>	Ngasang macha	LC	Classified	Cypriniformes	Sana khong	24° 48' 09.64" N, 93° 06' 39.77" E
<i>Labeo calbasu</i>	Ngathi	LRnt	Classified	Cypriniformes	Jiri river	24° 48' 0" N, 93° 7' 12" E
<i>Labeo gonius</i>	Khabak	LC	Classified	Cypriniformes	Jiri river	24° 48' 26.18" N, 93° 07' 20.70" E
<i>Puntius chola</i>	Phabou	LC	Unclassified	Cypriniformes	Sana khong	24° 48' 09.64" N, 93° 06' 39.77" E
<i>Puntius sophore</i>	Phabou	LC	Unclassified	Cypriniformes	Sana khong	24° 48' 09.64" N, 93° 06' 39.77" E
<i>Lepidocephalus guntea</i>	Ngakijou	LC	Classified	Cypriniformes	Jiri river	24° 48' 26.18" N, 93° 07' 20.70" E
<i>Labeo boga</i>	Ngaton	LC	Unclassified	Cypriniformes	Jiri river	24° 48' 0" N, 93° 7' 12" E
<i>Wallago attu</i>	Sareng	LRnt	Classified	Siluriformes	Sanakhong	24° 48' 09.64" N, 93° 06' 39.77" E
<i>Sisor rabdophorus</i>	Hangoi nga	EN	Classified	Siluriformes	Jiri river	24° 48' 0" N, 93° 7' 12" E
<i>Amblyceps mangois</i>	Ngachik macha	LC	Classified	Siluriformes	Sanakhong	24° 48' 09.64" N, 93° 06' 39.77" E
<i>Mystus vittatus</i>	Ngasep	LC	Classified	Siluriformes	Jiri river	24° 48' 28.49" N, 93° 06' 38.99" E
<i>Channa striata</i>	Porom	LRnt	Classified	Perciformes	Sanakhong	24° 48' 0" N, 93° 7' 12" E
<i>Channa punctate</i>	Ngamu	LRnt	Classified	Perciformes	Sanakhong	24° 48' 0" N, 93° 7' 12" E
<i>Channa orientalis</i>	Meitei ngamu	VU	Classified	Perciformes	Sanakhong	24° 48' 0" N, 93° 7' 12" E
<i>Badis badis</i>	Napet nga	VU	Classified	Perciformes	Jiri river	24° 48' 28.49" N, 93° 06' 38.99" E
<i>Nandus nandus</i>	Kharraobi	LC	Classified	Perciformes	Jiri river	24° 48' 26.18" N, 93° 07' 20.70" E

Note : A total of 21 species which are being used in aquariums, of which only 17 species are classified as ornamental fishes and the remaining 4 species have the potential to be used as ornamental fishes

Table 2. Sample identification results (homology comparison), based on GenBank and BOLD

Sl. No.	Sample/Accession number	Homology (%) in NCBI	Homology (%) in BOLD	Remarks
1.	FO2B(JQ713844)	99% with <i>Puntius sophore</i>	100% with <i>Puntius sophore</i>	AA
2.	BF6(JQ713845)	87% with <i>Polycentropis abbreviate</i>	99.33% with <i>Nandus nandus</i>	AB
3.	BF8(JQ713846)	100% with <i>Barbonymus gonionotus</i>	100% with <i>Barbonymus gonionotus</i>	AA
4.	DF1(JQ713847)	100% with <i>Labeo bata</i>	100% with <i>Labeo bata</i>	AA
5.	DF2(JQ713848)	100% with <i>Labeo calbasu</i>	100% with <i>Labeo calbasu</i>	AA
6.	DF3(JQ713849)	99% with <i>Labeo gonius</i>	99.13% with <i>Labeo gonius</i>	AA
7.	AUFO37(JQ713850)	100% with <i>Wallago attu</i>	100% with <i>Wallago attu</i>	AA
8.	AUJF39(JQ713851)	99% with <i>Labeo bata</i>	100% with <i>Labeo boga</i>	AB
9.	AUFO5(JQ713852)	95% with <i>Puntius brevis</i>	99.38% with <i>Puntius chola</i>	AB
10.	AUFO7(JQ713853)	100% with <i>Wallago attu</i>	100% with <i>Wallago attu</i>	AA
11.	AUFO27(JQ713854)	100% with <i>Sisor rabdophorus</i>	99.84% with <i>Sisor rabdophorus</i>	AA
12.	FO3C(JQ713855)	99% with <i>Mystus vittatus</i>	99.66% with <i>Mystus vittatus</i>	AA
13.	OF4(JQ713856)	99% with <i>Puntius sophore</i>	100% with <i>Puntius sophore</i>	AA
14.	OF16F(JQ713857)	93% with <i>Parapercis ommatura</i>	99.17% with <i>Glossogobius giuris</i>	AB
15.	OF19F	93% with <i>Lepidocephalus guntea</i>	unable to match any records	BB
16.	OF26F	93% with <i>Lepidocephalus guntea</i>	83.87% with <i>Lepidocephalus guntea</i>	BB
17.	OF29F(JQ713858)	98% with <i>Badis badis</i>	unable to match any records	AB
18.	OF31F(JQ713859)	99% with <i>Amblyceps mangois</i>	98.37% with <i>Amblyceps mangois</i>	AA
19.	AUFO17	92% with <i>Lepidocephalus guntea</i>	83.95% with <i>Lepidocephalus guntea</i>	BB
20.	AUFO12	93% with <i>Lepidocephalus guntea</i>	84.04% with <i>Lepidocephalus guntea</i>	BB
21.	AUFO35	99% with <i>Cirrhinus mrigala</i>	99.83% with <i>Cirrhinus cirrhosus</i>	AA
22.	BF1	100% with <i>Channa striata</i>	96.39% with <i>Channa striata</i>	AA
23.	BF2	100% with <i>Channa punctate</i>	99.23% with <i>Channa punctata</i>	AA
24.	BF3	100% with <i>Channa orientalis</i>	97.24% with <i>Channa harcourtbutleri</i>	AB
25.	FO1A	100% with <i>Channa punctate</i>	99.19% with <i>Channa punctata</i>	AA
26.	AUECO1	100% with <i>Rasbora daniconius</i>	97.54% with <i>Rasbora cf. danidia</i>	AB
27.	AUECO2	100% with <i>Esomus danricus</i>	unable to match any records	AB

Note : AA (confirmed by both databases), AB (results of the two databases do not match), BB (% similarity > 95%). Some sequences are unable to match with the database in BOLD

GenBank and BOLD, a large number of samples (12 species) were perfectly matched (98.37% - 100%) in both GenBank and BOLD. All these sequences thus showed definite identity and were assigned the respective species name. Six more sequences matched to different species names in the two databases. AUFO5 (*Puntius chola*) has 99.38% homology with *Puntius chola* in BOLD but it has 95% homology with *Puntius brevis* in GenBank. So, we consider our sample as *Puntius chola*. OF19F which matched 93% with *Lepidocephalus guntea* in GenBank was unable to match with any records in BOLD. Likewise, other 2 samples, OF29F and AUECO2 which matched 98% with *Badis badis* and 100% with *Esomus danricus* in NCBI (GenBank) respectively were unable to match with any records in BOLD. Another sequence BF3 showed 100% match with *Channa orientalis* in BOLD while it showed 97% match with *Channa harcourtbutleri* in GenBank. Similarly, AUFO35 (Accession number JQ713860) showed 99% match with *Cirrhinus cirrhosis* in BOLD and *Cirrhinus mrigala* in GenBank. Thus, the identity of these sequences is still in question and further samples of similar morphological symmetry have to be included to clarify the doubt.

The present study is the first of its kind being conducted to explore ornamental fishes of Northeastern India using DNA barcode technique. All the previous studies (Vishwanath *et al.*, 1998; Vishwanath and Laishram, 2004; Vishwanath and Shanta Kumar, 2005) on ornamental fishes were mainly based on morphology, habits, etc. Our current study will result in significant improvement of our knowledge concerning the systematics of the ornamental fish species found in Northeastern India, to facilitate monitoring changes in the geographic distribution of species that will probably occur in the future and ultimately to generate a DNA barcode library for all the ornamental fishes found in this region and hence making easy access to species identification.

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REVEALING GENETIC DIVERSITY OF *CLARIAS BATRACHUS* USING DNA BARCODE

Mohua Chakraborty, S.K.Ghosh,* Bishal Dhar and Bijoya Khomdram Devi
DNA barcode and Genomics Laboratory, Department of Biotechnology, Assam
University, Silchar-788011, Assam, India

ABSTRACT

Clarias batrachus is a species of freshwater catfish widely used for human consumption. Increasing demand of this species worldwide, coupled with its ability to survive in wide range of environmental conditions, has led to its introduction in many countries. This widespread translocation and distribution of *C. batrachus* have resulted in the rise of wide variety of haplotypes of this species. Nevertheless, there has been constant decrement in their population density in the last two decades. Thus, to endeavour conservation of the species we employ molecular technique of DNA barcoding in solving the standing problem of crypticism and haplotype sharing of the species. A better knowhow of the genetic makeup of the unique identifier region, that is, the 648 basepair region of COI DNA barcode will help to differentiate among closely related species and identify endemic species. In this study, a comparative analysis of *C. batrachus* from different regions in India and other parts of world shows presence of distinct haplotypes in different geographical locations. We also present a descriptive study of the various species of *Clarias* genus that have been barcoded in India till date. Our results also solve the dilemma of considering some species as synonymy of *C. batrachus*.

Key words : *Clarias batrachus*, cytochrome c oxidase subunit I (COI), genetic diversity, Kimura's two parameter (K2P), mean divergence, neighbour joining tree.

INTRODUCTION

The walking catfish, *Clarias batrachus*, is a species of freshwater air breathing catfish so named for its ability to "walk" across dry land, to find food or suitable environments. They normally inhabit in swamps, marshy and derelict waters (Lakra and Sarkar, 2007). The walking catfish is a native of South East Asia including Malaysia, Thailand, eastern India, Sri Lanka, Bangladesh, Burma, Indonesia, Singapore and Brunei (Kuang, 1986; Shrestha, 1978) and is also found in Philippines. Owing to its capability to adapt to wide range of environmental conditions, candidates of the genus *Clarias* has extensively travelled to many continents, adapting itself successfully and is now found throughout Asia and Africa.

* Telephone No: +919435372338, Fax: +91 3842270802, Email ID: drsankarghosh@gmail.com

Such introduction of new population of *C. batrachus* in addition to its existing wildtype species has resulted in enriching the ecosystem with wide variety of haplotypes of *C. batrachus*. However, their population has decreased significantly during the last two decades, owing mainly to imprudent development and reckless fishing. Conservation of this species is therefore the need of the hour. But, despite centuries of taxonomic inquiry, problems inherent to species identification continue to hamper the conservation of this species as *C. batrachus* for a long period of time (Ng and Hadiaty, 2011; Talwar and Jhingran, 1991). Many such populations of *C. batrachus* were originally identified as distinct species but later they were amalgamated with *C. batrachus* or considered as synonym of this species (Day, 1958; Talwar and Jhingran, 1991), though sufficient molecular data supporting this debate is lacking. There is also a significant lag in understanding the genetic diversity of the species. In such scenario, introduction of this species in a new environment may severely affect the existing population. Therefore, there is a need to understand the genetic composition of natural population of *C. batrachus* using molecular techniques to evaluate the latent genetic effects induced by hatchery operations. Among molecular methods, DNA barcoding has served as an effective tool in solving many distorted views of biodiversity (Hebert and Cywinska, 2003a; Hebert *et al.*, 2003b). Indeed, DNA barcoding surveys, using partial cytochrome c oxidase subunit I (COI) sequences, have revealed cryptic diversity across the animal kingdom (Dudgeon *et al.*, 2012; Janzen *et al.*, 2011; Mat Jaafar *et al.*, 2012; Puckridge *et al.*, 2012). For instance, previous DNA barcoding studies in other taxonomic groups have found as many as nine undescribed species embedded within a single known species of skipper butterfly (Hebert and Penton, 2004). Ward *et al.* (2005) had sequenced (barcoded) 655 bp region of the mitochondrial COI of 207 species of fish and recommended that COI barcode of all fish species could be generated from the same primer and all fish species could be differentiated by their COI sequence. Bucklin *et al.* (2011) calculated an average retrieval of 2 % new species in larger fish DNA barcoding studies, and they extrapolated this rate to about 600 overlooked or cryptic species to await discovery through similar studies. From the 31,000 species currently listed in the Catalog of Fishes, about 4000 have been described as new during the past 10 years (2000–2009), with 500 added in 2008 and 300 in 2009 (Eschmeyer *et al.*, 2012).

Thus, DNA barcode can be used as an effective tool to understand the genetic diversity of *C. batrachus*. In this study, we evaluate genetic diversity of Indian *C. batrachus* using mitochondrial cytochrome c oxidase subunit I (COI) sequences as DNA barcodes. Using distance based methods we attempt to resolve some of the key areas of discrepancies underlying the current taxonomic classification of this species. Moreover, our study will help to identify geographically isolated population of *C. batrachus*, which will further help in sustainable regulation of fishing practice of this economically important species.

MATERIAL AND METHODS

Data acquisition

A total of 50 COI barcode sequences of *C. batrachus* were mined from BOLD and GenBank. Among them, 12 sequences of *C. batrachus* were retrieved from BOLD and 38 sequences from NCBI. The two sets of sequences were checked for redundancy and a collection of 38 sequences of *C. batrachus* was used for final analysis. Further, these

databases were mined for available sequences of other species of *Clarias* genus in Indian subcontinent and six *Clarias dussumieri* and five *Clarias gariepinus* were retrieved. Sequences were included provided they had length greater than 540 bp with no missing nucleotides or gaps. All sequences were aligned using ClustalX. Probable erroneous sequences (with highly unlikely positions or extreme branch lengths, based on a neighbour-joining tree calculated with all sequences) were identified and omitted. The COI coding DNA sequence alignments were guided by pre-aligned COI protein sequences using MEGA5 software.

Data analysis

Genetic distances were calculated to quantify sequence divergences among individuals using Kimura's two parameter (K2P) models (Kimura, 1980), as implemented in MEGA 5.1 (Tamura *et al.*, 2011). Interspecific K2P distances were calculated for those species with at least two sequences, and intraspecific K2P distances were calculated between species in the entire data set. Genetic distances were analysed at species level in MEGA 5.1. Neighbour joining analyses were also conducted independently for COI using K2P distance model using MEGA 5.1. Node support was evaluated with 1000 bootstrap pseudoreplicates.

RESULTS AND DISCUSSION

We obtained 18 DNA barcode sequences of Indian *C. batrachus* deposited in NCBI, among which two sequences are also deposited in BOLD. These sequences belonged to specimens collected from different geographic regions in India. Of the 18 *C. batrachus* barcodes, five specimens belonged to Alibagh coast, Mumbai, Maharashtra and another four were from Mathabhanga, West Bengal. Further, two sequences were generated in our lab which were collected from Lala, Assam and these are the only barcode sequences of *C. batrachus* to be submitted in BOLD (Bhattacharjee *et al.*, 2012), because the remaining 7 sequences in NCBI geographic location is not specified. We also retrieved 20 other COI sequences of *C. batrachus* from different countries that have been barcoded till now (Table 1).

Distance analysis of COI barcode sequence based on K2P method of *C. batrachus* species from India showed large conspecific mean divergence (6.85 ± 0.76) % thus indicating either the presence of different haplotypes

of *C. batrachus* in India or presence of some mislabelled sequence. Geographically isolated populations of same species often show high genetic deviation. To explore the possibility of presence of distinct haplotypes of *C. batrachus*, the sequences were grouped according to different geographic locations (within India), from which they have been collected and

Table 1. Summary of *Clarias batrachus* sequences analysed

Name of species	Geographic location	NCBI	BOLD
<i>Clarias batrachus</i>	Thailand	13	0
<i>Clarias batrachus</i>	Philippines	5	4
<i>Clarias batrachus</i>	Vietnam	1	1
<i>Clarias batrachus</i>	India	18	2
<i>Clarias fuscus</i>	China	1	0
Unspecified		0	5
	Total	38	12

genetic variation within and between the groups were analysed (Table 2 and Table 3). We observed low conspecific mean genetic distance for *C. batrachus* species of a particular geographic location. Mean genetic distance of *C. batrachus* from Alibagh coast, Mumbai,

Table 2. Conspecific mean genetic divergence of different species of *Clarias* genus of different geographical locations

Species	Geographic location	Mean% Dist	S.E
<i>Clarias batrachus</i>	Thailand	0.12	0.08
<i>Clarias batrachus</i>	Philippines	0	0
<i>Clarias batrachus</i>	Vietnam	6.85	0.07
<i>Clarias batrachus</i>	India (all sequences)	N/A	N/A
<i>Clarias batrachus</i>	India (not available)	5.66	0.62
<i>Clarias batrachus</i>	India (Maharashtra ,Mumbai)	0.19	0.10
<i>Clarias batrachus</i>	India (Mathabhanga, West Bengal)	0.31	0.18
<i>Clarias batrachus</i>	India (Lala, Assam)	1.51	0.47
<i>Clarias dussumieri</i>	India (not available)	0.30	0.19
<i>Clarias gariepinus</i>	India (not available)	0.31	0.46

Table 3. Mean genetic distance between different samples of *Clarias* genus of different geographical locations

Sample 1	Sample 2	Mean% Dist	S. E.
<i>Clarias batrachus</i> India	<i>Clarias batrachus</i> Thailand	12.29	1.20
<i>Clarias batrachus</i> India	<i>Clarias batrachus</i> Philippines	11.37	1.16
<i>Clarias batrachus</i> India	<i>Clarias batrachus</i> Vietnam	8.95	1.04
<i>Clarias batrachus</i> Thailand	<i>Clarias batrachus</i> Vietnam	13.80	1.60
<i>Clarias batrachus</i> Philippines	<i>Clarias batrachus</i> Vietnam	12.33	1.52
<i>Clarias batrachus</i> Thailand	<i>Clarias batrachus</i> Philippines	2.51	0.63
<i>Clarias batrachus</i> India	<i>Clarias batrachus</i> India (Lala, Assam)	1.80	0.40
<i>Clarias batrachus</i> India	<i>Clarias batrachus</i> India (Mumbai, Maharashtra)	1.85	0.50
<i>Clarias batrachus</i> India (Lala, Assam)	<i>Clarias batrachus</i> India (Mumbai, Maharashtra)	0.93	0.30
<i>Clarias batrachus</i> India	<i>Clarias batrachus</i> India	12.69	1.50
<i>Clarias batrachus</i> India (Lala, Assam)	<i>Clarias batrachus</i> India	13.51	1.50
<i>Clarias batrachus</i> India (Mumbai, Maharashtra)	<i>Clarias batrachus</i> India	13.11	1.50
<i>Clarias batrachus</i> India	<i>Clarias batrachus</i> India (Mathabhanga, West Bengal)	12.92	1.50
<i>Clarias batrachus</i> India (Lala, Assam)	<i>Clarias batrachus</i> India (Mathabhanga, West Bengal)	13.63	1.50
<i>Clarias batrachus</i> India (Mumbai, Maharashtra)	<i>Clarias batrachus</i> India (Mathabhanga, West Bengal)	13.34	1.51
<i>Clarias batrachus</i> India	<i>Clarias batrachus</i> India (Mathabhanga, West Bengal)	0.87	0.30

Maharashtra, Mathabhanga, West Bengal and Lala, Assam were found to be 0.5%, 0.31% and 1.5% respectively, while remaining 7 individuals for which no location has been specified, show an overall mean of 0.15%. The mean distance between populations of Lala, Assam and Mathabhanga, West Bengal were found to be as high as 13.639 %. Many other interpopulation divergences also showed similar high range of conspecific divergence that lies in the range of congeneric divergence. However, not all populations exhibited high divergence, e.g., species from Lala, Assam and Alibagh coast, Maharashtra shared narrow divergence of 0.934%, thereby indicating that geographic isolation can be partly but not solely responsible for the observed high genetic divergence within Indian *C. batrachus* species.

Altogether, six sequences of Indian *C. batrachus* cluster separately from all other sequences of *C. batrachus* and diverge from the other Indian *C. batrachus* sequences by $13.21 \pm 1.25\%$. Moreover, with exclusion of these six sequences, conspecific divergence of remaining Indian *C. batrachus* sequences lowers to $1.21 \pm 0.25\%$. This indicates the presence of cryptic species diversity within Indian *C. batrachus* and that the six sequences might represent a different species. In previous taxonomic classification, many species showed conflict with *C. batrachus* and some are considered to be synonyms (Ferraris, 2007) like *C. assamensis*, *C. punctatus*, *C. marpus*, *C. magur*, *C. fuscus*, etc. (Day, 2011). Among these species many were traditionally found in India and some were introduced later (Talwar and Jhingran, 1991). Thus, barcode analysis indicates that discrepancy in conspecific mean divergence of Indian *C. batrachus* may be due to the presence of one or more of these species wrongly identified as *C. batrachus*. A comparison of DNA barcodes of the aberrant *C. batrachus* with barcode sequence of all other representative species of *Clarias* genus will verify whether these sequences belong to some previously described species or represent a latent or different species. However, *C. dussumieri* (six sequences in NCBI, 0 in BOLD) and *C. gariepinus* (five sequences in NCBI, 0 in BOLD) are the only species of *Clarias* genus other than *C. batrachus* for which barcode sequences are available from India. These two species cluster distinctly from both the clusters of *C. batrachus* and show divergence from *C. batrachus* in the range of congeneric divergence, thus validating that the aberrant sequences of *C. batrachus* is not any of the two barcoded species of *C. batrachus*. Further, taxonomic investigation and inclusion of more samples along with a comprehensive barcoding of all species of *Clarias* genus is required to resolve this issue.

Comparing barcodes of *C. batrachus* of different countries it reveals that populations of different countries also show high divergence between them (Table 3). *C. batrachus* sequences from Thailand and Philippines showed low conspecific divergence of 0 % and $0.121 \pm .08\%$ and formed single cohesive cluster in Neighbour joining tree. Further, sequences of the two countries form two distinct clusters (Fig. 1) with divergence between them being 2.5% (Table 3). This indicates that sequences of *C. batrachus* from these two countries represent unique haplotype specific for each country. However, narrow divergence in Thailand and Philippines (Table 2) is not conclusive of the diversity of *C. batrachus* in these countries as small sample size and inadequate sampling within these countries may result in the present observation. Barcode of a synonym species of *C. batrachus* from China of the name *C. fuscus* was retrieved from GenBank, and it was found to cluster closely with *C. batrachus* from Philippines (Fig 1) in the NJ tree with 0 % congeneric distance. This

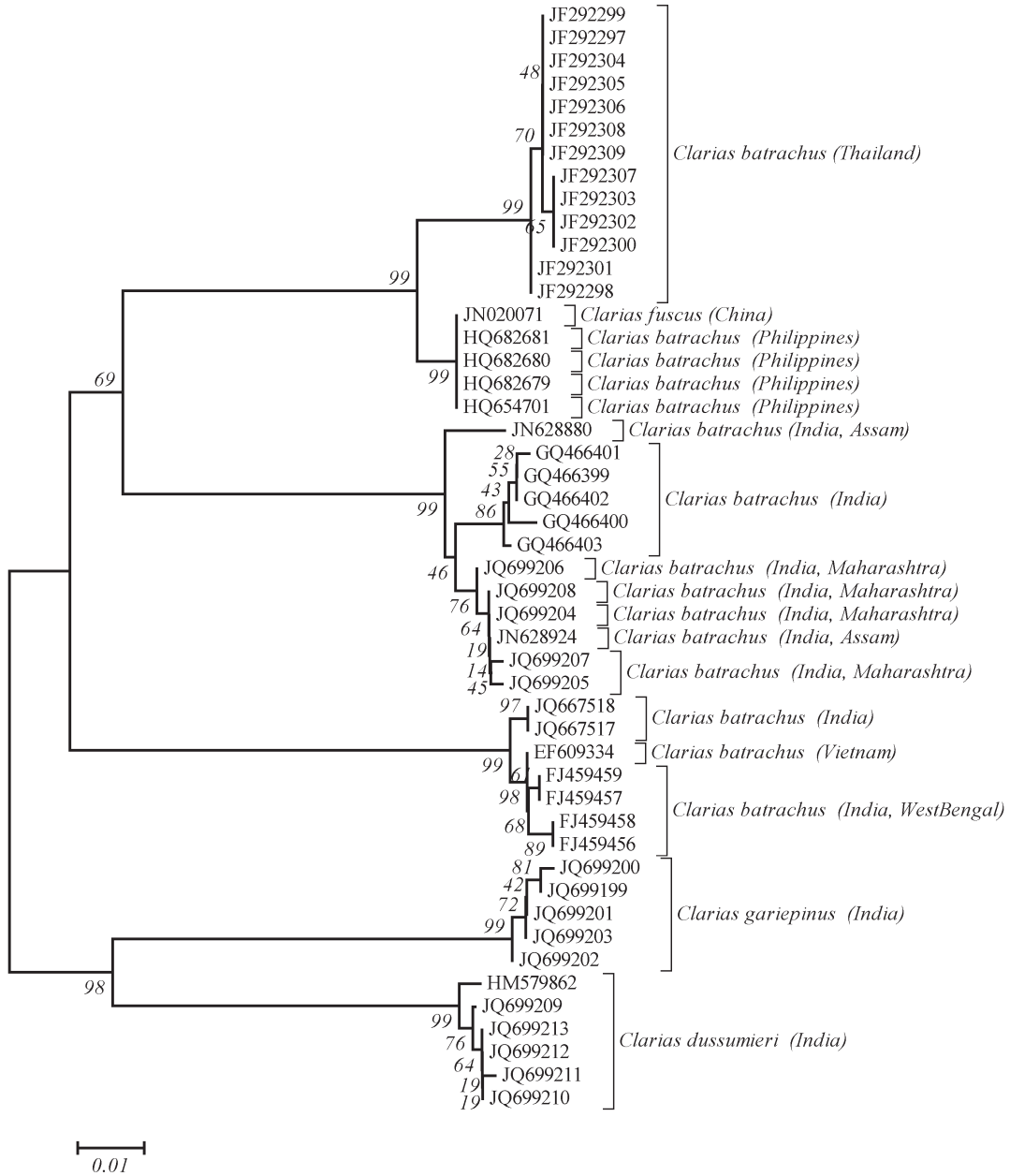


Fig 1 Neighbour joining tree (based on K2P parameter) representing clustering of Indian *Clarias batrachus* species with *C. batrachus* species of different countries and other species of *Clarias* genus barcoded in India till date. *Regional information within a country (if available) are included in parentheses.

supports the contention that *C. fuscus* is a synonymy of *C. batrachus*. Nevertheless, inclusion of more sequences of individuals sharing morphological symmetry with the specimen of *C. fuscus* is required to validate that these two species are synonymy and thus the two species can be amalgamated into single named species.

Further, even after exclusion of the six aberrant sequences of Indian *C. batrachus*, conspecific divergence of Indian *C. batrachus* sequences with the same named species of other countries shows high mean divergence of $10.51 \pm 1.25\%$. Similarly as observed above, all other sequences of *C. batrachus* show high conspecific mean divergence between populations of different countries. These observations suggest presence of unique haplotypes of *C. batrachus* with high genetic divergence between populations of different countries. This may be the result of high rate of introduction of *C. batrachus* species throughout the world, which have resulted in enriching the ecosystem with wide variety of haplotypes of *C. batrachus*.

CONCLUSION

This study reveals the presence of high genetic diversity of *C. batrachus* species across the world. Species of *C. batrachus* shows high range of divergence between different countries, which is easily traceable by COI barcodes. However, there is a significant lack of proper sampling of barcode sequences of all recorded populations of this economically important species in India and other countries which when done will definitely resolve the existing dilemma in this species. This is essential for proper regulation of import, export and introduction of a population to a new environment thereby ensuring sustainable fishing practice.

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