CHAPTER 2
REVIEW OF LITERATURE
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There have been several research articles, books and reviews were published from a long period of time. Many controversies are found in Indian turtle and tortoise systematics. To clarify this riddle many morphological and molecular approaches were involved and try to resolve this ambiguity. Hence, In Indian context we found limited molecular work have been finished to identified this animal and protect them in nature.

2.1 History of Indian turtle and tortoise:

The primary available works on reptiles of British India comprising of India, was ‘The Reptiles of British India’ by Gunther (1864). The work of Jerdon (1870) and Boulenger (1890) was mainly noted on Indian herpetofauna. Smith (1931) initiated the study on NE Indian Cheloniens with the publication of ‘The Fauna of British India, including Ceylon and Burma. Reptilia and Amphibia’. He proposed the distribution of Testudines in India as well as the state Assam. Subsequently, several studies have documented the diversity and distribution of turtle and tortoise in this region. He synonymized the new subspecies under *Trionyx gangeticus* and other two subspecies in *Lissemys punctata granosa* and *Kachuga tectum tentoria*.

Pritchard (1979), reported ‘Living Turtle of the World’ providing a systematic exposure of all the worlds turtle in, ‘Encyclopaedia of Turtle’ to providing the diversity of turtle and their many wonderful adaptations, taxonomy and natural history of Cheloniens. Daniel (1983) published ‘The book of Indian Reptiles’ which describes the natural history of reptiles commonly occurring in the Indian subcontinent.

The work of Biswas and Acharjyo (1984) on the aquatic Cheloniens of eastern regions of India is the only important work on freshwater turtle. He described one new subspecies, *Trionyx gangeticus mahanaddicus* and reported several other species like: *Trionyx leithi, Emyda punctata granosa* and *Kachuga tectum intermedia*.

Das (1990, 1991) also published a ‘Colour guide to the turtle and tortoise of the Indian subcontinent’. A few studies also indicate that though turtle trade is rampant in North India, South India and NE India, it is rather localized and difficult to quantify. ‘Turtle and tortoise of India’ and ‘A photographic guide to snakes and other reptiles of India’ were published by Das (1995) and Choudhury (1995). Nevertheless, several studies from India have investigated the breeding biology and reproductive ecology of turtle and tortoise (Bury 1979; Frazier and Das 1994; Das 2008).

Studies on turtle and tortoise in NE India have so far largely remained confined to inventorying of taxa and distributional aspects. Virtually no information is available on different aspects of chelonian ecology and biology in NE India. Choudhury 1995, reported the contents of the reptile type collection of the Zoological Survey of India, head quartered in Calcutta, with several regional stations in the country, which incorporates the holdings of the Asiatic Society of Bengal and the Indian Museum. In all, 556 primary and secondary types are present, including 289 name-bearing types (comprising 27 Chelonii).

Barman (1996) reported the occurrence of Indian peacock softshell turtle in this region. Several studies from South East Asian countries and other parts of the world have also looked into various aspects of Chelonian ecology and biology such as; habitat utilization patterns, niche divergence, food and feeding habits (Talukdar 1979; Dutta 1997; Datta 1998; Gupta 2002; Das and Gupta 2004). Further several field study and documentation was carried out in northern, western, southern as well as NE India by different taxonomic working groups and conservationist for knowing
the exact distribution of Indian turtle and tortoise (Pawar and Choudhury, 2000; Webb 2003). Adler, 2007 described the development of systematic reviews of the turtle of the world. Jensen and Das (2008) surveyed at two sites in Sarawak: Loagan Bunut National Park and Balai Ringin. Both soft shelled and hard shelled turtle were more active during overcast periods. Seasonality did not seem to affect soft shelled turtle activity, while hard shelled turtle were active 50.0% of the time during the dry South-west Monsoon from June to September. This literature was followed by Baruah and Sharma (2010) who published several papers on Chelonians distribution and described a few species from Indian subcontinent. Das et al. (2010 b) also contributed a book on ‘The discovery of Indian Turtle’ that gives information to the biology, ecology and conservation of the herpetofauna for a lay men point of view. The trade of freshwater turtle and tortoise is one of the major contributing factors for decline of chelonian diversity and density all over the world, especially in South and South-East Asian countries.

Ravindranath et al. (2011) indicated that majority of the districts in NE India are subject to climate induced vulnerability currently and in the near future. This is a first of its kind study that exhibits ranking of districts of NE India on the basis of the vulnerability index values. To protect the importance groups of the earth many initiatives were contracted in various time throughout the country.

Bohm et al. (2012) presented the first ever global analysis of extinction risk in reptiles, based on a random representative sample of 1500 species (16%of all currently known species). The proportion of threatened reptile species is highest in freshwater environments, tropical regions and on oceanic islands, while data deficiency was highest in tropical areas, such as Central Africa and Southeast Asian reptiles. Conservation actions specifically need to mitigate the effects of human induced habitat loss and harvesting, which are the predominant threats to reptiles. There have been other sporadic attempts to breed species in captivity at the Madras Crocodile Bank Trust but these have not yet amalgamated with conservation requirements or ensured the survival of entire India’s turtle population (Whitaker 2012).
2.2 Species specific review of all extant Testudines species in east and NE India:

The main objectives were “conservation action plan for Indian endangered turtle and tortoise” and prioritize important turtle areas for conservation action across India. To coordinate any conservation program, knowing the species-specific life history is most authoritative. The species wise reviews are given bellow:

Das and Pritchard (1990) and Dutta (1997) proposed that Melanochelys trijuga, is one of the most abundant Chelonian in the Indian subcontinent, with a distribution extending from Sri Lanka, through India and Burma, to western Thailand, although apparently excluding Bangladesh. Melanochelys trijuga is basically a pond turtle, and the Western Ghats appear to separate the essentially lowland ranges of Melanochelys coronata and Melanochelys trijuga. Das (2009 a) presented the observation on the natural history, distribution, morphology, ecology and exploitation of the freshwater and terrestrial Chelonains of Nepal. They included data on ten of the eleven Chelonian species record from Nepal and confirm the previously presumed presence of Melanochelys tricarinata and Aspideretes hurum. They documented a range extension for the flapshell turtle, Lissemys punctata, and provide new information on the life history and growth of the black pond turtle, Melanochelys trijuga, and the tricarinate hill turtle, Melanochelys tricarinata.

Engstrom et al. (2002) reported that Southeast-Asia holds the richest diversity of terrestrial turtle in the world, encompassing over 25 % of the world’s Chelonian species. At present a minimum of 12 to 20 million freshwater turtle and tortoise are consumed in Asia each year. The trade in turtle constitutes a large proportion of the trans-border wildlife trade between certain Asian countries. This trade seems virtually unregulated. Pritchard in 2000 finally cleared up the taxonomic nightmare and determined that Indotestudo forstenii was indeed a distinct species and not, as Crumly (1984) previously suggested a result of transplanted Indotestudo travancorica from India. Tobe et al. (2009) examined these clades based on variation in 1094 bp of the mitochondrial cytb gene, and found that Indotestudo travancorica, the species in question here, was found to be more closely related to Indotestudo elongata. The analysis offers no support for the
hypothesis that Indonesian populations of *Indotestudo forstenii* represent introductions of *Indotestudo travancorica* from India. The recognition of three nominal species (*Indotestudo elongata*, *Indotestudo forstenii* and *Indotestudo travancorica*) in the genus is thus warranted.

Das and Bhupathy (2009 a) described that the crowned river turtle, *Hardella thurjii*, is a large freshwater turtle, largely confined to lentic waters in the Indian subcontinent. The species shows extreme sexual size dimorphism, females attaining a length over three times that the males. Heavy exploitation for its flesh and extensive wetland development projects are among the factors suspected to have made the turtle rare in localities where it was once common, although there is some evidence that the species is difficult to observe in the wild. Das (2009 b) described that the tricarinate hill turtle, *Melanochelys tricarinata* is a small entirely terrestrial turtle from the deciduous and evergreen forest and riverine grasslands of the northern Indian subcontinent. The species appears to be rare, and is poorly known. Das and Singh (2009) described about the narrow-headed softshell turtle, *Chitra indica*, is an extremely large, highly aquatic species. It is widely distributed on the Indian subcontinent. Population sizes are unknown, but the species appears to be threatened by human exploitation and modification of its riverine habitat.

Das and Bhupathy (2009 b) described that the Indian black turtle, *Melanochelys trijuga*, is a medium sized, mainly still water species from northern, NE, and peninsular India. Six subspecies are currently recognized. The turtle has been introduced to some of the islands of the western Indian Ocean by seafarers. The species although in no immediate danger in India, is exploited in unknown numbers for food, and population declines have been reported from Sri Lanka. Das and Bhupathy (2010) reported that the spotted pond turtle, *Geoclemys hamiltonii*, is a medium sized freshwater turtle from lentic water bodies of the northern and NE Indian subcontinent. The species has been reported to be rapidly declining in Bangladesh, but populations in a few protected areas in India are apparently relatively abundant. Das (2010 a, b) reported that the Indian eyed turtle, *Morenia petersii*, is a small freshwater turtle with a fairly restricted distribution in northern, eastern and NE Indian, southern Nepal and Bangladesh. Das et al. (2009, 2010 a)
reported that the Assam roofed turtle, *Pangshura sylhetensis*, is a small freshwater turtle. It occurs primarily in running waters in the hills of NE India and northeastern and southeastern Bangladesh, where it appears rare and localized. Das et al. (2010 a, b) described that the Indian peacock softshell turtle, *Nilssonia hurum*, is a relatively abundant large riverine species that is found in rivers and reservoirs. It is distributed over eastern Pakistan, northern and central India, Bangladesh and Nepal. The species is heavily exploited for its meat and calipee throughout NE and eastern India and Bangladesh.

Binying et al. (2011) addressed the social issue of conservation and target the threats it faces. Moreover, many are sceptical about the effectiveness of local freshwater conservations. The lack of awareness the public has for the terrapin resulted in its triviality, and our solutions aim to help by increasing awareness of native species and allowing people to experience a personal connection with the terrapin. Schoppe and Das (2011) described that the Southeast Asian Box Turtle, *Cuora amboinensis*, is a small, semi-aquatic turtle, largely restricted to standing water bodies of Southeast Asia, from eastern India to Indonesia and Philippines. It has four currently recognized subspecies: *Cuora amboinensis amboinensis*, *Cuora amboinensis coura*, *Cuora amboinensis kamaroma* and *Cuora amboinensis lineate*. The species is currently listed as vulnerable by the IUCN Red List due to its heavy exploitation for the international food, pet and medicinal trade, and is also included in CITES Appendix II (UNEP-WCMC 2012). Fritz and Havas (2007), Baruah and Sharma (2010) and Praschag and Gemel (2002) reported the *Nilssonia nigricans* in a pond of Bangladesh.

Das and Gupta (2011) described that eight species of turtle belonging to the family Trionychidae (Reptilia: Chelonia) are known to occur in India and include *Nilssonia gangetica*, *Nilssonia hurum*, *Nilssonia leithii*, *Nilssonia nigricans*, *Chitra indica*, *Amyda cartilaginea*, *Pelochelys cantorii* and *Lissemys punctata* (two subspecies - punctata and andersonii). All the species barring *Nilssonia leithii* and *Pelochelys cantorii* have been reported from parts of Assam, Meghalaya and Mizoram in NE India. However, most of the records are from the Brahmaputra plains.
of Assam and adjoining areas in lower eastern Himalaya, parts of Meghalaya and Mizoram.

Turtle Taxonomy Working Group (2012) reported that five turtle species, *Cuora amboinensis*, *Cuora mouhotii*, *Cyclemys gemeli*, *Melanochelys tricarinata* and *Indotestudo elongata*, are reported for the first time from the Himalayan Kingdom of Bhutan. The record of *Cuora mouhotii* represents a significant range extension to the west; that of *Cuora amboinensis* represents a range extension across a political border from the same landscape in adjacent NE India; and those of *Cuora gemeli*, *Melanochelys tricarinata*, and *Indotestudo elongata* fill geographic gaps in their known distributions. Das et al. (2012a) listed a total of 10 species of amphibians and 42 species of reptiles from the area. Besides recording members of currently recognized species complexes, the study also documents species that were either conferred to closely related species or their identity remains to be ascertained. Deepak et al. (2011) and Whitaker (2012) reported that most threatened Chelonians occur in Asia including the two endemic Chelonians, *Indotestudo travancorica*, and the sympatric *Vijayachelys silvatica* in the Region of the Western Ghats. Phylogenetic relationships between the three species of Indotestudo, these being forstenii, elongata, and travancorica, have been in flux. Pritchard (2000) and Ives (2006) also described *Indotestudo forstenii* from Sulawesi and Halmahera, as a distinct species unrelated to *Indotestudo travancorica* and *Indotestudo elongata*, refuting information that *Indotestudo forstenii* were introduced from India to Indonesia. Schaffer and Morgan (2002) proposed the two subspecies of *Manouria emys* species and pointed their intergradation in Southeast Asia.

2.3 Molecular approaches for deciphering the Testudines systematic and evolutionary relationship:

Avise et al. (1992), Meylan (1987) and Shaffer et al. (1997) suggested a slowdown in mean micro evolutionary rate for turtle mtDNA. Within each of six species of Testudines sequence divergence estimates derived from restriction assays are consistently lower than expectations based on either the dates of particular geographic barriers with which significant mtDNA genetic clades appear associated
or the magnitudes of sequence divergence between mtDNA clades in non-turtle species that otherwise exhibit striking phylogeography concordance with the genetic partitions in turtle. Bowen et al. (1993) examined the nucleotide sequences from the *cytb* gene of mtDNA were employed to resolve phylogenetic controversies and to assess molecular evolutionary rates in marine turtle (Chelonioida) and constantly shared the molecular phylogenetic approaches of turtle and tortoise to the scientific community.

Gaffney and Meylan (1988) and Caspers et al. (1996) reevaluated the phylogenetic relationships among the major groups of amniotes vertebrates remain a matter of controversy. To discover the phylogenetic position of turtle in relation to mammals and birds, they have determined cDNA sequences for the eye lens proteins of the red-eared slider turtle (*Trachemys scripta elegans*). All sequences were analysed by three phylogenetic tree reconstruction methods (Neighbour-Joining, Maximum Parsimony, and Maximum Likelihood). Kitimasak et al. (2005) studied the Global phylogeography of the ridley sea turtle and comparative molecular phylogeography of North American softshell turtle (Apalone): implications for regional and wide-scale historical evolutionary forces.

Zardoya and Meyer (1998) noticed that the despite more than a century of debate, the evolutionary position of turtle (Testudines) relative to other amniotes (reptiles, birds, and mammals) remains uncertain. To address this question, the complete mitochondrial genome sequence of the African side-necked turtle (*Pelomedusa subrufa*) was determined. Phylogenetic analyses of the complete mitochondrial genome sequences supported the placement of turtle as the sister group of an alligator and chicken (Archosauria) clade.

Zardoya and Meyer (2001) determined the nucleotide sequence of the African side-necked turtle mitochondrial control region and it’s flanking *tRNA* genes. Several conserved motifs involved in the regulation of the mitochondrial genome replication process, including one conserved sequence block (CSB1), and three termination-associated sequences were identified. The potential usefulness of this microsatellite sequence for population level studies is enhanced by its unique localization in the
maternally inherited mitochondrial molecule (Vamberger et al. 2011). Walker et al. (1998) generated and employed sequence data from mtDNA to address the phylogenetic distinctiveness and phylogenetic position of *Sternotherus depressus*, the flattened musk turtle to all other musk and mud turtle in North America and also suggested that these species forms a well-supported monophyletic group separate from *Sternotherus minor*.

Cacccone et al. (1999) reported a phylogenetic reconstruction based on mtDNA sequences from Galapagos tortoise and Geocheleon from mainland South America and Africa. The split between *Geocheleon chilensis* and the Galapagos lineage probably occurred 6 to 12 million years ago, before the origin of the oldest extant Galapagos Island. They have sequences identical to Lonesome George, consistent with his being the last survivor of his subspecies. Gaffney (1975 b) and Rieppel and Reisz (1999) reported a critical re-examination of turtle relationships continues to support a sister-group relationship of turtle with a clade of marine reptiles, Sauropterygia, within crown-group Diapsida (Sauria). The high homoplasy index raises concerns about the phylogenetic information content of various morphological characters in broad scale phylogenetic analyses.

Weisrock and Janzen (2000) used a comparative analysis of partial *cytb* sequences to evaluate the evolutionary forces shaping wide-scale phylogeographic patterns of all three North American softshell turtle (*Apalone ferox*, *Apalone mutica*, and *Apalone spinifera*). The overall phylogeographic patterns are concordant with results from both extensive regional studies of south-eastern species, implicating historical variant processes during the *Pliocene* and *Pleistocene*, and investigations of more northerly distributed species, indicating a bottleneck effect of recent dispersal into postglacial habitat. The extensive phylogenetic structure and sequence divergences within both *Apalone mutica* and *Apalone spinifera* contrast sharply with most previous studies of turtle and with the hypothesis that turtle in general have slow rates of mtDNA evolution. Rieppel (2000) reviewed the neontological character evidence for turtle relationships and shows that most, but not all, of these characters are invalid in the reconstruction of turtle relationships within Amniota.
Hedges and Poling (1999) and Cao et al. (2000) suggested that turtle are closely related to archosaurs (birds + crocodilians), and a tree in which turtle are basal to other amniotes were rejected with high statistical significance. Therefore, they re-analysed their data using the maximum likelihood method, and evaluated the total evidence of the analyses of mitochondrial and nuclear data sets. McCord et al. (2000) placed *Geoemyda yuwooni* in a new monotypic genus, Leucocephalon, distinguished by its maxillary contact and lack of plastral hinge.

Zardoya and Meyer (2001) reported that recent morphological and molecular studies have radically changed our view of amniotes phylogenetic relationships, and evidence is accumulating that supports the diapsid affinities of turtle. Accepting these hypotheses implies that turtle cannot be viewed any longer as primitive reptiles, and that they might have lost the temporal holes in the skull secondarily rather than never having had them. They proposed that, Maximum likelihood mixed stock analysis was used to identify the natal origin of immature loggerhead turtle (*Caretta caretta*). These observations suggest that dispersal of loggerheads into benthic developmental habitats from the pelagic environment is not random.

Feldman and Parham (2001) and Engstromn et al. 2002 proposed that, the intense exploitation of turtle in Asian markets has contributed to declines in turtle populations across the continent. Individuals from Thailand have been described as a separate species, *Chitra chitra*, but this has not been universally accepted, and many sources consider *Chitra* monotypic. Phylogenetic analysis of sequence data from the mitochondrial *ND4* gene revealed three deeply divergent, monophyletic lineages within *Chitra*: *Chitra indica*, *Chitra chitra*, and a third unnamed form from Myanmar. McCord and Pritchard (2002) have presented a taxonomic revision of the Trionychid turtle genus Chitra, in which they describe one new species (*Chitra vandijkii*) from Myanmar, name two subspecies within *Chitra chitra* and consider *Chitra indica* to be monotypic across its wide range from Pakistan to Bangladesh.

Engstrom and McCord (2002) proposed that DNA sequence data from mitochondrial *ND4* gene, which support their taxonomic conclusions. There is deep
molecular divergence among the three recognized species of Chitra and a low level of geographically structured variation within *Chitra chitra* corresponding with mainland and island subspecies. In contrast, *Chitra indica* shows almost no molecular variation across its broad range from Pakistan to Bangladesh. Feldman and Parham (2002) examined the evolutionary relationships of emydine turtle using 2092 bp of DNA encoding the mitochondrial genes *cytb, ND4*, and adjacent *tRNAs*. Based on this phylogeny, and previous molecular work on the group, they suggested the following taxonomic revisions. Furthermore, the analyses show that neither kinetic-shelled nor akeinetic-shelled emydines form monophyletic groups.

Schwartz et al. (2003) described the development of nine microsatellite loci from the gopher tortoise and found these loci to be highly variable and thus likely to be applicable in parentage and population level analysis of *Gopherus polyphemus*. Barth et al. (2004), Fritz et al. (2005) compared 1036 bp of the mitochondrial *cytb* gene from all six Mauremys species with 16 other taxa, representing both currently recognized subfamilies of the Geoemydidae (Geoemydinae and Batagurinae) to contribute a comprehensive dataset towards resolving the conflicting Mauremys taxonomy and phylogeography. The results contradict this traditional zoogeographical scheme and the current taxonomy of the Geoemydidae.

Beheregaria et al. (2004) elucidated the history of diversification of giant Galapagos tortoise by using mtDNA sequences from 802 individuals representing all known extant populations. The endangered giant Galapagos tortoise represent a rapid allopatric radiation and further exemplify evolutionary processes in one of the world’s greatest natural laboratories of evolution. Stuart and Parham (2004), Spinks et al. (2004), Spinks and Shaffer (2006, 2007), Spinks et al. (2009) and Stephens and Wiens (2003, 2009) proposed the phylogenetic hypotheses for the turtle family Geoemydidae and focused the mitochondrial introgressions, numts, and their inferences from multiple nuclear loci.

Engstrom et al. (2004) represented a phylogenetic hypothesis and novel, rank-free classification for all extant 24 species of softshell turtle (Testudines: Trionychidae). The combined data set of nuclear and mitochondrial genome
provided complete taxonomic coverage for this globally distributed clade of turtle, which is in accord with many aspects of traditional softshell systematics including the monophyly of the Cyclanorbinae and Trionychinae. Fujita et al. (2004) proposed that, Introns have gained considerable popularity as markers for molecular phylogenetics. Maximum parsimony and maximum likelihood both demonstrated the polyphyly of Trionychoidea and the reciprocal monophyly of Australian/New Guinea and South American chelid turtle. This is the first study to resolve such relationships with strong statistical support, and suggested that R35 holds great promise for resolving additional persistent problems in the phylogeny of living turtle. They focused the systematics of turtle and tortoise using molecular phylogeny approaches. The existing systematics of softshell turtle species are then reconstruct and clustered under new tribes.

Kitimasak et al. (2005) proposed that, the distribution range and status of Chitra species in Thailand were investigated. *Chitra chitra* is so far known only from the Mae Klong and Chao Phraya river systems. Another species, *Chitra vandijki* was reported to occur in the Salween river system located along the Thailand-Myanmar border (McCord and Pritchard 2002). At present, the status of Chitra species is very rare everywhere and the natural population seem to be declining. Krenz et al. (2005) were sequenced >90% of *RAG-1* for 24 species representing all modern turtle families. *RAG-1* exhibited negligible saturation and base composition bias, and extensive base composition homogeneity. This research highlights the utility of molecular data in identifying issues of character homology in morphological datasets, while shedding valuable light on the biodiversity of a globally imperilled taxon.

Parham et al. (2006 a, b) reported the complete mitochondrial genome sequences of 10 species and a14 kb sequence from an eleventh species, as part of an on-going project to generate a mitochondrial database for terrestrial tortoise based on museum specimens. Two unusual genomic features are reported for the mtDNA of one tortoise *Mantidactylus tornieri*. They proposed that the wide-ranging spur-thighed tortoise (*Testudo graeca*) has recently been split into a complex of species. They sequenced a rapidly evolving mitochondrial marker for 30 specimens of
Testudo graeca from localities tied to the newly proposed morphology-based names in the Middle East. They strongly recommend that future systematic studies should strive to compare different data types to avoid misleading taxonomic changes. Le et al. (2006) included 32 Testudinid species (all genera and subgenera), and all species of Geochelone, representing 65% of the total familial species diversity), and both mitochondrial (12S rRNA, 16S rRNA, and cytb) and nuclear (Cmos and Rag2) DNA data with a total of 3387 aligned characters. Based on our systematic findings, they also proposed modifications concerning Testudinidae taxonomy.

Peng et al. (2005) proposed that the mitochondrial genome of the Chinese big-headed turtle, Platysternon megacephalum, was obtained using polymerase chain reaction (PCR). The entire mtDNA sequence, the longest mitochondrial genome in turtle reported so far, is 19161 bp. This mitochondrial genome exhibits a novel gene order, which greatly differs from that of any other vertebrates. Parham et al. (2006 a, b) and Fritz et al. (2006) proposed that the study robustly determines the phylogenetic placement of Platysternon and provides a well-resolved outline of major turtle lineages, while demonstrating the significantly greater resolving power of comparing large amounts of mtDNA sequence over that of short fragments. The duplicated control regions and gene rearrangements of the Platysternon mtDNA probably resulted from the duplication of part of the genome and then the subsequent loss of redundant genes. Praschag et al. (2006) described that systematic position of the rare Indian turtle Geoemyda silvatica is examined by a phylogenetic analysis of mtDNA sequence data (cytb gene) of most species of Geoemydidae previously. According to Bayesian analysis of mtDNA data, Melanochelys trijuga could be distantly related to Vijayachelys silvatica, whereas the morphological similarity of the other species probably is the result of a similar mode of life.

Engstrom et al. (2007) proposed that, molecular markers have proven to be a powerful tool for research on turtle. They also reported primers for 11 nuclear coding genes and introns and finally provided primer sequence, amplicon size, and number of observed alleles for 181 microsatellite loci from all major clades of living turtle. Jing et al. (2007) established a molecular phylogeny, with a 1725 bp fragment of mitochondrial DNA, using samples from three live individuals.
of *Cuora yunnanensis*, and found that the three newly discovered individuals and the old museum specimen of *Cuora yunnanensis* are very similar both in morphology and in mitochondrial DNA sequence, and thus the species is not extinct. The phylogenetic analysis also demonstrates that *Cuora yunnanensis* is not of recent hybrid origin, but rather represents a distinct evolutionary lineage.

Williams and Osentoski (2007) and Fritz et al. 2008 reviewed the genetic issues that need to be considered when establishing captive breeding colonies of tortoise and turtle for eventual re-introduction or population supplementation. Gjedrem and Baranski (2009) discussed how molecular methods and the concepts of evolutionarily significant units (ESUs) can be used to define breeding units for captive breeding colonies and determine their geographic origin for reintroduction. Fong et al. (2007) surveyed mitochondrial and nuclear DNA variation of the heavily exploited *Mauremys mutica* complex, a clade of Asian turtle that contains the endangered *Mauremys mutica* and critically endangered *Mauremys annamensis*. Haitao et al. (2007) and Zhang et al. (2008) proposed that, the Black Bridged Leaf Turtle, *Cyclemys atripons* is a poorly known species within the genus Cyclemys. They determined the complete nucleotide sequence of the *Cyclemys atripons* mitochondrial genome (mtDNA) and found it to be 16,500 base pairs in length, with the genome organization, gene order and base composition being identical to that of the typical vertebrate. Comparisons with three other Geoemydids showed that the *Cyclemys atripons* control region contained a highly variable region at the 3’ end composed of AT enriched tandem repeats containing a fifteen-unit variable number of tandem repeats (VNTRs).

Hsieh et al. (2008) reported the identification of fragmented turtle shells by *cytb* gene and Vargas-Ramirez et al. (2008) reported the eight extant Podocnemidid species are the last survivors of a species ancient group of turtle known to have existed since the Cretaceous. Phylogenetic relationships of all extant species were reconstructed from six mitochondrial and six nuclear DNA fragments in separate and combined analyses. The extant Podocnemis species evolved from the Late Eocene to the Middle Miocene, during a phase characterized by dramatic global cooling, and massive Andean uplift. Sterli (2008) presented here sheds new light on early turtle
evolution. An updated cladistics analysis of turtle shows that fossil turtle are not crown turtle, but stem turtle. This cladistics analysis also shows that stem turtle were more diverse than previously thought, and that until the Middle to Upper Jurassic there were turtle without the modern jaw closure mechanism.

Poulakakis et al. (2008) used mtDNA and microsatellite data obtained from museum specimens to show that the population on Floreana was evolutionarily distinct from all other Galapagos tortoise populations. Surprisingly, they found that these ‘non-native’ tortoise from Isabela are of recent Floreana ancestry and closely match the genetic data provided by the museum specimens. Le et al. (2006, 2007) described a new 220 million year old turtle from China, somewhat older than Proganochelys, that documents an intermediate step in the evolution of the shell and associated structures. Phylogenetic analysis places the new species basal to all known turtle, fossil and extant. The marine deposits that yielded the fossils indicate that this primitive turtle inhabited marginal areas of the sea or river deltas. Ives et al. (2008) examined morphological and genetic variation to determine if the nuchal scute is correlated with size or genetic divergence. The results indicate a strong correlation between size and nuchal scute such that turtle lacking a nuchal scute were larger than those possessing a nuchal scute. However, they also found no correlation between genetic divergence and presence/absence of nuchal scutes, and thus no evidence of genetically differentiated lineages.

Parham and Irmis (2008) and Reisz and Head (2008) described that during the Late Triassic, some 220 million years ago, and primitive turtle about 40 centimetres in length were preserved in sedimentary deposits in what is now southwestern China. These fossils are examples of a new species of a very early turtle, named Odontochelys semitestacea, and which will change ideas about turtle origins and the evolution of their striking body plan. Zhang et al. (2008) proposed that the complete mitochondrial genome from the Keeled box turtle (Pyxidea mouhotii) was determined. Nevertheless, the results strongly supported that 1) Pyxidea mouhotii and Cuora aurocapitata formed a monophyletic clade, whereas Cyclemys atripons was not closer to the Pyxidea- Cuora than to Chinemys reevesii, suggesting that Cyclemys and the Cuora group (containing Pyxidea) may have originated from two
ancestors; 2) the Geoemydidae with Testudinidae was a sister group rather than with the Emydidae. Naro-Maciel et al. (2008) focused the molecular marker for the identification turtle species and described evolutionary relationships of turtle: A molecular phylogeny based on nuclear and mitochondrial genes. Parham (2008) rediscovered the extinct Galapagos tortoise.

Alacs et al. (2009 a) proposed that, using genetic data and concepts when developing conservation strategies for turtle. Finally, they briefly discuss the Phylocode and DNA barcoding as examples of the new directions in which taxonomy may be moving. Werneburg and Sanchez-Villagra (2009) described the timing of events in organogenesis of diverse character complexes in all body regions is not uniform across amniotes and can be analysed using a parsimony-based method. Changes in the relative timing of particular events diagnose many clades of amniotes and include a phylogenetic signal. The clear signal of a basal position of turtle provided by heterochronic data implies significant convergence in either molecular, adult morphological or developmental timing characters, as only one of the alternative solutions to the phylogenetic conundrum can be right. Das and singh (2009) proposed that, the narrow headed softshell turtle, Chitra indica is an extremely large and highly aquatic species. It’s widely distributed on the Indian subcontinent.

Praschag et al. (2009) described the external morphology and habitat of Cyclemys gemeli, a recently discovered leaf turtle species from NE India. Further they assess the phylogenetic position of Cyclemys gemeli using mitochondrial cytb gene as well as nuclear DNA fragments. Spinks and Shaffer (2009), described that time calibrated phylogenetic analysis can provide basic insights into the tempo of turtle evolution and molecular phylogenetic analysis has confirmed that most families are demonstrably monophyletic, as are Cryptodira and Pleurodira. He also demonstrates that many of the most endangered clades are over 100 million years old and are represented by one or a few species, while the most species rich families tend to be relatively young. Wiens et al. (2010) showed widespread discordance between phylogenies based on mtDNA and nuclear DNA in a phylogenetic analysis of the turtle family Emydidae. The overall results illustrated that the potential
dangers of making inferences about phylogeny, speciation, divergence times and conservation from mtDNA data alone and suggest the benefits of using large numbers of unlinked nuclear loci. Chandler and Janzen (2009) augmented existing genetic datasets with nucleotide sequences from RAG-1, R35 and from the nuclear gene to address the phylogenetic relationships of the Chelydridae. The findings support for a sister group relationship between Chelydridae and either the clades Kinosternoidea (Kinosternidae + Dermatemydidae) or Chelonioidea (Cheloniidae + Dermochelyidae).

Baruah and Sharma (2010) proposed that, the paper reports 23 turtle species so far recorded from NE region of India, belonging to 3 families namely Geoemydidae (15 species), Trionychidae (6 species) and Testudinidae (2 species). Out of 23 species found in NE regions, 6 species namely Pangshura sylhetensis, Pyxidea mouhotii, Nilssonia nigricans, Chitra indicia, Manouria emys and Indotestudo elongata are listed by IUCN as the most endangered species of Asia. Chitra indica is on the new list of the 25 most endangered turtle species of the world. Another significant contribution was a taxonomic review of the softshell turtle of the genus Chitra, with a description of new taxa from Myanmar and Indonesia (Java).

Barley et al. (2010) used 14 nuclear loci to provide an in depth look at several of these troublesome nodes and infer the systematic relationships among 11 of the 14 turtle families. They also recover strong support for a sister relationship between the Emydidae and the monotypic big headed-turtle, Platysternon megacephalum. They also find strong support for a clade consisting of sea turtle, mud and musk turtle, and snapping turtle. The results emphasize the utility of multi-locus datasets in phylogenetic analyses of difficult problems.

Sterli (2010) contributed focuses on the former problem, exploring the phylogenetic relationships of extant and extinct turtle based on the most comprehensive phylogenetic dataset of morphological and molecular data analysed to date. This evolutionary scenario of a rapid diversification of modern turtle into
the major modern lineages is likely the reason for the difficulty in determining the interrelationships and the position of the root of crown-group turtle. Fritz et al. (2011) suggested that the closely related, but morphologically distinct genus Pelomedusa is paraphyletic with respect to Pelusios, and that Pelomedusa consists of nine deeply divergent lineages. Using three mitochondrial and three nuclear DNA fragments, they examined for the first time the phylogeny of Pelusios by molecular means.

Al-Mohanna and George (2010) suggested that the Neighbour-joining tree analyses with sequences available in the GenBank showed that it had a close relationship with those of the Atlantic colonies. For adult Loggerhead Turtle of the Atlantic colonies which undertake long distance migrations, the southern extension of Africa might be less formidable as a continental barrier to their passage into the Indian Ocean and subsequently into the Arabian area because of their temperate distribution. Wang et al. (2011) suggested the complete mtDNA sequence provide better resolution in species delineation of Testudines species. Angielczyk et al. (2011) assessed the contributions of function, phylogeny, and habitat to patterns of plastron shape variation in emydine turtle. They evaluated the fit of various evolutionary models to the shape data to investigate the selective landscape responsible for the observed morphological patterns.

van Dijk et al. (2000) proposed an annotated checklist of all recognized and named taxa of the world’s modern chelonian fauna, documenting recent changes and controversies in nomenclature, and including all primary synonyms, updated from previous four checklists (Turtle Taxonomy Working Group 2011, 2012). The diversity of turtle and tortoise in the world that has existed in modern times, and currently generally recognized as distinct and included on this checklist, now consists of 331 species. Turtle are among the most endangered of the major groups of vertebrates, surpassing birds, mammals, cartilaginous or bony fishes, and amphibians. Praschag et al. (2007 b), Praschag et al. (2011) and Hedges (2012) described that the position of turtle among amniotes remains in dispute, with morphological and molecular comparisons giving different results. Molecular studies
have not wavered as the numbers of genes and species have increased, but morphologists have been reluctant to embrace the molecular tree.

Fong et al. (2012) compiled a large cDNA/EST dataset for vertebrates (75 genes) to address these outstanding questions. Gene-specific phylogenetic analyses revealed a great deal of variation in preferred topology, resulting in topologically ambiguous conclusions from the combined dataset. After removing putatively biased sites, support emerged for a sister relationship between turtle and either crocodilians or archosaurs, as well as for a caecilian salamander sister relationship within Lissamphibia, with Lissamphibia potentially paraphyletic.

Drosopoulou et al. (2012) presented the complete sequence of the Caretta caretta mitochondrial genome is presented here. The genome comprises 16,440 base pairs, containing 37 genes (13 protein-coding genes, 22 tRNA genes, and 2 rRNA genes), and a control region, all organized similar to the majority of vertebrate mitogenomes. Furthermore, phylogenetic analyses among Testudines based on complete mitogenomes, as well as among marine turtle based on partial mtDNA sequences, are considered.

Parham et al. (2012, 2013) investigated a group of freshwater turtle (Trachemys) from a part of its range where it is purported to have undergone reticulation events from both natural and anthropogenic processes. Finally, they tested the impact of introgressed alleles using the multispecies coalescent in a Bayesian framework and show that studies that do not phase heterozygote sequences of hybrid individuals may recover the correct species tree, but overall support for clades that include hybrid individuals may be reduced. Liebing et al. (2012) and Lyson (2013) applied a novel molecular dataset, the presence versus absence of specific microRNAs, to the problem of the phylogenetic position of turtle and the root of the reptilian tree, and find that this dataset unambiguously supports a turtle in lepidosaur group. They find that turtle and lizards share four unique miRNA gene families that are not found in any other organisms genome or small RNA library, and no miRNAs are found in all diapsids but not turtle, or in turtle and archosaurs but not in lizards.
2.4 DNA Barcoding versus turtle & tortoise and its application in diverse approaches:

In Hebert et al. (2003 a) from the University of Guelph, Ontario, Canada, first established that the mitochondrial gene COI can serve as the core of a global bio identification system for animals by discriminating 200 closely allied species of Lepidopterians and proposed the compilation of public library of DNA barcodes that would be linked to named specimens. Hebert and his colleagues (2004 a) and Moritz and Cicero (2004) also sequenced DNA barcodes of 260 of the 667 bird species that breed in North America. They found that every single one of the 260 species had a different COI sequence. The sequences were either identical or were most similar to sequences of the same species.

DNA barcoding has several advantages over previous methods. One advantage is its availability. The standard DNA barcode region, a fragment of COI, is very efficient for species identification and has good discrimination power for most animal groups. The universal primer, originally designed for marine invertebrates, proved effective for many animal phyla Hebert et al. (2003 b). The first description of a new species using a DNA barcode from the holotype was by Meier et al. 2006, who used this method to describe a new species of Xenothictis (Lepidoptera: Tortricidae).

Thereafter, the Barcode of Life project was proposed to promote DNA barcoding as a global standard for sequence-based identification of eukaryotes. In 2004, this project was formally initiated by the establishment of the Consortium for the Barcode of Life (CBOL), which aims to develop a standard protocol for DNA barcoding and to construct a comprehensive DNA barcode library. Hebert et al. (2004 b) studied into the efficacy of DNA barcoding was focused on the neotropical skipper butterfly, *Astraptes fulgerator* in north-western Costa Rica.

Meyer and Paulay (2005) and Lambert et al. (2005) examined the possibility of using DNA barcoding to assess the past diversity of the earth's biota. The COI gene of a group of extinct ratite birds the moa, were sequenced using 26 sub fossil
moa bones. Ward et al. (2005) had sequenced (barcoded) for a 655 bp region of the mitochondrial COI of 207 species of fish. All species could be differentiated by their COI sequence, although single individuals of each of two species had haplotypes characteristic of a congener. Rubinoff (2006 a) described animal DNA barcodes, 600 to 800 base-pair segments of the mitochondrial gene COI, have been proposed as a means to quantify global biodiversity. DNA barcodes are most likely to provide potentially useful information for groups that are already well studied, and such taxa do not constitute the majority of biodiversity or those in most need of research attention.

Rubinoff (2006 b) observed that assignment of unknown individuals to species was impossible for 60% of the species, and if the technique had been applied, as in the previous study, to identify new species, it would have underestimated the species number in the genus by 75%. They attributed the failure of barcoding to the non-monophyly of many of the species at the mitochondrial level. Since then, many new species have been described with DNA barcodes from the holotype or paratypes, not only in arthropods, but also in other animals (Janzen et al. 2005).

Hajibabaei et al. (2006) compared the goals and methods of DNA barcoding with those of molecular phylogenetics and population genetics, and suggest that DNA barcoding can complement current research in these areas by providing background information that will be helpful in the selection of taxa for further analyses. Huang et al. (2007) and Kelly et al. (2007) compared the goals and methods of DNA barcoding with those of molecular phylogenetics and population genetics, and suggest that DNA barcoding can complement current research in these areas by providing background information that will be helpful in the selection of taxa for further analyses. Kress and Erickson (2008) suggested, DNA barcode consist of a standardized short sequence of DNA that in principle should be easily generated and characterized for all species on the planet. A remarkably short DNA sequence can contain more than enough information to resolve 10 or even 100 million species. Key to this objective is the widespread adoption of specimen imaging and reporting of identification 'confidence levels' as discussed in the new
protocol, which also reiterates the importance of a shared informatics workbench, the Barcode of Life Data system (Ratnasingham and Hebert 2007).

Seberg and Petersen (2009) further prove the efficiency of DNA barcoding technique by discriminating the Canadian fresh water fish species by developing cox1 sequences for 1360 individuals belonging to 190 of the 203 Canadian freshwater fish species. Ward et al. (2009) and Laskar et al. (2013) observed that fish in the related could be discriminated by barcoding and campaign DNA barcoding for all fishes and creat FISH-BOL. Ward et al. (2005) observed another example used DNA barcoding for the identification of cryptic species included in the on-going long-term database of tropical caterpillar life generated in Costa Rica. The molecular phylogeny is used to decipher the evolutionary origin of an unusual dietary habit of the hawksbill turtle. Furthermore, all of the marine turtle species are formally listed by the International Union for the Conservation of Nature and Natural Resources (IUCN) as threatened or endangered, and by enhancing phylogenetic understanding; genetic information may influence strategies for allocating finite management resources.

Linacre (2006), Naro-Maciel et al. (2010) and Tobe and Linacre (2010) sequenced a segment of the COI gene from all seven species in the Atlantic and Pacific Ocean basins. They established character-based DNA barcodes for each species using unique combinations of character states at 76 nucleotide positions. DNA barcoding of marine turtle is a powerful tool for species identification and wildlife forensics, which also provides complementary data for conservation genetic research. The Barcode of Life project entered a new phase with the launch of the International Barcode of Life project. The iBOL is a huge international collaboration of 26 countries that aims to establish an automated identification system based on a DNA barcode library of all eukaryotes.

Ratnasingham and Hebert (2007) suggested the ‘Barcode of Life’ Consortium is a worldwide initiative devoted to undertaking a molecular inventory of earth biodiversity. After having been demonstrated that the COI gene of the mitochondrial DNA (mtDNA) could be successfully used for identifying North
American bird species. Wong et al. (2011) developed and evaluated DNA barcodes for use in differentiating United States domestic and imported catfish species. They also suggest that as the United States heightens inspection and regulation requirements for seafood products, DNA barcoding will serve as an important tool in efforts to ensure consumer safety and fair international commerce.

Hausmann et al. (2011), Park et al. (2011) and Weigt et al. (2012) represents a DNA barcode data release for 3,400 specimens representing 521 species of fishes from 6 areas across the Caribbean and western central Atlantic regions. Merged with their prior published data, the combined efforts result in 3,964 specimens representing 572 species of marine fishes and constitute one of the most comprehensive DNA barcoding ‘coverages’ for a region reported to date. The FISH-BOL campaign currently has barcoded for the COI gene about 8,000 of the 31,000 fish species currently recognised (Ward et al. 2009).

Stoeckle et al. (2011) described the potentiality of BLAST results for identification of any species. Michel et al. (2012) used next-generation sequencing to obtain seven new transcriptomes from the blood, liver, or jaws of four turtle, a caiman, a lizard, and a lungfish. They used a phylogenomic dataset based on 248 nuclear genes for 16 vertebrate taxa to resolve the origins of turtle. Relaxed molecular clock methods estimate the divergence between turtle and archosaurs around 255 million years ago. These results provide a phylogenetic framework and timescale with which to interpret the evolution of the peculiar morphological, developmental, and molecular features of turtle within the amniotes. Keshavmurthy et al. (2013) showed, by analysing COI, from 241 Stylophora pistillata, widely used coral ‘lab-rat’ species samples across this range, that this taxon in fact comprises four deeply divergent clades. On the basis of the fossil record of these four clades diverged from one another 51.5- 29.6 myr, and should be recognised as four distinct species.

DeSalle et al. (2005) first described the useful of barcode sequences in Testudines but further, all species could be differentiated by their COI sequence, although single individuals of each of two species had haplotypes characteristic of a
congener. DNA sequence based analysis has been proven potential in the forensic investigation of wildlife by Dawnay et al. (2007). Vargas et al. (2009) represent five out of the seven recognized species of sea turtle (Testudines) occur on the Brazilian coast. The observed values were on the same range as those already described for other animal groups. The family Dermochelyidae and Cheloniidae are being 19-fold higher than the mean divergence observed within species and obtained species-specific COI barcode tags that can be used for identifying each of the marine turtle species studied. Lohman et al. (2009), Russello et al. (2010), Reid et al. (2011), Rhodin et al. (2011) and Lourenço et al. (2012) observed that large number of phylogenetic studies are carried out for constructing evolutionary clade of turtle based on morphological and molecular data, which provides novel COI barcode sequences for 174 turtle species. Honda et al. (2002 a, b) observe that the new protocols might help to promote DNA barcoding of reptiles and the established library of reference DNA barcodes will facilitate the molecular identification of Madagascan reptiles. This technique might be useful to easily recognize undescribed diversity, to resolve taxonomic problems, and to monitor the international pet trade without specialized expert knowledge.

Borisenko et al. (2008) described the complete mtDNA sequences and amino acid analysis of COI from Aedes aegypti most of the SNPs occurred as synonymous transition within the COI gene segment, novel approach to phylogeny reconstruction from protein sequences. In 2004, Feldman and Parham described the molecular clocks in reptiles and further, Near et al. (2005) described assessing concordance of fossil calibration points in molecular clock studies by using turtle. By using this calibration data each divergence node should be given a relaxed molecular divergence value. They present a new cross-validation method that identifies inconsistent fossils when multiple fossil calibrations are available for a clade and apply our method to a molecular phylogeny of living turtle with fossil calibration times for 17 of the 22 internal nodes in the tree. This cross-validation procedure identified seven inconsistent fossils.