Decoding Evolution of Native Fishes in Garhwal Himalaya using Molecular Markers and DNA Barcoding

Madhu Thapliyal, Bipin Sati, Ashish Thapliyal, K. K. Joshi

Abstract: As we are moving forward into the modern era of science, several new technologies have revolutionized various branches of science. Techniques of biodiversity conservation, fish biology etc. has also adapted to modern techniques. For a long time, most of the researches in taxonomy, including fisheries science were based on morphology and traditional methods. After the decade of 90’s, slowly several molecular markers like RFLP, RAPD, SNP’s etc. made inroad into taxonomy and fisheries. Molecular markers have several applications in the field of livestock improvement and understanding population dynamics to name a few. Since the 2004, a specific molecular marker, generally known as DNA Bar-coding for species identification, came up. This molecular marker is a part of mitochondrial genome that encodes for Cytochrome C Oxidase Unit I (also called as COX or COI). It is advantageous because it has been tested across several animal species and it can differentiate species very well. This marker has also been used as a forensic tool to identify the species. In the current paper, we have used this molecular marker to decode evolution of native fishes of Garhwal Himalayan region. Over 350 barcodes were developed and these barcodes were used to for phylogenetic analysis.

Key words- Molecular Markers, DNA Bar-coding, Evolution, Himalaya, Breeding, livestock

I. INTRODUCTION

Himalaya has diverse and extensive network of fresh water rivers, streams, lakes etc. All these fresh water bodies harbor diverse aquatic fauna with fishes being the most extensively studied. There are many fish species reported by many authors in Uttarakhand. It is suggested that the native fish species of Himalayan region might be one of the earliest inhabitant of these fresh water systems and hence they are a good model to study evolution unfolding. These fishes have been well documented. However, prior to the year 2000, most of the studies were based on morphological characters and books like “Day Fauna” were served as the “KEY” for identification of fishes. All these so called “KEYS” were extensive illustrations of each species. About 2500 species of fishes have been reported in India and approximately 930 of these are fresh water fishes. The Himalayan region of India harbor’s about 225 of these fresh water fishes. Various researchers have reported up to 50 different fish species from Garhwal Himalayan region.

In Uttarakhand, most of the fresh water fishery resources are contributed by the River Yamuna or River Ganges. Fishes of Rivers Ganges (and is tributaries) has been well documented by fishes of River Yamuna in Garhwal Himalaya have not been well known except some contributions. There are numerous morphological based studies but there are only few report investigating fish species using molecular markers. Molecular markers are also being used used for assessing biodiversity using environmental DNA and meta-genomics (Krehenwinkel et. al., 2019; Adams et. al., 2019; Xing et. al. 2020). In about last three decades, the scenario of most of the Himalayan region as changed due to fast changing ecology of upland waters. Impact of anthropogenic activity on genome of fish species is among the interest in Himalayan region. The genetic variation in Himalayan region due to the regular floods in rivers and Dam constructions still not reported. Attempts have also been made to generate the DNA barcode & Population genetics of fishes, but most of the attempts are limited to major rivers i.e. the Ganges and the Yamuna (Thapliyal et al., 2013). The molecular markers based on DNA are helpful to provide evolutionary relationship among different populations and cryptic species identification. The current paper is an attempt to investigate these changes using molecular markers specially focusing on DNA barcoding.

II. WHY MOLECULAR MARKERS

During several studies on morphological characters, research encountered a dilemma. There were several individuals which looked alike or had only small variations. A good example is that of Schizothorax species. It needs an expert to identify (ID) the two species of Schizothorax namely S. progastus and S. richardsonii and even after identification there could be queries about the ID. This happens in several species that they look alike but they are actually different species genetically. The latest example is of Giraffe (Petzold&Hassanin, 2020). To solve this issue, molecular markers emerged and as they are specific sequences of DNA, these studies when coupled with morphometric studies were considered better option for species level identification. Introduction of molecular biology techniques in fisheries had a huge impact on the entire fishery research. Through application of these techniques we can figure out the variations in specific regions of genome. We can also develop a marker for desired characters and identify species based on DNA Barcode which is somewhat similar or just like a product barcode.

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III. MOLECULAR MARKERS

Molecular marker is a specific sequence located on a specific gene. During the process of new progeny formation, inheritance of specific character occurs and these molecular markers stay together with the desired character. Hence the name molecular markers as the desired character can be followed by just following the molecular marker sequence. The interest in the DNA sequence based molecular markers had started as soon as the DNA model was presented by Watson and Crick and this model was awarded a Nobel Prize. This was followed by a rapid development of new technologies and methods like Polymerase Chain Reaction and DNA Sequencing. Once the sequencing of genome started, it opened an entire new ear of molecular markers and even a change in single nucleotide in a gene could be followed – technically called as Single Nucleotide Polymorphism.

IV. WHY MITOCHONDRIAL MARKER:

Besides the nuclear DNA, the eukaryotic mitochondria also has an extra DNA. This mitochondrial genome codes for 37 genes (two rRNAs, 22 tRNAs and 13 polypeptides).Mitochondrial DNA is of interest because of its unique features. These features are: a) This DNA is maternally inherited, b) it is a haploid molecule, c) there is no recombination process, d) there is no repair mechanism during DNA replication process e) there are several mitochondria in a cell and so it can be isolated and targeted easily, f) there are no introns in mitochondrial genome and g) mitochondrial genome is not too big and the optimum size makes it a favorite h) The COI marker can be used as a universal marker across entire animal species.The COI marker was first reported by Dr. Paul Hebert from University of Guelph, Canada as a molecular marker that can be used effectively to develop molecular database based catalogue of various animals inhabiting different regions (Hebert et al., 2003; Hebert et al., 2004). The sequences of these markers can also be compared using different available software’s of sequence alignment and analysis.

V. STUDIES IN GARHWAL HIMALAYAN REGION OF UTTARAKHAND :-

As Garhwal Himalayan region is the origin point of River Ganges and River Yamuna, it is important to understand the evolutionary context of fauna inhabiting these river. Attempts have been made to generate the DNA barcode & study population genetics of fishes in these rivers. Besides these river, there are several small tributaries that also inhabited by many species. In the entire study – one of the molecular marker called as COI – Cytochrome C Oxidase Unit I has been used. A specific region of this gene is sequenced and the bases are represented as colour codes and hence the name DNA Barcode (Fig. 1).

Example: GenBank Accession numbers **NCBI ID JN965201**

![Fig 1. The concept of DNA Barcode. A sequence is converted to a barcode.](Image)
Table 1 – Molecular markers and their description

| S.No. | Marker                        | Details                                                                                                                                 |
|-------|-------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|
| 1.    | **RFLP** (Restriction Fragment Length Polymorphism) | In this method, one or more restriction enzyme(s) are used to cut a DNA isolated from the desired samples. The DNA digested by these restriction enzymes is then run on a gel which gives a unique banding pattern. These patterns are used in an analysis called as RFLP (Restriction Fragment Length Polymorphism). RFLP analysis is used in population studies (Ferguson et al., 1995). |
| 2.    | **RAPD** (Random Amplification of Polymorphic DNA) | RAPD technique is a PCR based technique. Several primers are used on the same DNA sample and then the amplified regions profile is developed. (Hadrys et al., 1992). |
| 3.    | Dloop region                   | There is a region in mitochondrial region which is a non-coding region. This region is called as The D-loop region. Variations of this region are mapped in case of studies using D-loop as a marker. |
| 4.    | **VNTRs** (Variable number tandem repeats) | When the eukaryote genome was analyzed, it was surprising to note that there were several unique segments of sequences that were repeated several times (from 10 to 100 or more, O'Reilly and Wright, 1995). These repeated units can be of two types – first one called as mini-satellite DNA (9–65 bp long), and second called as microsatellite DNA (4–8 bp long). (Magoulas, 1998). |
| 5.    | COI                           | The mitochondrial genome COI gene is an approximate 656 bp region. The gene encodes part of the terminal enzyme of the respiratory chain of mitochondria. |
| 6.    | Cytocrome b                    | Cytocrome b is a component of electron transport gene and is used in some studies. This gene is too long (about 1,140 bp) and sometimes the DNA sequencing data of longer genes is cumbersome to handle. |
| 7.    | 16s rRNA                      | 16S rRNA gene has been used extensively for bacterial identification. |
| 8.    | ATPase 6/8                     | A region of mitochondrial ATPase gene                                                                                                   |

Table 2. Some of research papers in the Uttarakhand region on molecular marker.

| Species                                      | References                  | Marker used          | Year | Place/Area |
|----------------------------------------------|-----------------------------|----------------------|------|------------|
| Dawkinsia Tambraparniei                       | karuppiahkannan             | Cytocrome b          | 2014 | Uttarakhand|
| Labeogonius                                   | Grishma Tewari.             | RAPD                 | 2013 | Uttarakhand|
| S. richardsonii, T. putitora, B. Bendelisis and G. Gotyla, Danio | Thapliyal M                | COI                  | 2013 | Uttarakhand|
| S. richardsonii, T. putitora, B. Bendelisis and G. Gotyla | Himani Pandey.             | 16SRNA               | 2013 | Uttarakhand|
| Barilius bendelisis                           | A. K. Mishra.               | RAPD                 | 2012 | Uttarakhand|
| S. richardsonii and S. progastus.             | Suresh Chandra.             | COI                  | 2012 | Uttarakhand|
| Golden Mahseer Tor putitora, Snow trout, Schizothorax richardsonii, Indian trout, Raimamus bola Garra, Garragotyla | G.K. Sivaraman.            | RAPD, 12S rRNA       | 2012 | Uttarakhand|
| Barilius bendelisis                           | Seema Sah.                  | cytocrome b          | 2011 | Uttarakhand|
| Schizothorax richardsonii                    | Ashoktaru Barat.           | cytocrome b          | 2011 | Uttarakhand|
| Eutropichthys vacha                          | Gyan Chandra.               | RAPD                 | 2010 | Uttarakhand|
| Tor putitora                                  | Mamta Singh.                | 45S and 5S           | 2009 | Uttarakhand|

VI. MATERIAL AND METHODS

Study site: The present study was a 200 kilometre radius of Garhwal Himalaya (30N; 78E approx.) Uttarakhand. Sampling sites included Bakot to Ponta Sahib in River Yamuna and from Bhatwari to Rishikesh in river Ganges.
The DNA was isolated from fish fin (Wizard Genomic DNA Purification Kit, Cat# A1120, Qiagen Integrity). The isolated DNAs was then checked on 1% Agarose gel and quantified (Nanodrop 1000 spectrophotometer, Applied bio system). Method used was as per Thapliyal et. al., 2013. In short, Samples were subjected to PCR using universal primers (FF2d (forward): TTCTCCACCAACCACARGAYATYGGFR, FF1d (reverse): CACCTCAAGGTGTCCGAARAAYCARAA) The thermal cycler program was initial Denaturation at 95°C for 5min followed by 35 cycles of 95°C for 30sec of Denaturation, 55°C of annealing for 30sec and 72°C of extension for 1min and final extension of 72°C for 7min and then the samples were stored at 4°C. The samples were then run at 1.5% of Agarose gel for their quality check. The sample showing one clear band after PCR samples were sorted and purified with EXO1-SAP (Exonuclease1 and Shrimp Alkaline Phosphatase: USB Corp) with the temperature conditions suggested by manufacturer. The purified PCR amplicons were then ladled with Big Dye Terminator v3.1 (Applied Bio systems) by cycle sequencing, with each side labelled separately. The cycle sequencing PCR reaction contained Ready reaction mix (2.5x) 0.5µL, Dilution Buffer 1.75µL, Template (200ug/µL)1µL, Primer (0.8pMol/µL). The cycle sequenced amp icons were then purified with Big Dye (R) X Terminator (TM)(Big Dye Terminator v3.1 clean up Applied Bio systems, USA) each side labelled separately and were sequences on ABI 3130 DNA genetic analyser.2µL, MQ Water4.75µL. The cycle sequencing conditions was Initial Denaturation of 960C for 1min followed by 35 cycles of Denaturation 960C for 10sec, annealing 500C for 5sec, extension 600C for 4min and then the samples were stored at storage temperature of 40C. The sequences were then obtained and analysed in the Sequence Scape software v2.7 for possibilities of indels(Applied Biosystems 3130 Genetic Analyzers).

VII. DATA ANALYSIS

DNA sequence were also submitted to Gene bank (accession numbers included in appendix online tools). MEGA program (XXX) was used for sequence alignment and further interpretation.

VIII. RESULTS

About 350 GenBank submissions have been made during the entire period of study starting from 2013. There were some interesting observations that are becoming evident from our study that the distribution pattern of species, especially the *Schizothorax* species, needs to be redefined based on molecular data. More data is also being added so that statistical validation can be carried out.Some of these submissions of various molecular markers are:
| S.No. | Name of the Species            | Voucher No | NCBI Accession No |
|-------|-------------------------------|------------|------------------|
| 1     | Bariliusbarna                 | RS05       | JN965191         |
| 2     | Bariliusbarna                 | GM01       | JN965190         |
| 3     | Bariliusbendelisis            | KR01       | JN965192         |
| 4     | Bariliusbendelisis            | KR07       | JN965196         |
| 5     | Bariliusbendelisis            | KR06       | JN965195         |
| 6     | Bariliusbendelisis            | KR02       | JN965194         |
| 7     | Bariliusbendelisis            | GM02       | JN965204         |
| 8     | Bariliusbendelisis            | KR04       | JN965212         |
| 9     | Bariliusbendelisis            | KR03       | JN965193         |
| 10    | Bariliustileo                 | GM07       | JN965198         |
| 11    | Bariliustileo                 | GM08       | JQ692874         |
| 12    | Chaguniuschagunio             | GM10       | JN965199         |
| 13    | Garragotyla                   | BS55       | JN965210         |
| 14    | Garragotyla                   | HD09       | JN965211         |
| 15    | Garragotyla                   | HD10       | KC473939         |
| 16    | Garragotylagotyla             | RS07       | JN965200         |
| 17    | Macrognathusspancalus         | BS123      | KC473940         |
| 18    | Puntiustrochomus              | KR10       | JN965201         |
| 19    | Puntiusticto                  | GM12       | JN965202         |
| 20    | Puntiusticto                  | GM11       | JN965203         |
| 21    | Schizothorax progestus        | UM01       | JN965205         |
| 22    | Schizothorax progestus        | UM02       | JQ692872         |
| 23    | Schizothorax progestus        | RS01       | JQ692870         |
| 24    | Schizothorax progestus        | HD08       | JQ692873         |
| 25    | Schizothorax sp.              | HD07       | JQ692871         |
| 26    | Tor chelynoides               | UM04       | JN965207         |
| 27    | Tor chelynoides               | RS04       | JN965206         |
| 28    | Tor putitora                  | GM05       | JN965209         |
| 29    | Tor putitora                  | UM05       | JN965197         |
| 30    | Tor sp.                       | BSS01      | KC473941         |
| 31    | Tor tor                       | BS153      | KC473942         |
| 32    | Tor tor                       | GM06       | JN965208         |
| 33    | Acanthocobitisbotia           | GPCR 281AB | KR809714         |
| 34    | Acanthocobitisbotia           | GPCR1AB    | KU043312         |
| 35    | Acanthocobitisbotia           | GPCR2AB    | KU043313         |
| 36    | Acanthocobitisbotia           | GPCR3AB    | KU043314         |
| 37    | Acanthocobitisbotia           | GPCR4AB    | KU043315         |
| 38    | Badisbadis                    | GPCR 141BB | KR809715         |
| 39    | Badisbadis                    | GPCR 282BB | KR809716         |
| 40    | Badisbadis                    | GPCR 284BB | KR809717         |
| 41    | Badisbadis                    | GPCR 287BB | KR809718         |
| 42    | Badisbadis                    | GPCR5BB    | KU043316         |
| 43    | Badisbadis                    | GPCR6BB    | KU043317         |
| 44    | Badisbadis                    | GPCR7BB    | KU043318         |
| 45    | Badisbadis                    | GPCR8BB    | KU043319         |
| 46    | Bariliusbarna                 | GPCR 435BB | KR809719         |
| 47    | Bariliusbarna                 | GPCR9BB    | KU043320         |
| 48    | Bariliusbarna                 | GPCR10BB   | KU043321         |
| 49    | Bariliusbarna                 | GPCR11BB   | KU043322         |
| 50    | Bariliusbarna                 | GPCR12BB   | KU043323         |
| 51    | Bariliusbendelisis            | GPCR 113BB | KR809720         |
| 52    | Bariliusbendelisis            | GPCR 114BB | KR809721         |
| 53    | Bariliusbendelisis            | GPCR13BB   | KU043324         |
| 54    | Bariliusbendelisis            | GPCR14BB   | KU043325         |
|   | Species              | Accession Number | GenBank ID |
|---|----------------------|------------------|------------|
| 55| Bariliusbendelisis   | GPCR15BB         | KU043326  |
| 56| Barilusiavagra       | GPCR112BV        | KR809722  |
| 57| Barilusiavagra       | GPCR115BV        | KR809723  |
| 58| Barilusiavagra       | GPCR270BV        | KR809724  |
| 59| Barilusiavagra       | GPCR272BV        | KR809725  |
| 60| Barilusiavagra       | GPCR273BV        | KR809726  |
| 61| Barilusiavagra       | GPCR274BV        | KR809727  |
| 62| Barilusiavagra       | GPCR385BV        | KR809728  |
| 63| Channagachua         | GPCR142CG        | KR809729  |
| 64| Channagachua         | GPCR16CG         | KU043327  |
| 65| Channagachua         | GPCR17CG         | KU043328  |
| 66| Channagachua         | GPCR18CG         | KU043329  |
| 67| Channagachua         | GPCR19CG         | KU043330  |
| 68| Channapunctata       | GPCR146CP        | KR809730  |
| 69| Channapunctata       | GPCR20CP         | KU043331  |
| 70| Channapunctata       | GPCR21CP         | KU043332  |
| 71| Channapunctata       | GPCR22CP         | KU043333  |
| 72| Channapunctata       | GPCR23CP         | KU043334  |
| 73| Cyprinuscarpio       | GPCR223CC        | KR809731  |
| 74| Cyprinuscarpio       | GPCR225CC        | KR809732  |
| 75| Cyprinuscarpio       | GPCR293CC        | KR809733  |
| 76| Cyprinuscarpio       | GPCR294CC        | KR809734  |
| 77| Cyprinuscarpio       | GPCR295CC        | KR809735  |
| 78| Cyprinuscarpio       | GPCR50CC         | KR809736  |
| 79| Danioodevario        | GPCR236DD        | KR809737  |
| 80| Danioodevario        | GPCR237DD        | KR809738  |
| 81| Danioodevario        | GPCR24DD         | KU043335  |
| 82| Danioodevario        | GPCR25DD         | KU043336  |
| 83| Danioodevario        | GPCR26DD         | KU043337  |
| 84| Garragotyla          | GPCR144GG        | KR809739  |
| 85| Garragotyla          | GPCR27GG         | KU043338  |
| 86| Garragotyla          | GPCR28GG         | KU043339  |
| 87| Garragotyla          | GPCR29GG         | KU043340  |
| 88| Garragotyla          | GPCR30GG         | KU043341  |
| 89| Garralamta           | GPCR145GL        | KR809740  |
| 90| Garralamta           | GPCR31GL         | KU043342  |
| 91| Garralamta           | GPCR32GL         | KU043343  |
| 92| Garralamta           | GPCR33GL         | KU043344  |
| 93| Garralamta           | GPCR34GL         | KU043345  |
| 94| Lepidocephalichthysguntea | GPCR280LG     | KR809741  |
| 95| Lepidocephalichthysguntea | GPCR147LG     | KR809742  |
| 96| Lepidocephalichthysguntea | GPCR35LG     | KU043346  |
| 97| Lepidocephalichthysguntea | GPCR36LG     | KU043347  |
| 98| Lepidocephalichthysguntea | GPCR37LG     | KU043348  |
| 99| Lepidocephalichthys sp. | GPCR289Lsp.   | KR809743  |
| 100| Lepidocephalichthys sp. | GPCR38Lsp.   | KU043349  |
| 101| Lepidocephalichthys sp. | GPCR39Lsp.   | KU043350  |
| 102| Lepidocephalichthys sp. | GPCR40Lsp.   | KU043351  |
| 103| Lepidocephalichthys sp. | GPCR41Lsp.   | KU043352  |
| 104| Mystusvittatus       | GPCR288MV        | KR809744  |
| 105| Mystusvittatus       | GPCR42MV         | KU043353  |
| 106| Mystusvittatus       | GPCR43MV         | KU043354  |
| 107| Mystusvittatus       | GPCR44MV         | KU043355  |
| 108| Mystusvittatus       | GPCR45MV         | KU043356  |
| 109| Nemacheilusmontana  | GPCR58NM         | KR809745  |
| 110| Nemacheilusmontana  | GPCR46NM         | KU043357  |
| 111| Nemacheilusmontana  | GPCR47MV         | KU043358  |
| 112| Nemacheilusmontana  | GPCR48MV         | KU043359  |
|     | Species                       | Accession   | Accession   |
|-----|------------------------------|-------------|-------------|
| 113 | Nemacheilus montana          | GPCR49MV    | KU043360    |
| 114 | Pseudecheneis salutaca       | GPCR197PS   | KR809746    |
| 115 | Pseudecheneis salutaca       | GPCR292PS   | KR809747    |
| 116 | Pseudocheneis salutaca       | GPCR63PS    | KR809748    |
| 117 | Pseudocheneis salutaca       | GPCR50PS    | KU043361    |
| 118 | Pseudocheneis salutaca       | GPCR51PS    | KU043362    |
| 119 | Pseudocheneis salutaca       | GPCR52PS    | KU043363    |
| 120 | Puntius chelynoiides         | GPCR196PC   | KR809749    |
| 121 | Puntius chelynoiides         | GPCR170PC   | KR809750    |
| 122 | Puntius chelynoiides         | GPCR171PC   | KR809751    |
| 123 | Puntius chelynoiides         | GPCR172PC   | KR809752    |
| 124 | Puntius chelynoiides         | GPCR195PC   | KR809753    |
| 125 | Puntius chelynoiides         | GPCR221PC   | KR809754    |
| 126 | Puntius chelynoiides         | GPCR262PC   | KR809755    |
| 127 | Puntius chelynoiides         | GPCR263PC   | KR809756    |
| 128 | Puntius chelynoiides         | GPCR267PC   | KR809757    |
| 129 | Puntius chelynoiides         | GPCR331PC   | KR809758    |
| 130 | Puntius chelynoiides         | GPCR387PC   | KR809759    |
| 131 | Puntius chelynoiides         | GPCR390PC   | KR809760    |
| 132 | Puntius chelynoiides         | GPCR430PC   | KR809761    |
| 133 | Puntius chelynoiides         | GPCR431PC   | KR809762    |
| 134 | Puntius chelynoiides         | GPCR432PC   | KR809763    |
| 135 | Salmotrutta                   | GPCR121BT   | KR809764    |
| 136 | Salmotrutta                   | GPCR124BT   | KR809765    |
| 137 | Salmotrutta                   | GPCR126BT   | KR809766    |
| 138 | Salmotrutta                   | GPCR128BT   | KR809767    |
| 139 | Salmotrutta                   | GPCR1BT     | KR809768    |
| 140 | Schizothorax plagiosomus     | GPCR101SP   | KR809769    |
| 141 | Schizothorax plagiosomus     | GPCR53SP    | KU043364    |
| 142 | Schizothorax plagiosomus     | GPCR54SP    | KU043365    |
| 143 | Schizothorax plagiosomus     | GPCR55SP    | KU043366    |
| 144 | Schizothorax plagiosomus     | GPCR56SP    | KU043367    |
| 145 | Schizothorax progastus       | GPCR105SP   | KR809770    |
| 146 | Schizothorax progastus       | GPCR162SP   | KR809771    |
| 147 | Schizothorax progastus       | GPCR356SP   | KR809772    |
| 148 | Schizothorax progastus       | GPCR4SP     | KR809773    |
| 149 | Schizothorax progastus       | GPCR9SP     | KR809774    |
| 150 | Schizothorax progastus       | GPCR100SP   | KR809775    |
| 151 | Schizothorax progastus       | GPCR131SP   | KR809776    |
| 152 | Schizothorax progastus       | GPCR160SP   | KR809777    |
| 153 | Schizothorax progastus       | GPCR227SP   | KR809778    |
| 154 | Schizothorax progastus       | GPCR374SP   | KR809779    |
| 155 | Schizothorax progastus       | GPCR97SP    | KR809780    |
| 156 | Schizothorax sinnatus        | GPCR110SS   | KR809781    |
| 157 | Schizothorax sinnatus        | GPCR111SS   | KR809782    |
| 158 | Schizothorax sinnatus        | GPCR57SS    | KU043368    |
| 159 | Schizothorax sinnatus        | GPCR58SS    | KU043369    |
| 160 | Schizothorax sinnatus        | GPCR59SS    | KU043370    |
| 161 | Schizothorax richardsonii    | GPCR1SR     | KU695217    |
| 162 | Schizothorax richardsonii    | GPCR2SR     | KU695218    |
| 163 | Schizothorax richardsonii    | GPCR3SR     | KU695219    |
| 164 | Schizothorax richardsonii    | GPCR4SR     | KU695220    |
| 165 | Schizothorax richardsonii    | GPCR5SR     | KU695221    |
| 166 | Tor putitora                 | GPCR151TP   | KR809783    |
| 167 | Tor putitora                 | GPCR382TP   | KR809784    |
| 168 | Tor putitora                 | GPCR383TP   | KR809785    |
| 169 | Tor putitora                 | GPCR384TP   | KR809786    |
| 170 | Tor putitora                 | GPCR51TP    | KR809787    |
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