REVIEW OF LITERATURE
2. Review of Literature

2.1. The ancient river civilization

As part of nature, society arises out of an ecological basis that we cannot overlook. Most of civilization, both in its flourish and decline, reflects how people are able to manage the ecosystems and their natural resources in which they live. Human civilization was derived from its first culture known as agriculture, our ability to work with the land (Desai 2013). This depends particularly on fresh water that is found in rivers, and flat land that can be easily irrigated. Therefore, rivers are the nuclei of human settlement since ancient times and from the civilizations of Egypt, China, Mesopotamia and Indus valley (India). The formation of such civilization was possible only because of the great river systems around which they developed. These rivers not only made these civilizations possible, but also played important role in expansion of human invention or any special ethnic type who migrated there. However, so far this history is trying to define political, economic and racial concerns in a non-ecological way. Our account of ancient history particularly that of India, has not afforded an adequate regard to ecological factors. It has put too much weight on migration, in which people live and rely upon for developing their way of life. India, on the other hand, had a massive nexus of numerous great rivers from the Indus in the West to the swamplands of the Gangetic delta in the East (Frawley 2003). It had
both a warm subtropical climate and seasonal abundant rains. This river region included relatively dry areas from the northwest to the very wet regions of eastern India affording an abundance of crops both in type and quantity (Frawley 2003). The Indian River system was much larger in size having better climatic conditions than perhaps all the other three river regions put together. Also, there is no other river system in the world having such diverse habitat that could serve to create such an agricultural diversity or cultural richness.

These rivers could be considered as the nerves of the nation and it’s worthy to maintain sound ecological conditions for any civilization. Unfortunately, many civilizations from Roman culture to recent Western European societies have used these river resources according to their need by impounding with small or large weirs or dams. Likewise, in most of the arid or dry parts of the world, these streams and rivers have been modified or diverted to provide water for agriculture and wellbeing of human society. To the present day, these efforts of impoundments, deviation and canalization have left few rivers undisturbed. These environmental impacts of hydraulic engineering along with variety of agricultural, domestic and industrial chemicals must be added to the hazards of the contamination of the waters. In addition to this, non-existent river basin management, deforestation, farming of marginal hill slopes and mining has increased erosion and silt loads of rivers resulting in rapid modification of lowland reach of rivers. These deviations not only alter the environmental conditions affecting the life cycles of fishes, but also alters the quality and quantity of the water, in which they live (Welcomme 1985).
2.2. **Account on river biodiversity**

The rivers of the world have incredible biodiversity (Allan & Flecker 1993), with some recognized as ‘Global Biodiversity Hot Spots’ (Myers et al. 2000), despite the fact that these rivers contain only a minuscule proportion (0.0006% by volume) of the world’s freshwater (Shiklomanov 1993). It is considered rich in diversity (Poff et al. 2001), particularly in vertebrates including amphibians and fish and invertebrates including insects and molluscs, even though, our knowledge in freshwater biodiversity is deficient (Dudgeon et al. 2006; Allan and Flecker 1993). However, freshwater ecosystems, including rivers are represented as world’s most endangered ecosystems (Dudgeon 1992). Indeed, declines in biodiversity tend to be greater in fresh waters than in most other habitats (Sala et al. 2000). The ecological attributes of the river largely relies on biogeochemical processes, while morphology of the river is strongly influenced by geology and climate (Imhof et al. 1996; Ward 1998). Thus, this implies that the rivers are important sources to study the impact of growing civilization in their vicinity that can be directly accessed using a biodiversity aspect of the specific river system. Particularly, fish community studies can be correlated to habitat degradation and related perils. These communities provide the basis for fisheries presumably from the earliest phases of human occupation belonging to river valley. As there number of uses competing with fisheries increased, some of the long established fisheries disappeared, while others are on their way to extinction (Welcomme 1985). Since, from last two decades there has been an increase in interest in general studies of fish communities that were lagging behind
on those of rivers, lakes and reservoirs. The studies and practical concerns for management of river fisheries began in the North America and Europe towards the end of the last century that supported sport fisheries and led to physical improvement in fish stocking. Subsequently, systematic studies on large rivers started by earliest contribution of Antipa (1910), whose original work on the Danube was continued by other workers until it became one of the most widely studied work on world’s major river. Antipa concluded that the Danube rivers fishery production was directly proportional to the extent and duration of flooding in any particular year (Botnariuc 1968) is equally applicable to all other flood rivers investigated. Also, from work of Danube it was exemplified that the floodplain must be treated as an integral part of the larger system rather than considering it in isolation (Balon 1967, Botnariuc 1967). Later, some intensified work commencing studies of the Volga River after creation of the cascade reservoirs in the system by some Russian workers is available with part of literature in form of translation. Detailed studies of the Mississippi-Missouri system has been further delayed and obtained in the last two decade (Welcomme 1985). Modern work on these and other temperate rivers now deals with many ecological and biological issues especially those with the conservation of riverine habitats.

The Systematic study of the fisheries ecology began from one of the tropical river Niger in the laboratory of Central Delta (Blanc et al. 1955), who clarified much of the taxonomy and biology of fishes through numerous publications of Daget associated with the study of this river in Niger, Benin and Nigeria. The Nile Sudd River
in Sudan has been studied by a series of missions including the Jonglei Investigation (Mefit-Babtie 1983). Also, there were intensive but short term duration studies on the Kafue River by the Universities of Idaho and Michigan, they focus mostly on the biology of the fishes of the Kafue flats (Welcomme 1985). During the 1970’s the ORSTOM team studied the Yaeres floodplain of the Logone River. Some workers from Botswana Society have been gathering information on the fisheries and general ecology of the Shire River, the Okavango delta (Smith 1976; Gilmore 1976; Thompson 1976; Fox et al. 1976) and components of the Zaire River.

Most of the river systems in the South America have been examined to a certain extent. Bonetto and his team had done numerous work and have provided a large amount of information on the Parana River and its tributaries, while Godoy (1975) have extensively worked on the Brazilian Mogi Guassu tributary. The Amazon staff have studied the area around Manaus, while Peruvian authorities funded with the FAO project have collected information on the same river at the level of Iquitos. On the Orinoco River the work began by Mago-Leccia (1970) which was further continued by Novoa and his co-workers. Lowe-McConnell studied Rupununi River based on the ecology of tropical river fish communities.

The studies on Asiatic rivers is limited to the work of Chevey and Poulain (1940) on the Mekong River, who have contributed to the general understanding of large tropical systems. Otherwise occasional studies have been carried out on rivers in Peninsular Malaysia, Borneo, India, Sri Lanka and the Mesopotamic River. Studies of freshwater fishes in the Indian subcontinent have been limited to scattered works
on commercial fisheries and even these have been largely restricted to some of the major river systems like the Ganga and Yamuna. In parts, globally, the faunal assemblages of many riverine ecosystems have not been rigorously assessed. In some cases, the only information known about a given fish species is its name and a basic morphological description (Welcomme 1985). In other cases, Hogan (2011) signified that there is a lack of taxonomic clarity that hinders development of effective conservation strategies. In the developing countries where the regions are difficult to access e.g. South American and Asian forested regions, some species still have to be discovered (Kang et al. 2009; Junk & Soares 2001; Dudgeon 1992). Unlike rest of the fields (Wheeler 2004), the interest in fish taxonomy and systematics appears to be declining (Chakrabarty 2010), as well as fish biodiversity in running water cannot be maintained, which is further limiting our ability (Wheeler 1995).

It is necessary to rectify the information on the true range of fish biodiversity from rivers and should not be excused for inaction (Cookie et al. 2012). In case of marine realm, the cataloguing of species has been considered important, which resulted in the formation of ‘Census of Marine Life ‘a global network of researchers that was engaged to assess and explicate the diversity, distribution, and abundance of life in the oceans (Yarnick and O’Dor 2005). Beyond having a species list with basic taxonomic information on diversity, distribution and abundance of fish species, it is necessary to effectively conserve the knowledge of fish natural history. It is a prerequisite to obtain basic information about food preferences (Butler and Wooden 2012) and critical habitat requirements (McRae et al. 2012; Hahn et al. 2011, Spindler
et al. 2012; Knight et al. 2012) for many riverine fishes including those that are endangered (Cookie et al. 2012). In order to protect those critical habitats (Khedkar et al. 2012; Knight et al. 2012) it is necessary to understand which habitats are used for feeding (Butler and Wooden 2012; Spindler et al. 2012), for spawning and at different life stages (McRae et al. 2012). Taking these factors into consideration that have been influencing the growth, reproductive success, offspring development (Kemp 2011) and survival of fishes (Hasler et al. 2011), it is essential to undertake an extensive research on fish-environment relationship.

2.3. Indian River biodiversity scenario

The studies on aquatic biota of Indian subcontinent could be traced back to the early nineteenth century when British ruled the country, extensive surveys were on freshwater fauna were undertaken by Hamilton (1822), McClelland (1839) and Day (1873, 1878). In the early of 19th century, E. Hackel (1988) had examined and published fish fauna from Kashmir valley (Das and Subla 1963). The 20th century brought renaissance in Indian Ichthyology through considerable contribution of researchers and eminent directors from Zoological survey of India (ZSI) namely; Annandale (1916-1924), Sewell (1925-1933) and Hora (1937-1942), an ichthyologist par excellence, ecologist and fish taxonomist. Now, the surveys followed most of the areas beyond British India (Afghanistan, Tibet, China, Mongolia and Southeast Asia) under the observation of Annandale who became the first Director of the Zoological Survey of India in 1916. Annandale was accompanied by several prominent Indian
scientists at the Indian Museum or worked in the universities at Calcutta, Madras and Bombay. He studied all kinds of habitats such as lakes, swamps, ponds, thermal springs, streams, torrents, rivers, coastal lagoons and estuaries were surveyed, which included detailed observations on water quality and related habitat characteristics, and their relationships with the organisms (Annandale 1915–24, 1918, 1919, 1921, 1923; Annandale et al. 1921; Annandale & Chopra 1924; Annandale & Rao 1923; Arora 1931; Chopra 1927; Gurney 1907; Hora 1922; Prashad 1916, 1919, 1922, 1923; Preston 1909, 1914; Sewell 1924, 1934). These detailed field studies based on the collection and description of the fauna were done till late 1920s, that had generated enough data to initiate zoogeographic analysis and detailed field studies on the environmental relationships of various organisms (Gopal and Zutshi 1998). Among these studies, Hora made a noticeable contribution on biogeography, evolution, ecology, physiology and general biology of fishes and thus, laid the foundations of Indian fishery science (Hora 1921, 1923, 1930, 1933, 1934, 1935a, b). The distribution of flora and fauna including fishes from peninsular India and Malaysian region were explained in Satpura hypothesis, propounded by Hora (1937a, b, 1944). Also, study on impact of dams on fisheries was of most concerned to Hora (1940, 1942), far ahead of his time, a subject which is now much debated. Also, contribution to fish ecology was made by Das (1927); Pearse (1932) and Khan (1924, 1934). The detailed limnological studies of eleven high altitude lakes in the Ladakh-Tibet region were undertaken in the Yale North India Expedition in 1932, along with those studies several lakes in Kashmir were also investigated (Hutchinson 1933, 1937a). After this Expedition, in 1934 Swedish Expedition to Burma and British India was followed. The
descriptions of biota collected during these two expeditions continued to appear over the next two decades (Alexander 1946, 1954; Brehm 1936, Brehm and Woltereck 1939; Edmondson and Hutchinson 1934; Drouet 1938; Hora 1936, Hutchinson 1937b; Mukerji 1936; Guignot 1954; Kiefer 1939; Kimmins 1957, Lieftinck 1948; Ochs 1940; Prashad 1937).

2.4. Studies undertaken during post-independence developments of India

The scenario of scientific research after the independence of India was improved by establishing several research institutes and Universities in every State. The Central Inland Fisheries Research Institute was established at Barrackpore (West Bengal) in 1959, previously named as Inland Fishery Research Station of Calcutta in 1947, was utilized with the main objective of assessing all inland fishery resources of the country, which further evolved suitable methods for their conservation and optimum utilization. The Institute and its several research stations throughout the country contributed not only to the fishery science but also to the understanding of ecology and production processes in rivers, estuaries, reservoirs, floodplain lakes, lagoons and other aquatic habitats (Sinha 1997). The university departments of Zoology started studies on different aspects of aquatic and fauna with greater focus on exploration of all kinds of aquatic environments. Ichthyology emerged as a major discipline in many universities (Das 1962); closely followed by the studies on zooplankton which are major constituents of fish food.
Later, the interest in inland aquatic environments grew rather rapidly. Several thousands of publications during the past fifty years reflect the widespread interest in hydrobiological research in the country. However, a closer scrutiny brings out phases of episodic research activity which can be readily correlated with the major international programmes that have been initiated from time to time. The researchers switched from one area of research to another without focusing intensively on one or gaining adequate insight of any one field. The studies do not appear to have been driven by the need to understand the aquatic ecosystems and their biota, or by considerations of national interests but largely by factors governing the availability of financial resources and the desire to swim in the mainstream of international scientific research. One can readily identify certain periods when the focus of investigations shifted to a new area followed by a spurt in activity whose peak declined as rapidly as it was achieved (Gopal and Zutshi 1998). The first two decades after independence represented a period of continuity with the pre independence studies with rapid expansion into unexplored areas. The investigations of fishes diversified into areas such as food and feeding behaviour, reproductive biology, physiology (especially of airbreathing fishes), factors affecting growth and production of capture fisheries, and cultivation of freshwater and brackishwater fishes. Zoologists and fishery biologists also took a lead in studies of phytoplankton and zooplankton communities and their seasonal dynamics in all habitats. Phycological research on systematics, physiology, cytology, and to a smaller extent, ecology was pursued at the universities and promoted by the Indian Council of Agricultural Research (Kachroo 1960).
2.4.1. Major Scientific contributions

The studies on freshwater ecosystem mostly emphases on lentic water bodies belonging to certain geographical areas, like Ganga and Yamuna belonging to northern region of India studied by Hamilton (1822) followed by Krishnamurti et al. (1991) and Sarkar et al. (2011). The northeastern rivers by Sen (1985); Nath and Dey (1989). The rivers of peninsular India were studied by Daniels (2002), also, the rivers from Western Ghats region were studied by (Kumar et al., 1998). Similarly, Godavari River was studied by David (1963) and Khedkar et al. (2014). The Krishna River was studied by David (1963) and Jayaram et al. (1995). The river Narmada belonging to north-western region of India was firstly studied by Hora and Nair (1941) followed by Karmchandani et al. (1967), Dubey (1984); Rao et al. (1991), Unni (1997); Desai et al. (1992); Arya et al. (2001); Bakawale et al. (2006); Vyas et al. (2006); Verma and Kanhere (2007). Several such studies have been reported from past few decades, regarding fresh water diversity from rivers of India (Trivedy 1988, 1990).

2.5. Taxonomy and distribution

Substantial contribution of researchers from Zoological Survey of India continued even after the independence to yielded ample information on the flora and fauna of India’s inland waters, which seems to be scattered in their publications (Biswas 1949a). Large number of new species have been discovered and named so far, however the efforts to compile information on the biota have been at the best half-hearted. The research institutes like Indian Council of Agricultural Research and
Zoological Survey of India had started publishing series of volumes on systematics. An overview of Indian fauna has been published by the Zoological Survey of India (Anonymous 1991). Fishes of India have been well documented (Jayaram 1981; Jhingran 1992). Out of the 2,500 species of freshwater fishes that have been recognized in the Indian subcontinent, 930 are categorized as freshwater species by Talwar and Jhingran (1991), 852 species by Jayaram (1999) and 452 species by Menon (1999). Much of the early study on the freshwater systems of the Indian subcontinent started with the works of British officers working for the East India Company, who took great interest in the natural history of the region. Some early contributions were those of Hamilton (1822) in 'The Fishes of the Ganges' and by others like McClelland (1839) and Jerdon (1849). Some of the most important contributions to such studies were made by Francis Day in his Fishes of India (1873-1878). Substantial literature is now available on the identification and systematic of freshwater fishes of India, starting with Hora’s contributions between the 1920 - 1950s and the most recent texts by Talwar and Jhingran (1991) and Jayaram (1999). The Indian fish fauna comprises the endemic fish families’ forming 2.21% of the total bony fish families of the Indian region. The endemic fish species are found in India are 223, representing 8.75 per cent of the total fish species known from the Indian region. The richest diversity is found in the region of Western Ghats in India with respect to endemic freshwater fishes (Jayaram 1999) representing almost 40 fish families, of which 25 families contain commercially important species. In parts, freshwater fishes 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.

2.6. Note on taxonomic impediments

The rate of taxonomic studies from past two decades have been declined such that taxonomists themselves have become ‘endangered species’ if not already extinct (Gopal and Zutshi 1998). Field studies on aquatic communities generally identify the taxa at the generic level only (in case of aquatic insects and oligochaetes, mostly at the family level only). The identification and nomenclature system developed by Carl Linnaeus in 18th century based on morphological characters is followed by taxonomist even today after almost 230 years, that relays upon specific characters of individuals classifying them into hierarchy of phyla, division, class, order, family, genera and species. Although, this method is extremely useful in several cases to assign organisms to well defined categories, the use of morphological characters for species identification procedures has several disadvantages. As, this system leads to ambiguous delimitation because Linnaean categories are widely known to be subjective (Griffiths 1974, 1976; Minelli 2000; Ereshefsky 2002; Laurin 2005). There is a considerable morphological plasticity between organisms of the same species which may lead to incorrect identifications when employed for species identification. For instance it is difficult to differentiate eight species of Barbus from Iberian Peninsula based on morphological features only (Callejas and Ochando 2001). In parts, most of the factors like; interbreeding capabilities of the organism, ecological context and genetic similarities delimit species recognition. Taken together it is
necessary to develop multidisciplinary taxonomic approach that includes morphological and molecular data (Krzywinski and Besansky 2003). The morphological studies and molecular approach employing genetic markers (DNA markers) would certainly help taxonomists to resolve ambiguities at great extent, which is popularly known as molecular taxonomy, DNA taxonomy, etc.

2.7. Molecular taxonomy

The molecular methods based on the analysis of proteins such as, protein sequencing, protein electrophoresis, isoenzyme analysis, immunological reactions, etc. were employed in past few decades for species identification (Bolin et al. 1995; Skarpeid et al. 1998). However, these methods were having some limitations; proteins are degraded rapidly in samples under stress conditions, risk of cross reactions with proteins from closely related species and differential expressions of specific tissues (Skarpeid et al. 1998, McManus 1996). Consequently, protein based methods in species identification were circumvented with the advent of DNA over proteins. DNA can provide more information than protein due to degeneracy of the genetic code and possess large non-coding stretches. Furthermore, assessment of species was done by number of DNA markers, such as RAPD, RFLP, SSCP, DGGE, ALFP, sequencing and PCR specific primers. Focusing on the past decade, Teletchea (2009) revealed three methods competent for fish species identification, i.e., RFLP, sequencing and use of PCR specific primers. During past few years, advances in PCR and sequencing technology have led to rapid development reducing the chemical and
operational costs, it became a common tool for molecular based species identification. This method has been advocated under the FINS (Forensically Informative Nucleotide Sequencing) and proven as a most efficient and cost effective method for identifying animals (Bartlett and Davidson 1992). The identification of animal species using molecular genetics approach involves analysis of specific genes from mitochondrial DNA (16S rRNA, 12S rRNA, cytochrome b and cytochrome oxidase subunit-1) and nuclear DNA (18S, 28S, 5.8S and 5S rRNA). However, mitochondrial DNA and ribosomal RNA were considered as better target for analysis, as rRNA are slowly evolving genes, often do not differ among closely related organisms, but they are required in recovering ancient relationship, providing insights of cellular life (Woese 2000). On the other hand, mtDNA evolve rapidly to overwrite the trace of ancient affinities, but regularly reveal divergence between closely related species. The mitochondrial DNA could provide a record of evolutionary history within species, thereby linking population genetics, systematics and establishing the field of phylogeography. John Avise was the first to employ mt DNA to recognizing diversity among the animal species. Further, mt DNA assisted in identification of animals by accurately discriminating them in an appropriate taxa using specific genes (16S rRNA, 12S rRNA, cytochrome b and cytochrome oxidase subunit 1 (COI)). In 2003, mitochondrial fragment, COI was elected as a standard tool for molecular taxonomy and identification of animals (Ratnasingham and Hebert 2007). The total length of COI gene in vertebrates is about 1,540 bp, and a region about 650 bp long commencing near the start of the COXI reading frame was nominated as the barcode region or “Barcode of Life”.
2.7.1. DNA Barcoding

DNA barcoding is a micro genomic identification system developed by Paul Hebert, from University of Guelph at Canada in 2003, which discriminates life through the analysis of small segment of genome to diagnose the biological diversity. This system was brought up by an analogy of supermarket scanner that uses black stripes of the Universal Product Code (UPC) barcode to identify the item. The DNA barcode has similar concept, where four alternate nucleotides at each position creates 415 (one billion) alternate codes, a 100-fold surplus required to discriminate the diversity of life on our planet. It was demonstrated by using a very short sequence of Mitochondrial DNA relative to the entire genome that differentiated animal species, further revealing low genetic divergence within species than the divergence between species (Hebert et al. 2003a). This fact initiated a proposal, to standardize DNA based species identification by analyzing a uniform segment of the mitochondrial genome. With this approach, a library of sequence from taxonomically verified voucher specimens serves as DNA identifiers for species, in short, DNA barcodes (Hebert et al. 2003a).

The identification of biological species through this concept can be achieved by comparing the barcode sequence with a library of reference barcode sequences derived from known individuals. The specimen is identified if the unknown sequence closely matches to the reference sequence in barcode library. For instant, if the unknown sequence of the specimen is divergent from a known specimen with more than 2% for vertebrates and 3% for invertebrates it is likely that this is a different
species with 95% probability. Interestingly, Ward et al. (2008) made a revelation in identifying a second species of Asian sea bass (*Lates calcerifer*) based on COI sequence divergences. Avise and Walker (1999) also found that sister species usually showing pronounced mtDNA divergence, in other words sequence divergence is larger among the phylogeographically different species than within the species which were recognized by taxonomist as species. Taking advantage of this fact, taxonomic revisions at the species level now regularly include analysis of mtDNA divergence (Avise and Zink 1988, Gill and Slikas 1992, Murray et al. 1994, Banks et al. 2000, 2002, 2003). Another proposed method for distinguishing species is 10x concept, which suggest that if a pair of sequences diverges by at least 10 times the average divergence of a species group then the specimen will be a different species defining the barcode gap. This method was examined by Langhoff et al. (2009), Ward (2009), Lukhtanov et al. (2009), and Meyer and Paulay (2005) revealing that varation between and within species did not always find a distinct gap between species. Thus, this method of differentiating between species will not function with less divergent cryptic species (Ward 2009). Meyer and Paulay (2005) and Ward (2009) found that neither the 2% and 3% set divergence nor the 10x concept performed without making mistakes in delineating species.

Some studies have shown that sequence data from gene region other than COI can also be utilized as DNA barcode, the most significant species level research being in mitochondrial cytochrome b than in COI gene (Johns and Avise 1998; Page and Huges 2010; Sevilla et al. 2007; Teletchea 2009). Cytochrome b may be more
discriminating, and perform better than COI for specimen identification in some mammal species (Tobe et al. 2010). Consequently, there are large number of sequences for cytochrome b gene available on GenBank for fishes (Johns and Avise 1998; Page and Huges 2010; Sevilla et al. 2007; Teletcha 2009), but in contrast to the barcode application of COI, many of the cyt b data in GenBank frequently lack the associated voucher specimens essential for a reference library, and are not from consistent regions of the 1,140 bp gene (Broughton et al. 2001; Dawnay et al. 2007; Ward et al. 2009). Recently Fish-bol initiative for DNA barcoding of fishes has revealed that COI has overtaken cyt b in terms of number of sequences on GenBank (Becker et al. 2011; Ward et al. 2009). The COI barcodes have shown adequate resolution of even closely related species, and especially so for many fishes (Ward 2009; Ward and Holmes 2007).

DNA barcoding has now been demonstrated as an effective tool for identification in different animal groups (Hebert et al. 2003a, b; Hebert et al., 2004, Ward et al. 2005; Hajibabaei et al. 2006a; Smith et al. 2006). Also, a reference library of DNA barcodes is available on BOLD (Barcode of Life Data Systems; www.boldsystems.org; Ratnasingham and Hebert 2007, 2013). BOLD is a public database portal providing integrated environment for assembly and application of DNA barcodes, representing 2.1 million sequences for more than 0.1 million species all over the world.
2.7.2. Molecular phylogenetics

The species discrimination using sequence data has revolutionized phylogenetic or cluster analysis over the past two decades (Pagel 1999). This involves targeting a specific group for analysis (e.g. family), collection of primary information regarding morphology and geographic location and acquisition of DNA sequence data. These methods are discrete methods of data analysis that resolve phylogenies more accurate at deeper levels (Austerlitz et al. 2009), but are computationally more demanding (Baldauf 2003; Nei 1996). However, if we consider typical DNA barcode datasets, they are larger than most phylogenetic datasets, so NJ clustering is the most popular (Casiraghi et al. 2010; Goldstein and Desalle 2011). The species are confirmed based on their monophyletic relationship to the query sequence (Goldstein and DeSalle 2011). Thus, tree based methods have been repeatedly criticized (Little and Stevenson 2007; DeSalle et al. 2005; Goldstein and Desalle 2011; Meier et al. 2008; Will and Rubinoff 2004).

In case of character based methods each nucleotide is used as an independent source of information (DeSalle et al. 2005) that relays on shared similarity rather than overall similarity (Little 2011). This could work better for closely related taxa with few sequence information separating species (Lowenstein et al. 2009). It was implemented via CAOS program as the most common character based diagnostic method (Sarkar et al. 2008). Therefore, these character based methods are superior over distance approaches (DeSalle et al. 2005; Rach et al. 2008; Lowenstein et al.
2009). However, there have been few studies comparing the two approaches directly (Rach et al. 2008; Zou et al. 2011; Reid et al. 2011).

### 2.7.3. Fish barcoding

The DNA barcoding and molecular phylogenetic approach unified with traditional taxonomy discriminated fishes firstly from Australia by generating 754 barcodes for 207 species (Ward et al. 2005). Further, DNA barcoding became a reliable species identification tool that provoked Fish Barcode of Life Campaign (FISH-BOL) initiative ([http://www.fishbol.org](http://www.fishbol.org)). Within a decade, Ward (2009) catalogued 6000 species of barcoded fishes, which included 400 species from the New Zealand, 207 Australian commercial marine fish species, 250 species of marine fish from South African waters and 100 species of fish from Pacific Canada. In India, DNA barcodes of more than two hundred Indian marine fishes has been initiated and developed since 2006 by Persis et al. (2009) and Lakhra et al. (2011). Recently, Chakraborty and Ghosh (2013) have assessed 1383 barcode sequences of 175 Indian freshwater fish species from public databases available on BOLD and GenBank. Many studies covering larger geographic areas reported cryptic or potentially new species (Reviewed from studies of Pereire et al. 2013; Weigt et al. 2012; Steinke et al. 2009; Mabragan et al. 2011), with an average retrieval of 2% from those identified and classified so far (Bucklin et al. 2011). In this respect, Bucklin et al. (2011) extrapolated at that rate and revealed about 600 overlooked or cryptic species to be awaited for discovery. The Catalog of Fishes has currently listed 31,000 fish species, of which about 4000 have been described new during the past 10 years (2000–2009), with 500
added in 2008 and 300 in 2009 (Eschmeyer 2010). Today, Fish Barcode of Life (FISH-BOL) campaign has targeted 32,257 species, of which 10,185 have been barcoded and uploaded so far.

In addition to the species identification, DNA barcoding has been used for identification of processed fish products (Cohen et al. 2009, Lowenstein et al. 2009, 2010), with the U.S. Food and Drug Administration (FDA) recently validating DNA barcoding as an identification tool for marketplace seafood (Becker et al. 2011; Stoeckle 2012; Yancy et al. 2008). The DNA Barcoding approach was also applied for fish inspection in biosecurity and quarantine procedure by Armstrong and Ball (2005), but has yet to be scrutinized for many fishes in the aquarium trade (Collins et al. 2012a). Other applications for fisheries management and conservation have also been demonstrated (Holmes et al. 2009; Ogden 2008; Steinke et al. 2009; Wong et al. 2009).

2.8. Genetic diversity studies

Besides, generating DNA barcodes for identification of organisms it has also provided distorted views of biodiversity. Indeed, DNA barcoding surveys using partial cytochrome c oxidase subunit I (COI) sequences have demonstrated genetic diversity and species diversity from different geographic regions. Studies on genetic diversity of fishes that took place in different regions and among different genera showing great diversity in sequence divergence (Ward et al. 2008; Hubert et al. 2008; Ward et al. 2005). Moreover, to address the issues related to genetic variability,
typical population genetic studies determining population structure of species belonging to specific ecological system should be undertaken. This could be extended by selecting appropriate genetic marker. The selection of a suitable marker system for a population genetics survey depends upon the topic of interest defining the population level study.

2.9. **Molecular markers in population studies**

Selection of appropriate marker is prerequisite to reach to the conclusion in any population genetic study. The models of population such as haplotype diversity, haplotype network, gene flow, analysis of molecular variance and isolation by distance models can potentially provide useful information in this regard. As, mitochondrial DNA markers are haploid and maternally inherited, they are frequent targets for analysis and have made a particularly strong contribution to population studies (Avise 2004). However, markers like COI usually do not have sufficient phylogenetic signal to resolve these issues, especially at deeper levels (Hajibabaei 2006a). Subsequent makers from mitochondrial genome like cytochrome-b (coding region), D-loop (non-coding region) and microsatellites could be employed to determine relationship among individuals of a species.

The use of cyt-b and D-loop for population level studies has been from past two to three decades, the first set of versatile primers was published for portions of cytochrome b gene, the small ribosomal RNA gene and the major non-coding region
i.e. D-loop (Meyer 1994). These primers were found to amplify homologous portions of mtDNA in all classes of vertebrates and many other animal phyla (Kocher et al. 1989). The cyt-b gene was thought to be variable enough for population questions and conserved enough for deep phylogenetic questions (Kocher et al. 1989; Irwin et al. 1991; Edwards et al. 1991; Kornegay et al. 1993, Meyer and Wilson 1990). However, recent publications point out that cyt-b possess base compositional biases, rate variation between different lineages early saturation of third codon positions, and limited variation in the first and second codon position resulting in little phylogenetic information for deep evolutionary questions (Meyer 1994). The shortcomings of cyt-b could be circumvented by using alternate maker. The D-loop region might be better choice and offer good advantage than cyt-B. The D-loop or non-coding region possess certain conserved bases, but large parts are highly variable having rate of mutation five times higher than the rest of the organelle genome (Larizza et al. 2002; Horai and Hayasaka 1990; Vigilant et al. 1991). Also, it is the fastest evolving region in the mitochondrial DNA of vertebrates and invertebrates and therefore more sensitive than protein loci as a marker to score intraspecific variations of many organisms (Caccone et al. 1996; Avise 2000). This makes mt D-loop an effective marker for resolving the intraspecific evolutionary relationship among the individuals and population structure of the species belonging to different geographical locations. Along with D-loop, microsatellite marker could be used, as microsatellites are justifiably considered as the ideal markers for a wide range of applications in fisheries and aquaculture.
Moreover, D-loop region has been analyzed in several studies to define population structure and genetic variation through various methods like isolation by distance with a Mantel test (IBD Web service; Jensen et al. 2005), F-statistics and Analysis of Molecular Variance (Excoffier et al. 1992; Weir 1996; Weir et al. 1984) haplotype frequency and network structure, further revealing population connectivity in Indo-pacific reef fishes (Horne et al. 2008), Sub-tropical reef fish (Van der Meer et al. 2012), genetic variation in sword fishes (Alvarado Bremer et al. 2005; Alvarado Bremer et al. 1995, 1996; Rosel and Block 1996; Reeb et al. 2000), big eye tuna and blue fin tuna (Alvarado Bremer et al. 1997, 1998).