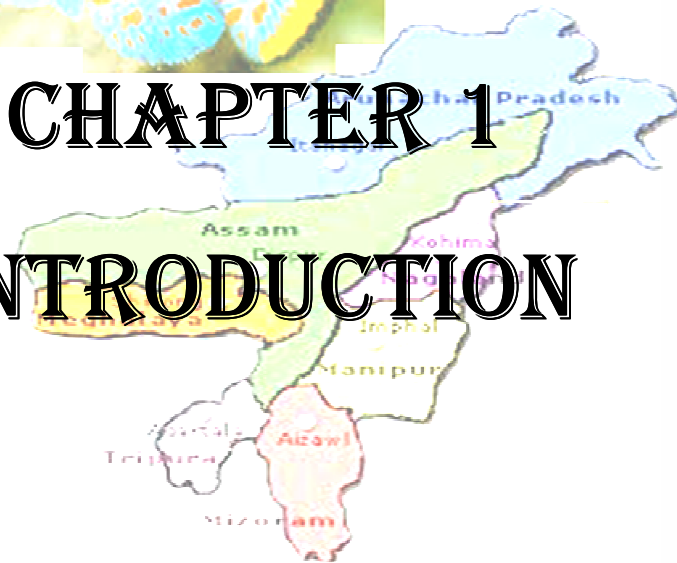




CHAPTER 1

INTRODUCTION



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1.1 FISH DIVERSITY

The biological diversity is a valuable natural resource of a nation. Sampling, identifying, and studying biological specimens are among the first steps toward protecting and benefiting from biodiversity. Knowledge about biodiversity and the ability to identify organisms that comes with it are global public goods. It has been estimated that Earth is a home for about 10 million species of plants and animals, but human brain can learn to recognize few thousand species which is only a small fraction of biodiversity (Mora et al. 2011), so it has become very necessary to identify and catalogue the global biodiversity reserve to protect it from non-judiciary exploitation.

The fishes stand for a diverse group of vertebrates with a record of around 32,000 extant species worldwide (Froese and Pauly 2012). They exhibit massive variations in their morphology, in the habitats they occupy and in their biology. The fishes in general, unlike other vertebrates, are considered paraphyletic i.e. all representatives have not arrived from a common ancestor and different members have evolved independently (Lecointre and Le Guyader 2007a, Nakatani et al. 2011). Their body shape is mostly flattened and typical characteristics being, presence of lateral line sense organ and paired and unpaired fins supported with fin rays. Fishes are mainly categorized as freshwater or bony (teleosts) and marine or cartilaginous (elasmobranches) based on the habitat specificity. They principally serve as major food item due to possession of high protein and poly-unsaturated omega 3-fatty acid (Oehlenschlager 2012) and also being used as ornamental component (Lawrence and Mason 2012), which is the one of the largest hobby in the world. Besides that, many fishes provide excellent thrills to anglers and thus are also important component of angling tourism. Thus fishery resource is an important component of biodiversity and therefore their detailed diversity study together with protection strategy is an absolute necessity.

1.2 ORNAMENTAL FISH

Ornamental fishes usually mean eye-catching colourful and interesting fishes of various characteristics, which are kept as pets in confined space of an aquarium or a garden pool for fun and fancy. They are usually kept in glass aquarium and hence popularly known as “Aquarium Fishes”. These living jewels need not always have bright colours, as sometimes their peculiar characteristics such as body colour, morphology, mode of taking food etc. may also add to their attractiveness. Most of the small food fish which are treated as unwanted for conventional farming have good potency as ornamental fishes.

Aquarium fishes are categorised based on vivid colour pattern (colourful), morphologically uniqueness (peculiar), or based on behaviour (semi-aggressive, community, non-community). However, it can be broadly categorized into two categories, classified and non-classified ornamental fishes (Mahapatra et al. 2004). The small fish like *Botia dario*, *Danio dangila*, *Puntius shalynius*, etc. are classified types of ornamental fish, which can be reared in an aquarium throughout their lifespan. On the other hand, some larger food fishes like, *Rita rita*, *Channa marulius*, *Labeo gonius* etc. are treated as ornamental fish in their juvenile stage and are termed as non-classified ornamental fish.

1.2.1 DISTRIBUTION OF ORNAMENTAL FISHES

The present distribution of fishes has been shaped by millions of years of changes in the global water cycle. In relation to climate change, the nature of surface freshwater systems has evolved continuously, at various spatial and temporal scales. Many of the surface freshwater systems have, therefore, been short-lived; their fauna and flora usually vanished when the systems disappeared, or were able to survive by developing adaptations to the changing circumstances. The double processes of speciation and extinction have interacted with climatic and geological events that have both isolated fish populations and provided opportunities for migration and colonization into new habitats (Berra 2001).

Freshwater ornamental fishes comprise a diverse group, with up to 150 families of ornamental fish reported. One of the common families is the Cyprinidae (Teleostei: Cypriniformes), the record of 333 species from this group are in the aquarium trade (Hensen 2010). The global diversity is far higher, however, at over 2,400 species (Nelson et al. 2016). Many, such as the barbs, danios and rasbora are popular aquarium and pond fishes, being ubiquitously available at low prices from aquarium and general pet-retailers. In particular, the danios and barbs are frequently promoted as being suitable for beginner aquarists.

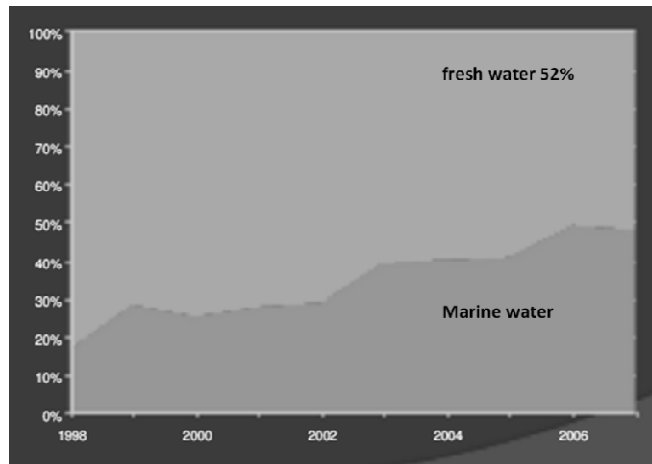


Figure 1.1 Fresh water fishes constitute a major share in Ornamental fish trade.

Biogeographical distribution freshwater fishes are, respectively 4,035 species (705 genera) in the Neotropical region, 2,938 (390 genera) in the Afrotropical, 2,345 (440 genera) in the Oriental, 1,844 (380 genera) in the Palearctic, 1,411 (298 genera) in the Nearctic, and 261 (94 genera) in the Australian (Lévêque et al. 2008).

1.2.1.1 ORNAMENTAL FISH IN INDIA

Our country has a rich and unique biodiversity with a variety of indigenous ornamental fishes. There are a total of 34 biodiversity hotspots across the world. Out of these 3 are located in India. A biodiversity hotspot is a biogeography region with a significant reservoir of biodiversity that is under

threat from humans. Indian waters possess a rich diversity of ornamental fishes, with over 300 varieties of indigenous species.

It has already been reported that around 287 fish species in Western Ghat India, among them 92 exotic fish species, 44 ornamental shrimps including its commercial value, such as food fish and ornamental fish. Among the native freshwater species to be traded, the largest number of species belongs to the family Cyprinidae which is around 98 species, 16 species were found to belong to families Bagridae and Balitoridae. 12 species were found to belong to the families Channidae, Cobitidae and Sisoridae (Jayalal and Ramachandran 2012). The Western Ghats including the west-coast of Peninsular India are well known for their richness in biodiversity of freshwater species. The region has enormous potential for endemic, cultivable and ornamental fish species. Of the 287 species of freshwater fishes, 155 are considered ornamental fishes, of which 117 are endemic to the Western Ghats. As a part of exploration of the Western Ghats for fish species diversity studies, Sharavathi River, this flows through Shimoga and Uttara Kannada districts in Karnataka (NBFGR, 2014-15).

North east India forms a part of two out of the biodiversity hotspots listed by conservation International, the Himalayas and Indo-Burma (Goswami et al. 2012). The Himalayas are the home of the world's highest mountains and deepest gorges. The mountains rise abruptly, resulting in a diversity of eco-systems. This region is endowed with a variety of brilliantly coloured ornamental fishes of around 422 species, belonging to 133 genera and 38 families. The region, northeast India, is distinctive in having certain endemic genera of fishes, viz., *Aborichthys*, *Akysis*, *Badis*, *Bangana*, *Chaca*, *Conta*, *Erethistoides*, etc, (Goswami et al. 2012).



Figure 1.2 Map of India (Satellite Image) showing Biodiversity hotspots where the ornamental fishes are harvested (source Google)

Table 1.1 Native Indian Fresh water fishes traded as Ornamental fish

#	Family	Native species (freshwater)	#	Family	Native species (freshwater)
1	Adrianichthyidae	1	17	Erethistidae	9
2	Ambassidae	7	18	Gobiidae	5
3	Amblycipitidae	1	19	Heteropneustidae	1
4	Anabantidae	2	20	Mastacembelidae	3
5	Anguillidae	1	21	Moringuidae	1
6	Aplocheilidae	4	22	Mugilidae	1
7	Badidae	5	23	Nandidae	2
8	Bagridae	16	24	Notopteridae	2
9	Balitoridae	2	25	Olyridae	1
10	Belonidae	1	26	Osphronemidae	6
11	Chacidae	1	27	Pangasiidae	1
12	Channidae	12	28	Schilbeidae	1
13	Chaudhuriidae	1	29	Siluridae	3
14	Cichlidae	3	30	Sisoridae	10
15	Cobitidae	26	31	Syngnathidae	3
16	Cyprinidae	98	32	Tetraodontidae	4

[Source: Jayalal and Ramachandran 2012]

1.2.1.2 ORNAMENTAL FISH IN NORTHEAST INDIA

North east India, comprising eight states, viz. Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura provide unique ecological variations. The biodiversity present in the freshwater ecosystems in this region, (Himalayas and Indo-Burma) are both highly diverse and of great provincial importance in terms of the livelihood and the economic importance of the people living around it (Allen et al. 2012, Goswami et al. 2012).

This region has a drainage system of the river Ganga in the north and northeastern part of Bengal, the Brahmaputra and the Barak system in Assam, Meghalaya, Tripura and some parts of Mizoram, Manipur and Nagaland. The eastern part of Manipur, as well as the central plain is drainage system primarily of the Chindwin river system, while the southern part of Mizoram, has the Koladyne river system (Allen et al. 2012). It has already been reported that 422 fish species from North East India, belonging to 133 genera and 38 families (Table 1.2), including its commercial value, such as food fish, ornamental fish, available in the different states of north eastern India (Goswami et al. 2012, Ponniah and Sarkar 2000, Talwar and Jhingran 1991).

Due to the diversity of topographic and climatic features of North-Eastern states of India, this region is rich in endemic fish. These endemic species are attracting hobbyists both locally and globally. The highest number recorded from Assam, followed by Arunachal Pradesh, Meghalaya, Manipur, Tripura, Nagaland, Mizoram, and Sikkim (Mahapatra et al. 2004).

Maximum diversity is observed in the family Cyprinidae which is represented by 154 species. Families Anguillidae, Engraulidae, Heteropneustidae, Chacidae, Aplocheilidae, Syngnathidae, Sciaenidae, Osphronemidae, Ophichthidae, Pristigastiridae and Tetraodontidae are represented by a single species each. Some of the fishes having greatest adaptation to hill stream mode of life as described by Hora (1944) are found in the region. They are: *Psilorhynchus*, *Garra*, *Glyptothorax*, *Pseudocheineis*, *Pseudolaguvia*, *Myerglanis*, *Exostoma*, *Erethistes*, *Parachiloglanis*, *Pareuchiloglanis*.

Table 1.2. Number of fishes identified from different families from North Eastern States.

SI No	Family	No. of species	SI No	Family	No. of species
1	Notopteridae	2	20	Belonidae	2
2	Anguillidae	1	21	Apocheilidae	1
3	Clupeidae	5	22	Syngnathidae	1
4	Engraulidae	1	23	Synbranchidae	4
5	Cyprinidae	154	24	Mastacembelidae	4
6	Psilorhynchidae	6	25	Chaudhuridae	2
7	Balitoridae	48	26	Chandidae	5
8	Cobitidae	20	27	Scianidae	1
9	Bagridae	28	28	Nandidae	8
10	Siluridae	10	29	Cichlidae	2
11	Schilbeidae	8	30	Gobiidae	3
12	Pangasiidae	2	31	Anabantidae	2
13	Amblycipitidae	7	32	Salmonidae	2
14	Akysidae	2	33	Belontidae	6
15	Sisoridae	49	34	Osphronemidae	1
16	Erethistidae	15	35	Channidae	11
17	Claridae	3	36	Ophichthidae	1
18	Chacidae	1	37	Pristigasteridae	1
19	Mugilidae	2	38	Tetraodontidae	1

[Source: Goswami et al., 2012]

1.3 ORNAMENTAL FISH TRADE: GLOBAL VS NATIONAL SCENARIO

Aquarium fish keeping has become the second most accepted hobby and fishes suitable for keeping in aquarium obtain high economic value across the globe; leading to increased growth in global ornamental fish trade. Ornamental fish production is among the leading cash crops of the United States of America aquaculture economy, whose retail value of the fish trade is more than US\$1,000 million as reported. But, few studies exist to document this trade. Using import and export documents the trends in total values for the U.S. trade in ornamental fish was reported. Also, it was determined the number and value of the most commonly imported ornamental fish. Freshwater fish accounted for approximately 96% of the total volume and 80% of the value of the imports. Most freshwater ornamental fish were farm-raised and imported from Southeast Asia

(Chapman et al. 1997). Production of animals for the aquarium hobbyist trade is a rapidly growing sector of the aquacultural industry, and it will continue to become more important as restrictions are placed on collecting animals for the wild. Currently, approximately 90% of freshwater fish traded in the hobbyist industry are captively cultured. However, for marine ornamentals, the reverse is true as only a handful of species is produced via aquaculture technology (Tlusty 2002).

It is reported that all countries of the European Union are the principal market place for ornamental fish; on the other hand, the United States is the single largest buyer of ornamental fish in the world (FAO 1996-2005). While exact figures on the value of the ornamental fish trade do not exist precisely, the value of ornamental fish imported into different countries worldwide is just about \$278 million US dollars (FAO 1996-2005). Pet industry surveys have estimated the aquarium industry worth over \$1,000 million USD (Livengood and Chapman 2009). The number of fish species of ornamental relevance is almost equally divided between freshwater and saline water species. However, the utmost volumes in the trade, around 90-96%, are of freshwater origin and are produced in commercial aquaculture facilities (Cato and Brown 2008).

The aquarium fish trade is a large, bio-diverse, global industry. The worldwide pet trade covers a wide diversity of freshwater and marine organisms. While depending on a frequent supply of healthy, vibrant aquatic animals, few sustainability initiatives exist within this sector (Tlusty et al. 2013) worth billions of US\$ and involving 5300 freshwater and 1802 marine fish (Hensen 2010). The global export of ornamental fish as increased from US\$ 176 million in the year 1998 to US\$ 337 million in 2008 and US\$ 371.42 million in 2012 (FAO, 2008-12). Among the exporters, Singapore continues to be the biggest exporter and also remains the Asian hub for ornamental fish while, US, EU and Japan are the major importers with Czech Republic having a prominent place in EU in ornamental fish trade (Jayalal and Ramachandran 2012).

The Indian ornamental fish export is very little in global share (less than 1%) but pulsating with 20% rise per year with annual income of US\$ 1.2 million (MPEDA).

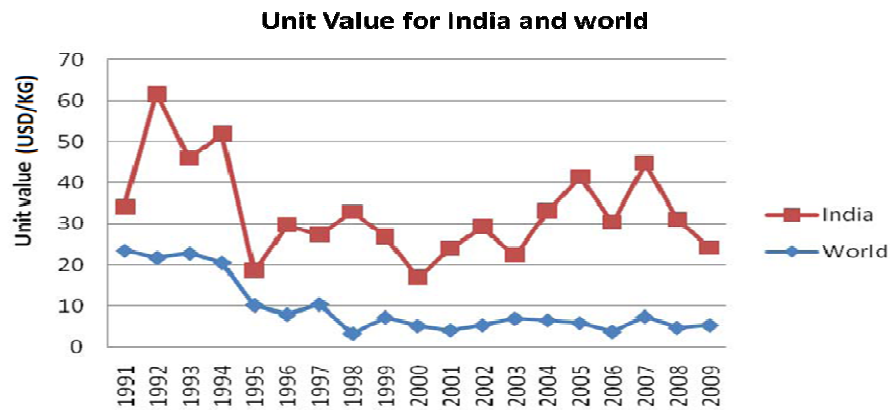


Figure 1.3 Unit value (USD/KG) of the Ornamental fish exports from India and Globally during 1991-2009 (Rani et. al. 2014)

The ornamental fish exports from India showed a growing trend and an exponential growth over the years (1991-2009). India’s share in world ornamental fish trade oscillated and remained less than one percent for most of the years. Its share in world market ranged from 0.12% to 1.16% during 1991- 2009. India gained highest market share of 1.16% during the year 2007. In 2008 it had a share of 0.64 percent which again declined to 0.39% in 2009 (Rani et al. 2014).

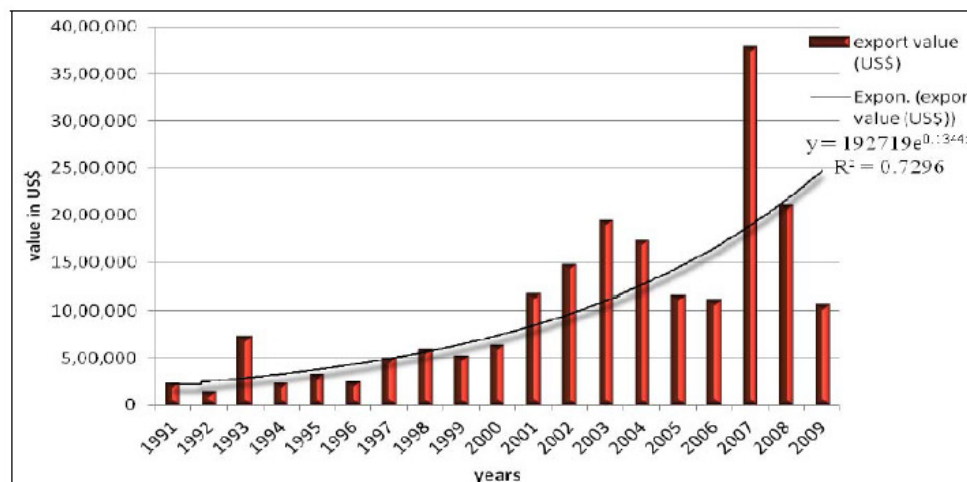
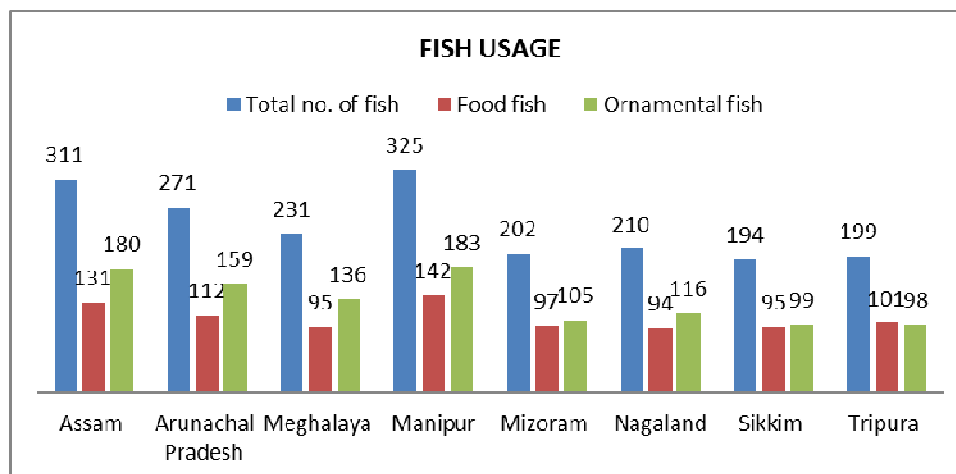


Figure 1.4: Ornamental fish exports from India and trend line during 1991-2009 (Rani et al. 2014)

The North-eastern (NE) region of India contributes the lion's share of Indian aquarium fish trade (Bhattacharya and Choudhury 2004). This region of the country is important in view of large-scale production from capture fishery underlined by the existence of innumerable rivers, rivulets and lentic water bodies, which harbour a diverse fish fauna. There are about 267 species belonging to 136 genera of fresh water fishes inhabiting in NE India (Ponniah and Sarkar 2000). Out of which, 54.32% possess either of the three values as food for human, component in angling tourism or aquarium fish trade, and are thus potential resources for the growth of economy. About 80% of the total ornamental fish trade is rooted from wild catch and is contributed by this region of India via Kolkata Airport (Das and Biswas 2009, Kalita and Deka 2013).



Figures 1.5 State-wise fish individual numbers and their uses as both food and ornamental fish. (Goswami et al, 2012)

Besides, ornamental fish traders always intend to publicize uncommon or unseen species in the trade in order to attract hobbyists. In the process, some traders adopt an unfair practice, like, use of synthetic dyes to develop color in those species which are originally colourless thus claiming them to be ornamental and traded by different names to the clients. On the other hand, many endemic fish species are being traded from the wild harvest, due to lack of established species-specific culture or breeding, and serve as a threat to biodiversity from harvest pressure. Despite several regulatory enactments in India, aquarium fishes are traded largely without endowment to the government and mostly from wild

capture. In the process, many threatened species, (IUCN listed) are being sold due to the problem in identification that arises due to the utilization of multiple commercial names and thereby enhancing the risk of jeopardizing of those species from the wild. The use of such trade names rather than scientific names is commonly practiced in ornamental fish market, and this has proven to be always misleading in identification as there may exist various trade names for a single species. So it has become very necessary to catalogue the ornamental fish reserve and its diversity in this region which trades globally and monitor the trade of threatened or restricted species (Dhar and Ghosh 2015).

A



B



Figure 1.6 A. Ornamental Fishes under trade (Google). B. Endangered Species of *Puntius denisonii* exported from India under the trade name Red Line Torpedo Barb (source WWF, India)

1.4 MORPHOTAXONOMY IN ORNAMENTAL FISH IDENTIFICATION

Taxonomic impediments have remained the biggest barrier in freshwater fish diversity studies as in case of other taxonomic groups. In recent times, taxonomy at the species level has tended to be neglected within ecological researches. The identification of organisms within communities to species level is one of the greatest constraints in terms of time and costs in ecological studies.

Some studies have suggested that working at a taxonomic level higher than species does not result in an important loss of information (Taxonomic sufficiency) (Giangrande 2003). Taxonomy has always been considered a marginal science even during the pioneer descriptive period of ecology, and traditionally has received little financial support. The result was the production of many misidentifications and erroneous records. Before the widespread use of digital taxonomy, the developing experimental ecological approach has led to an improvement in scientific methods, but concurrently to a reduction in the number of expert taxonomists for many groups. Further cases of loss of voucher specimen, independent identification in different geographical location and lack of uniform database over a long history of taxonomy has resulted in many conflicting taxonomy.

Before the widespread use of digital taxonomy, the developing experimental ecological approach has led to an improvement in scientific methods, but concurrently to a reduction in the number of expert taxonomists for many groups. Biodiversity, particularly 'species richness' has long been thought to influence temporal variability. Efforts to clarify the temporal variability relationship and to demonstrate the lack of such a relationship should continue. Such information is essential in order to maintain the ecological function despite the loss of component species, an important topic not only to ecologists but also to policy makers. Many species appear to have overlapping niches, and as such it could be argued that it is not essential for all species to be present. In contrast the crucial role of keystone species has been embraced in conservation biology as a tool to help highlight species requiring priority for protection (Berra 2001).

Freshwater fishes in India are a poorly studied group since information regarding distribution, population dynamics and threats is incomplete, and most of the information available is from a few well-studied locations only. Threats to Indian freshwater fishes are physical in nature, such as habitat destruction, fragmentation, poisoning, pollution, pesticides, destructive fishing, and other kinds of human interference. Trade is an important contributing factor in threatening some freshwater fish taxa in India. This is mainly because of unsustainable harvest, poor scientific practices in fishing and an ever-growing demand. Moreover, the actual number of fish species found in India is still not accurately known because of taxonomic impediments (Hoagland 1996b) arising due to lack of exploration, indiscernibility among some alike species, and ambiguity in taxonomic keys. As a result, many species have been considered as cryptic some of which may also be latent (Darshan et al. 2013). Furthermore, due to lack of proper morphological description with respect to sexual dimorphism, allopatric population, etc., the statuses of a few nominal species have been contentious (Darshan et al. 2013, Kottelat and Lim 1995, Roberts 1992). In India, in addition to the absence of an updated compiled checklist of freshwater fishes, the identification keys for many valid species have not been updated (Jayaram 1999, 2010, Talwar and Jhingran 1991). During the last decade, many new species have been described in India (Talwar and Jhingran 1991). However due to lack of an updated compiled checklist or database, the newly discovered species are often overlooked in further assesment using taxonomic and molecular methods.

1.5 MOLECULAR MARKERS IN SPECIES IDENTIFICATION

Since the onset of modern biotechnological methods, principle being gene sequencing, scientists have realized that the changes and rearrangement in the nucleotide sequence of DNA lay down the backbone of molecular evolution which in turn leads to evolution in its true sense and discrete variation pattern among organisms. Thus, evolution as it proceeds; the initial changes accumulate at the molecular level which in long term leads to visible morphological variations. The above reason thus brings the conclusion that even if two

organisms are morphologically alike, they may bear substantial variation at the molecular level and the phenotypic similarity or dissimilarity between organisms is not a true reflection of actual variation. This dilemma condition often leads to misinterpretation and is a major drawback in biodiversity research when morphological keys are the basis. To dig the fact further, enthusiasts inclined to explore variation at the molecular level in inventorying and understanding biodiversity. More than 40 years ago, starch gel electrophoresis of proteins was first used to identify species. Nearly 30 years ago, single gene sequence analysis of ribosomal DNA as well as other genes, rDNA, allozymes, etc. was being used to investigate evolutionary relationships at a high level (Woese and Fox 1977), and mitochondrial DNA approaches dominated molecular systematics in the late 1970s and 1980s (Avice 1994). The mitochondrion is the organelle associated with the supply of energy for a cell survival. It contains its own DNA and encodes 37 genes: 13 for subunits of respiratory complexes I, III, IV and V, 22 for mitochondrial tRNA (for the 20 standard amino acids, plus an extra gene for leucine and serine), and 2 for rRNA.

Early studies of mtDNA analyzed restriction fragment length polymorphisms (RFLPs). Whole mtDNA were digested with specific endonucleases and the products were separated by using gel electrophoresis. Restriction sites were then mapped and their presence or absence was scored (Dowling 1996). Previously RFLP studies had been a popular approach in quantifying degree of divergence within and among populations. However, in applying this approach to species and higher systematic questions, the homology of restriction site characters became less certain. A better approach for these comparison involved direct analysis of DNA sequences. In coding regions, the variation in DNA sequences were evaluated among first, second and third codon positions and at the amino acid level in order to increase potential phylogenetic utility at higher systematic levels. The relative strength of the phylogenetic signal with the codon position and between the nucleotide and amino acid level were critically evaluated by Naylor and Brown (1998).

1.6 DNA BARCODING AS MOLECULAR IDENTIFICATION TOOL

For decades scientists have been trying to develop an effective tool for proper identifying and cataloguing the biological wealth of the globe. DNA barcoding is emerged as a new tool for taxonomic research. The DNA barcode is a very short, standardized DNA sequence in *COI* gene of mitochondrial DNA which provides a way to identify the species to which a plant, animal or fungus belongs. Any biological sample of closely related species which are very difficult to distinguish due to morphological similarity, eggs, larvae, juvenile form, etc which cannot be identified based on morphological studies can be easily identified and characterized by DNA barcoding. DNA barcoding is a powerful molecular tool for species level identification and characterization of any biological sample by using a short stretch of DNA sequence (658 bp) derived from 5' end of *COI* (*Cytochrome c oxidase I*) of mitochondrial DNA (Hebert et al. 2003a, Hajibabaei et al. 2007a, Lévêque et al. 2008). Mitochondria are energy-producing organelles in plant and animal cells, and have their own genome. Twenty years of research have established the utility of mitochondrial DNA sequences in differentiating among closely related animal species. Four properties make mitochondrial genomes especially suitable for identifying species. While each cell typically contains only 2 copies of nuclear DNA sequences, the same cell encompasses 100-10,000 mitochondrial genomes. Recovering mitochondrial DNA sequences succeeds much more often than nuclear sequences, especially from small or partially degraded samples. Greater success with smaller samples means lower processing costs. Sequence differences among closely related animal species average 5 to 10-fold higher in mitochondrial than nuclear genes. Thus, shorter segments of mitochondrial DNA distinguish among species, and because they are shorter, less expensively.

The primary aspect of DNA barcoding is the development of reference data source for each taxa of the World and thus creation of a reference database. It is the most vital step and involves contribution of renowned taxonomist and also inclusion of the reference specimens in the regional/national museum which are identified and curated by various experts, and their barcode sequences as

standards or reference for those taxa. Nevertheless, keeping in mind that every resource is not catalogued in the museums therefore, new collection and exploration are also paid equal importance. As the museum specimens maintains some standard data's, new collections of specimens were undertaken maintaining some standards records such as, collector name, collection date, geographical location, elevation/depth, collection gear, notes on habitat and microhabitat, sex of specimen, life stage, specimen imaging, identifier etc. Practically cataloguing the total biodiversity of Earth in a museum is not feasible and even if it can be done gradually with time the specimens gets deformed as no fixatives can guarantee total preservation of the samples. Under this circumstances the specimen information are maintained in database (Ratnasingham and Hebert 2007). The secondary aspect of DNA barcoding involves accession of the reference data by the researcher or taxonomist for subsequent analysis and interpretation.

One the other hand the most decisive issues regarding DNA barcoding is how accurately it can identify species. Generally, the accuracy mainly depends on the extent of overlap between the divergences of interspecific vs intraspecific variation. The larger the “gap” between intra and interspecific differences in genetic distance (often called the barcode gap), the more successful is the species identification. Undeniably, some early studies reported very high identification success and the presence of a distinct barcoding gap. Initially a mean intraspecific divergence of 10 times was proposed as the standard threshold for differentiating species. However, the barcoding gap is sometimes misinterpreted and should be quantified as the difference between intraspecific and minimum congeneric distances instead of using mean values. When intra- and interspecific distances are widely overlapped, DNA barcoding-based identification is not effective. Overlap can be caused by several factors, including large genetic diversity in a species. Another major cause is paraphyly or polyphyly of species that appear to be closely related. Indeed, it is estimated that one-fourth of animal species are not monophyletic. Species may appear to be polyphyletic or paraphyletic in phylogenetic analyses due to incomplete lineage sorting of mitochondrial DNA,

introgression or incongruence in the definition of morphological species. Two groups of organisms may share the same DNA barcode(s) but may not belong to the same species, in particular, if they have diverged very recently. Such situations are rather common. These points show the limitations of the DNA barcoding method depending on a single region of mitochondrial DNA. In such cases, supplemental analyses combined with other traits, such as nuclear genes, are required. Another factor that may lead to overlap is the incongruence between molecular data and the traditional definition of species, in particular when a group is poorly studied taxonomically. Such cases may be improved by integrative taxonomic revisions that combine genetic and morphological data.

1.6.1 DNA BARCODING OF FISHES

The taxonomic impediments associated with fishes aroused due to lack of proper morphological description with respect to sexual dimorphism, allopatric population, biasness in identification keys etc. prompted the launch of an international Fish Barcoding of Life initiative - FISH-BOL (<http://www.fishbol.org/>) with the aim of barcoding all fishes. The Fish Barcode of Life Initiative is a worldwide effort to co-ordinate an assembly of a standardized reference sequence library for all fish species, one that is derived from voucher specimens with authoritative taxonomic identifications. The benefits of barcoding fishes include facilitating species identification for all potential users, including taxonomists; highlighting specimens that represent a range of expansion of known species; flagging previously unrecognized species; and perhaps most importantly, enabling identifications where traditional methods are not applicable. The FISH-BOL is a wing of CBOL and at present stores DNA barcode records of nearly 30,000+ species of marine, freshwater and estuarine fish of the world. The various uses of the FISH-BOL library take account of detecting consumer fraud, aiding fisheries management, improving environmental investigations (food web system) and assisting with taxonomic revisions (Ward 2012). This procedure additionally allows application in monitoring fish products for health safety (Lowenstein et al. 2010) and in the regulation of the

exploitation of fish species under aquarium trade (Dhar and Ghosh 2015, Wong et al. 2011).

In 2005, *COI* barcode sequence from 207 species of fish was sequenced (Ward et al. 2005). All species could be differentiated by their *COI* sequence. In 2011, another group developed and appraised DNA barcodes for use in delineating native United States and imported catfish species (Wong et al. 2011) and suggested to heighten the inspection and regulation requirements for fish products where DNA barcoding will serve as an important tool in efforts to ensure consumer safety and fair international commerce. On the other hand, April et al. (2011) established DNA barcode reference library for more than 80% of the named freshwater fish species of North America. This study successfully delineated 90% of known species using barcodes. Furthermore, it exposed numerous independently evolving lineages within described species, which directs to the presence of morphologically cryptic diversity. From the 752 species analyzed, this survey flagged 138 named species that represent as many as 347 candidate species, which suggests a 28% increase in species diversity. It had been demonstrated that an average retrieval of 2% new species in larger fish DNA barcoding studies (Bucklin et al. 2011), where the authors 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 new during the past 10 years (2000–2009), with 500 added in 2008 and 300 in 2009 (Eschmeyer et al. 2012). Many sequences of Indian freshwater fishes have been submitted to the database and few studies have addressed problems specific to certain groups. In 2014 the DNA barcoding analysis comprising the entire Indian fresh water fish was undertaken (Chakraborty and Ghosh 2014), it was found that most of the species were successfully delineated except for few where it showed the presence of some cryptic diversity.

1.7 DNA BARCODING IN PHYLOGENY

DNA barcoding aimed to decipher an efficient method for species-level identifications and, it contributed impressively in taxonomic and biodiversity

research (Hajibabaei et al. 2007b). As the number of DNA barcode sequences accumulates, however, these data will also provide a unique 'horizontal' genomics perspective with broad implications. Though the barcode sequences have been analyzed mainly by reconstruction of phylogenetic tree such as NJ or MP, these barcode-based trees are used both to identify species and to draw attention to overlooked and new species; they can also help to identify candidate exemplar taxa for a comprehensive phylogenetic study. Barcode of Life projects created a perfect taxonomic sampling environment for conducting phylogenetic studies on different branches of the Tree of Life and this sampling has been shown to be a key factor in obtaining a robust phylogeny.

The first comprehensive study targeting the entire reptile fauna of the fourth-largest island in the world was presented by Nagy (2012), where the biodiversity hotspot of Madagascar is compared with available multi-gene phylogenies. It was found that DNA barcoding correctly assigned most samples to species, genus and family with high confidence and the analysis of fewer taxa resulted in an increased number of well supported lineages.

In 2009, a study conducted on the Neotropical avifauna which is more diverse than that of any other biogeographic region (Kerr et al. 2009). The understanding of patterns of regional divergence is limited and critical examination of this issue is currently constrained by the limited genetic information available. This study begins to address this gap by assembling a library of mitochondrial *COI* sequences for Argentinian birds and comparing their patterns of genetic diversity with those of North American birds. Deep genetic splits were observed in at least 21 species, though distribution patterns of these lineages were variable. Fifteen of the 32 species of waterbirds showed low genetic divergence. The study like this showed the approach in which a broad-ranging analysis of sequence diversity in a single gene region can also deliver insights concerning the diversification of faunas. Interestingly, incidences of deep intraspecific divergences as well as low genetic divergence or barcode sharing are of great importance to our understanding of avian speciation.

Phylogenetic relationships among the commercially important Indian sciaenids were evaluated by Lakra et al. (2009). The taxonomic ambiguity, and systematic position of the Malabar snakehead, *Channa diplogramma*, was resolved by Benziger et al. (2011) using morphological and molecular genetic information like specific *COI* barcode, 16S, etc, in addition to making an attempt to understand its phylogenetic relationships, evolution time of its most recent common ancestor and evolutionary biogeography. A detailed study of Indian catfishes was undertaken by (Bhattacharjee et al. 2012), where the phylogenetic relationships of different catfish were studied with an effective barcode gap devised for the entire catfish groups. This study also solved the status of the *Mystus horai* and confirmed the former to be the junior synonym of *M. vittatus*. Taxonomic discrepancy of Mahaseer fish was studied and resolved by (Laskar et al. 2013). The taxonomic validity of Northeast Indian endemic Mahseer species, *Tor progeneius* and *Neolissochilus hexastichus*, had been disputed repeatedly. This disagreement is mainly in the recognition of the species based on morphological characters. Consequently, both the species have been obscured for many decades. Altogether, 28 different mahseer specimens including paratypes were studied from different locations in Northeast India, and extensive study on the morphometric characters were carried out for the species along with the *COI* barcode sequence. This combined approach substantiated the identification of *N. hexastichus* to be a true species, and tentatively regarded *T. progeneius* to be a synonym of *T. putitora*. Hence DNA barcode showed its efficacy in solving species status of two disputed species.

1.8 DNA BARCODING UNCOVERS THE ILLEGAL TRADE

DNA barcoding also gained its importance in the identification and authentication of the biological samples/products extensively used in trade. Seafood authentication and food safety concerns are a growing issue in today's global marketplace, although traditional morphology-based identification keys and existing molecular approaches have limitations for species identification (Wong and Hanner 2008). It is a rapid, cost-effective and broadly applicable molecular diagnostic technique for this purpose. The present case study was able

to provide species matches of >97% sequence similarity for 90 of 91 samples tested when matched with Barcode database. Twenty-five percent of the samples were potentially mislabeled, demonstrating that DNA barcodes are already a powerful tool for the identification of seafood to the species level. Similarly in 2012, DNA barcoding revealed a high incidence of fish species fabrication on the South African market (Cawthorn et al. 2012). It was demonstrated that 95% samples was clear-cut categorized into correct species. However, 10 of 108 (9%) samples from wholesalers and 43 of 140 (31%) from retailers were identified as different species to the ones indicated at the point of sale. Although some cases of mislabelling were potentially unintended due to misapplied market nomenclature, a far bigger section represented serious and seemingly deliberate acts of fraud for the sake of greater profits. This study has highlighted that the existing legislation pertaining to seafood marketing in South Africa is inadequate or poorly enforced and requires urgent revision.

The authenticity of Natural Health Products remains an important legal, economic, health and conservation issue. Here, the use of DNA barcoding for the authentication of 95 health products from animal and plant species of varying form and origin, revealed success rates of up to 83% and 88% in shark and ginseng products, respectively. It was confirmed that half of the samples commercially tagged as Korean ginseng were actually American ginseng. This shows the utility of DNA barcodes for the real-world authentication of Natural Health Products. Recently, Seafood mislabeling in food market was detected in India (Nagalakshmi et al. 2016). Samples were authenticated by comparing the *COI* gene sequences with public reference taxonomic databases. The study revealed 22% of seafood mislabeling prevailing in Indian domestic market.

In the recent years there were some reports of illegal trade of threatened species of parrot (Gonçalves et al. 2015). Forensics faced many questions during the investigation process which was solved through barcoding technique. It was the case of a man who was caught in a Brazilian airport trying to travel with 58 avian eggs. The man claimed to be carrying quail eggs, but authorities suspected those to be the eggs of parrots. Soon after the the embryos' species was identified

based on the *COI* and 16S ribosomal DNA. The results based on both the genes, indicated that 57 embryos were of parrots (*Alipiopsitta xanthops*, *Ara ararauna*, and the *Amazona aestiva/A. ochrocephala* complex), and 1 was an owl. This kind of approach can help to design species-specific anti-poaching strategies, and demonstrate how DNA sequence analysis is a powerful conservation tool.

1.9 RESEARCH GAP

DNA Barcode technology has already proved its efficacy in species identification (Hajibabaei et al. 2007a, Hebert et al. 2003a) even in case of any uncertainty in identification of the specimens and studying biodiversity, like Fish (April et al. 2011), Lepidoptera (Hausmann et al. 2011), Coral reef fish (Hubert et al. 2010), Medicinal plants (Chen et al. 2010), spruce budworm food web (Smith et al. 2011), Parasitoid flies (Smith et al. 2006), Fresh water Fish (Valdez-Moreno et al. 2009, Ward et al. 2009, Ward et al. 2005), Aquarium fish (Collins et al. 2012, Steinke et al. 2009), Catfish (Bhattacharjee et al. 2012, Wong et al. 2011), overlooked marine fish (Zemlak et al. 2009), Fish (Zhang 2011, Zhang and Hanner 2012), Indian fresh water fish (Chakraborty and Ghosh 2014). As stated in previous section there are also evidences where *COI* barcode sequence is proved to be useful in authentication of commercial biological items used in trade like Tuna sushi (Lowenstein et al. 2009), Tea (Stoeckle et al. 2011), Herbal food supplement (Little and Jeanson 2013), etc. and in addition for trade monitoring for threatened animals (Luo et al. 2013), Frozen fish items (Cutarelli et al. 2014). But there are lack of data in trading fishes as pets and their ecological status. Therefore, the study was undertaken with the aim of the study is to create a DNA barcode library of ornamental fishes endemic to NE India for proper identification and molecular cataloguing of ornamental fish reserve in this region and the combined approach of morphotaxonomy and *COI* barcode sequence as a reliable species identification tool to categorize the ornamental fish species being traded from NE region of India with maximum precision where there is a hurdle in distinguishing species even if the specimen is falsified using multiple trade names. The following objectives are:

1.10 OBJECTIVES:

- 1. Collection and vouchering of the ornamental fish samples from Northeast India.**
- 2. Development of species specific genetic marker as DNA barcode for the collected specimens.**
- 3. DNA barcoding approach to detect traded ornamental fishes from Northeast India.**
- 4. Barcode sequence analysis including Codon Usage, Transition/ Transversion pattern in the barcode sequence.**
- 5. Phylogenetic analysis of different ornamental fishes based on barcode sequence.**