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Review Article

MOLECULAR ECOLOGY OF FRESHWATER TURTLES AND FUTURE PROSPECTIVE

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Abstract

The distribution and diversity of turtles now reflect the lengthy and complex evolution of the taxonomy, which represents an old group of tetrapod vertebrates in terms of evolutionary history. Freshwater turtles represent the majority of the 365 species, and they mostly live in tropical and subtropical regions. In Southeast North America, Emydidae (Family) of turtle's diversity is high, as well as Geoemydidae and Trionychidae (Family) in the Indo-Malayan region. While *Pelomedusidae* are mostly found in Africa, *Chelidae* are primarily found in the Neotropics and Australia. Most species of the genus are endemic to a particular region or even to a single location. The majority of freshwater turtles suffer varied degrees of threat, mostly from habitat changes and collection. With the use of morphological and molecular data, the majority of phylogenetic trees for different turtle species have been generated using deoxyribonucleic acid (DNA) techniques and procedures. The complete mitochondrial DNA (mtDNA), dehydrogenase subunit 4 (ND4), cytochrome b (Cyt b), carapacial ridge (CR), and cytochrome c oxidase subunit I (CO I) genes of freshwater turtles were sequenced by using universal PCR and long-PCR methods. Along with CR sequences of freshwater turtles, the composition and structure of the control region of diverse species were compared and analysed. Functional domains in the regulatory area, as well as their conserved sequences, were determined based on sequence similarities to other turtles. The mitochondrial regulatory regions and flanking sequences of diverse freshwater turtle species were recovered using Long-PCR and gene-specific primers. To clarify the genetic links between the fresh water turtle species that share the same habitat type, a tree was created based on Cytochrome b sequencing data and the PCR- Restriction fragment length polymorphism (RFLP) pattern.

Keywords: Complex evolution, Phylogenetics, Phylogenomics, Tetrapod vertebrates, Cytochrome b

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1. INTRODUCTION

The turtles are used as trade for different purposes on large scale and mostly adult turtles are captured because of their unique features. Scientists recognize seven living species of sea turtles, which are grouped into six genera (Harvey *et al.*, 2019). The scientific name recognizes the genus and species, while the common name usually describes a physical feature of the turtle. Freshwater turtles are members of the order Testudines in the class Reptilia, and they play an important role in aquatic ecosystems as scavengers, carnivores, and herbivores (Kayani *et al*, 2015b). It is a terrestrial or aquatic reptile with toothless jaws, horny beaks and a body protected by a special bony or cartilaginous shell (Pritchard, 2017). These are mostly used for eating and medicine in Asian countries, particularly China and Southeast Asia, because their shells are also highly effective at purifying blood and curing many diseases (Kayani *et al*., 2015a; Lo *et al*., 2006; Zuberi, 2007). Pakistan is referred to as "the land of many lands" since it contains two of the world's six zoogeographic zones. The fauna of Pakistan is represented by 1100 marine fish species

and 198 fresh-water, 174 mammalian species, 668 birds, 24 amphibians and 195 rep-tiles (Ali *et al.,* 2018). In Pakistan, freshwater turtles include black pond turtle (*Geoclemys hamiltonii*), Indian roofed turtle (*Pangshura tectum*), brown roofed turtle (*Pangshura smithii*) and crowned river turtle (*Hardella thurjii*) while softshell turtle species include Indian flapshell turtle (*Lissemys punctata andersonii*), Ganges softshell turtle (*Nilssonia gangeti-cus*), Indian narrow-headed softshell turtle (*Chitra indica*) and Indian peacock soft shell turtle (*Nilssonia hurum*) (Ali *et al.*, 2018).

The turtle population is on great threat due to their late maturity, high mortality rate of juveniles and great longevity (Van Dijk *et al.,* 2000). The matrilineal phylogeography of stinkpot turtle (*Sternotherus odoratus*) was investigated by analyzing of restriction site of mitochondrial DNA (Walker *et al.,* 1997). They reported phylogeographic differentiation in restriction sites of mitochondrial deoxyribonucleic acid (DNA) between stinkpot turtle and another co-distributed species *Sternotherus minor* morphologically and genetically which elevate issues of subspecies designations in test dine systematic (Walker *et al.,* 1995; Johnson *et al*., 2022).

In order to examine the muscle, blood and shell of turtles that have been sold for the Chinese three-keeled turtles (*Chinemys reevesii*) are in the list in the China Pharmacopoeia, the mitochondrial 12S rRNA gene employed (Wu *et al.,* 1998). The bleached and skinned turtle shell specimens could all be identified using this procedure. In order to run PCR and determine the ideal PCR conditions, two pairs of universal primers for the mitochondrial genome 12S rRNA (L: 1373, H: 1478) and cyt b (L: 14181, H: 15149) were used (Lo *et al*., 2006). In Florida and Louisiana 36 turtle food products were reported which include *Macrochelys*, they used the mtDNA cyt b gene (256 bp) and CR (394 bp), but molecular studies revealed that the majority of these products actually contained *Chelydra serpentine* and only a small amount of *Apalone spp*. (soft shell turtles). It is advised to conduct this research to prevent or lessen the impact of overexploitation in species (Roman and Bowen, 2000).

Comparative analyses of incomplete cytochrome b sequences were used to assess the evolutionary factors influencing the broad-scale phylogeographical patterns of three North American soft shell turtles (Chen *et al*., 2022; Weisrock and Janzen, 2000). The overall phylogeographic patterns are consistent with results from both extensive regional research of southeast species, which implicate historical vicar mechanisms during the Pleistocene and Pliocene, more northern species study indicates a limiting impact on habitat dispersion during a glacial period. Additionally, it is identified a brand-new strong genetic breakage among populations in the southeast and northeast regions of both *A.spinifera* and *A mutica*. The finding highlights the importance of utilizing widespread genera to assess phylogeographic patterns on both a local and a global scale. The substantial evolutionary structure and genetic dissimilarities found in both these *A.mutica* and *A.spinifera* stand in stark contrast to the majority of earlier research on turtles as well as the theory that turtles as whole exhibit slow rates of mtDNA evolution.

The Blanding's turtle (*Emydoidea blandingii*) has seven microsatellite loci (Osentoski *et al.,* 2002; Guinto, 2021). The rate of polymorphism, which ranges mostly from 3-15 alleles with recorded heterozygosities among 0.31 to 0.95. These loci may be useful in all the other turtle species, when the cross-species amplification is recommended. According to changes in 863 base pairs of the mitochondrial 12S and 16S rRNA genes, they determined *Mauremys*

freshwater turtles' phylogenetic relationships to many other batagurid taxa, as well as those of other genera (Phillips *et al*., 2022; Honda *et al.,* 2002). The species of the genus *Chinemys*, strongly suggests that *Mauremys* is not monophyletic and closest association among *Mauremys japonica* and *Chinemys reevesii*. As evidenced by multiple possible *synapomorphies* with other *batagurine* genera, current morphological examinations of genera *batagurid* concluded that *Chinemys* is subfamily member of *Batagurinae* while *Mauremys* is a primitive species of the subfamily *Geoemydinae*. As a result, it is possible that the structural characteristic values used to designate *Mauremys*, which represent plesiomorphy and morphological characteristic shared by *Chinemys* and some other *batagurine* genera are the result of converged. The similar relationship group among *Mauremys mutica* and *Mauremys annamensis* that has been explicitly or implicitly claimed by a lot of other publications on the basis of the morphological data that was also not confirmed by our observations. *M. annamensis*, on the other hand, was shown to be most similar to *Mauremys iversoni*, a species that was thought to be the most diverged among some of the East Asian *Mauremys* by earlier writers. The majority of the phylogenetic trees for various turtle species were built utilizing DNA approaches and procedures that included morphological and molecular data (Simmons and Hart, 2007).

DNA-based analysis tools can be used to establish classification and provide information on the geographic origin of seized species in order to monitor illegal trafficking (Sahajpal *et al*., 2021; Randi, 2003). Typically, species are determined by their morphology. But in the other hand, typical diagnostic features are not present in seizures that typically involve particles of cooked meat, eggshell, carapace shell, or turtle shell powder. The best forensic procedures use genetic approaches to identify damaged and altered samples and to identify species, populations, and regional origins. By using genetic techniques, it is possible to distinguish between items obtained through legal trade and those obtained through illegal tactics and to determine the tag animals maternities and paternities, which is crucial to monitor the activities of certified turtle breeders. The use of molecular methods in animal research is still in its infancy. Only a few molecular approaches have recently been used to solve freshwater turtle and tortoise difficulties (Alacs, 2009).

In pet shops juveniles of extraordinary species of the chelonians are misused as articles of trade investigated (Sollund, 2019; Ceballos and Fitzgerald, 2004), which ultimately results in extinction of endangered and rare species (Cheung and Dudgeon; 2006 Gamble and Simons, 2004). Large number of these chelonians is regularly collected for making jelly and powder by grinding their outer shells which is assumed to be beneficial for human long life and virility so after words sold (Van Dijk *et al*., 2000; Hsieh *et al*., 2006; Lo *et al*., 2006). Molecular methods were used to determine species composition in powdery turtle shell (Lo *et al.*, 2006). Phylogenetic relationship of sixty-five reconstructed species and subspecies which represent all twenty three genera of *Geoemydidae* by gathering a seventy-nine taxon of nuclear and mtDNA data set (Spinks *et al.*, 2004). They identified three major clades: *Testudinidae* and *Geoemydidae* from the old world and *Rhinoclemmys*, a geoemydid species from South America (Ascarrunz *et al*., 2021).

The evolutionary relationships of nine species of *Mauremys* using COI and ND4 genes of mtDNA of 1539 bp and three adjacent tRNA genes are reported (Feldman and Parham, 2004). It was suggested that *Mauremys japonica, M. reevesii, M.*

nigricans and *M. sinensis* formed *monophyletic clade,* as do *M. annamensis and M. mutica*. But they reported that *M. mutica* is paraphyletic with respect to *M. leprosa, M. rivulata, M. annamensis* and *M. caspica* to Asian texa did not form a sister monophyletic group. The 1723 bp of mtDNA of museum specimen of *Cuora yunnanensis* collected from China before 1908; by using DNA method which was considered to be extinct (Parham *et al.,* 2004). From other turtles that were known, they represented a separate lineage. DNA sequencing technology and procedures have helped to address the majority of evolutionary problems among turtle groups (Krenz *et al.,* 2005), certain contentious issues are still extant (Parham *et al.,* 2006).

The formation of the ribs is drastically altered in the turtle shell, which is an evolutionary innovation. To produce a carapace, the turtle ribs laterally develop into the dorsal dermis, unlike those of other amniotes. The carapacial ridge (CR), which runs along the lateral edge of the carapacial primordium, is thought to be crucial for the patterning of the carapace. They used RT-PCR and comparative cDNA analysis using microbeads to thoroughly screen for genes show especially in the CR of the *Pelodiscus sinensis* in order to identify the development mechanisms involves in this structure (Rice *et al*., 2015). Sp5, adenomatous polyposis coli down-regulated 1 (APCDD1), cellular retinoic acid-binding protein I (CRABP-I), and lymphoid enhancer-binding factor 1 orthologs were all found (LEF-1). Despite the fact that all of these genes are shared by the major vertebrate species, an examination of their coding sequences in comparison to those of mouse and chicken indicated that the turtle lineage has generated de novo expression of these genes in the regulatory area. The control region ectoderm showed nucleus localization of b-cateninprote, which suggests that canonical Wnt signaling initiate's carapace development. This finding occurred in conjunction with the expression of LEF-1. These results show that the turtle shell was acquired through co-optation of pre-existing genes rather than through the production of novel genes (Kuraku *et al*., 2005; Rice *et al*., 2015).

The polymerase chain reaction (PCR) and DNA sequencing technology are frequently used to characterize and identify fresh water turtle species based on their DNA for phylogenetic analysis and forensic identification (Kayani *et al*., 2015c). Using DNA sequencing using 17 pairs of primers, the entire mitochondrial genome of the *Pelodiscus sinensis* turtle was discovered (Stuckas and Fritz, 2011; Peng *et al.,* 2006). The entire genome was 17364 bp long and contained 37 genes along with a non-coding regulatory section that was similar of other vertebrate genomes. The results demonstrated that *Cheloniidae* and *Bataguridae* have closer phylogenetic relationship. The molecular techniques for investigating a CITES-listed threatened turtle (*Kachuga tecta*) was found in Taiwan's shell preparations (Hsieh *et al.,* 2006). The largest molecular data used to determine the phylogenetic position of Platysternon in taxonomy of turtle (Parham *et al.,* 2006). Additionally, they compared the big-headed turtle's full mitochondrial genome sequence to the almost complete mitochondrial genome sequences of two additional relevant turtles (*Platysternon megacephalum*). The resultant phylogenetic revealed that *Testudinoidea,* the class that included pond turtles and tortoises, is where *Platysternon* belongs and that it is not linked to *Chelydrids* (*Baek et al.,* 2021).

PCR was used to identify and describe *Lissemys punctata*, which was discovered in the Rawalpindi, Islamabad area. Lp|SamPK had a perfect genetic match with Gen Bank accession No. EF558363.

With a bootstrap of 60%, the sister relationship of this clade to another clade made up of six sequences downloaded from GenBank was poorly supported. With less than 40% genetic diveristy to the present studied fresh water turtle (Lp|SamPK), the maximum nucleotide genetic diversity was observed between Lp|SamPK and Genbank downloaded sequence (FR850644), which forms a clade with FR 850643. The findings indicated that molecular techniques can be used to identify fresh water turtle species in broad sense and endangered species in particular, as well as keep them in their proper taxonomic position (Kayani *et al.*, 2015).

The mitochondrial genome of the Chinese big-headed turtle was shown to include a unique gene sequence that is distinct from all other animal species using the PCR method (Peng *et al.,* 2006). Phylogeny and taxonomy of all species and subspecies of South and Southeast Asian turtles which are *Callagur, Hardella, Batagur, Pangshura and Kachuga* by sequencing cytochrome b gene of mtDNA was studied (Zheng *et al*., 2013; Praschag *et al.,* 2007). The result showed three major clades first one is *Callagur, Kachuga and Batagur* (lage riverine species) second is *Pangshura* (monophylum) and third is *Hardella* that is a clade comprising *Pangshura, Batagur, Kachuga and Callagur* is polyphyletic. They recommended placing all *Kachuga, Callagur and Batagur* are in one genus.

The turtle conservation genetics working group especially focused on conservation genetics which placed an emphasis on identifying control tactics and forensic research on endangered animals, cryptic lineages and gene flow across natural populations i.e., captive breeding, in-situ and ex-situ have provided an important way against extinction by which propagation of most endangered turtles is carried out and repatriated into the wild (Sigouin *et al*., 2017; Williams *et al.,* 2007).

Phylogeny of turtles using data and developed a set of super tree based on mtDNA and nuclear DNA which represented "backbone tree" of turtles and phylogenetic relationship among three families *Trionychidae, Geoemydidae,* and *Testudinidae,* which helped in true Tree of life for turtles (Iverson *et al.,* 2007). Later the taxonomic issues are successfully resolved with the help of neutral molecular markers and morphological characteristics, because they are less flexible and may be selected for advantages over morphological traits (Crawford et al., 2015; McGaugh *et al.*, 2007).

The thirty-three damaged turtle species shells from 2002 that were submitted by the COA, one complete ventral turtle shell was recognized (Hsieh *et al.,* 2008). These specimens' cytochrome b genes' incomplete sequences were amplified and analyzed. Of the thirty-four sequences, there are fourteen haplotypes (Mohd Salleh *et al.,* 2022). They identified the species with the closest similarity (greater than 98%) in the EMBL databank for the sequences of the nine haplotypes comprising twenty samples. The thirty-four broken turtle shells had an overall identification rate of 58.8%. There was one for CITES Appendix I, eighteen for CITES Appendix II, and one for CITES Appendix III. This demonstrated that the findings of identification of species using this procedure may be certain and trustworthy. By scanning the EMBL databank, the remaining haplotypes had reduced similarity, and their precise species could not be determined. This was a result of the EMBL databank's incomplete information, and it demonstrated the necessity of expanding the turtle database for species identification (Mohd Salleh *et al.*, 2022).

A well-optimized PCR-RFLP method was used to identify five Indian freshwater

turtle species, including three hard-shelled turtles which are *Geoclemys hamiltoni, Kachuga dhongoka,* and *K. Aspideretes* gangeticus and two soft-shelled turtles including *Lissemys punctata* and *Aspideretes gangeticus* (Rohilla and Tiwari, 2008). Its value in the taxonomic separation of these species was discovered through nucleotide sequence changes in the PCR-amplified mitochondrial cyt-b genes from the five species. To clarify the genetic relations among these species that live within a similar particular habitat, a tree was created using information from the cytochrome-b genome and the PCR-RFLP pattern. When examining phylogenetic relationships or even species differentiation, the PCR-RFLP of mitochondrial 16S rDNA genes seemed less significant in contrast (Chiari *et al*., 2012). The molecular technique used (PCR-RFLP) is also simple, quick, reproducible and accurate as a result, it may be used for species identification on a regular basis, which is critical for the management and conservation of threatened Chelonian species. The genetic structure of "eyed" turtles (*Sacalia quadriocellata* and *S. bealei*) from China, Vietnam, Laos and Hongkong studied by using cyt b gene of 1140 bp of mtDNA for construction of their phylogeny (Shi et al., 2008). Results showed that the two species of *Sacalia* contain four district mitochondrial clades: The findings revealed that the two *Sacalia* species contain four district mitochondrial clades: one matched to *S. bealei*, and the other three corresponded to morphologically diagnosable populations of *S. quadriocellata,* with the species from Laos and Vietnam forming sister clades (Ouso *et al*., 2020).

Long-PCR was used to amplify the entire genomes of the CR of four different species of mtDNA, including *Cyclemys atripons, Cuora aurocapitata, Cistoclemmys galbinifrons* and *C. flavomarginata* and 980 bp, 1379 bp, 1722 bp and 1207 bp were the respective lengths (Zhang *et al.,* 2009). They evaluated by comparing the CR structure using *Pyxidea mouhotii's* CR sequence (DQ659152), and they were able to recognize three functional domains (TAS, CD, and CSB) in which the conservation sequences (CSB-1, CSB-2, CSB-3 TAS and CSB-F,) were also effectively recognized based on their sequence similarity to those of other turtles. These five turtles share the same CSB-2 and CSB-3 sequences, and four of them share the same CSB-1 sequence, although *Cy. atripons* has one base transversion (T A) (Jiang *et al*., 2011). We examined the VNTR sequences, also known as microsatellites, that are found at the 3' end of CR. Tandem repeat patterns come in seven different varieties, with copy numbers ranging from four to forty-eight. With the exception of *Cy. atripons*, all five of the turtles have the "TATTATAT" repeats and are finished by TA. The findings of the CR structural study showed that while the Cuora, Cistoclemmys, and Pyxidea are quite close to Cyclemys, they are not exactly the same. We used the MP, ML, and BI techniques to generate the molecular phylogenetic trees, utilizing *Indotestudo forstenii* (DQ080044) and *Indotestudo elongate* (DQ080043) as outgroups and the CR sequences (1123bp) that removed the microsatellite markers at the 3' end of CR. According to the findings, which are in line with an analysis of the CR structure of the five turtles, the Cuora group, which consists of *Cistoclemmys, Pyxidea* and Cuora has a strong case for being monophyletic. This group is closely related to *Mauremys* and *Chinemys* but is not related to Cyclemys (Jiang *et al*., 2011).

A female (*Mauremys reevesii*) and a male (*Mauremys sinensis*) turtle were mated to generate the two hybrid turtles. The mitochondrial (ND4) and nuclear (R35) DNA of the two probable hybrid turtles were sequenced, and it was discovered that these two turtles are hybrids with independent

origins (Fong and Chen, 2010). The phylogenetic position of the pig-nosed turtle (*Carettochelys insculpta)* was studied for the first time by sequencing the whole mtDNA using the LA-PCR method, which comprised of 16439 bp length and similar to those of other turtles and vertebrates with the exception of an ATC start codon in NADH4 gene (Xiong *et al.*, 2010). They concluded that the Carettochelyidae and Trionychidae clade, which developed as a result of the earliest evolutionary tree breaks, was indeed the sister member of the genus to all cryptodiran turtles (Suzuki *et al*., 2013).

Using long-PCR and PCR method, sequenced the entire mtDNA CR, NADH, ND4, Cyt b and CO I genes of three Asian freshwater turtles (*Mauremys mutica, M. japonica* and *Ocadia sinensis*) and the structure and composition of CR in the five species were analyzed and examined in conjunction with CR sequences from *Chinemys reevesii*. Using sequence similarities to other turtles, three functional domains in CR and found conserved sequences (Jiang *et al.,* 2011). Nucleotide divergences between and CR of 11 Cytochrome b, CO I, and ND4 turtle species were compared using transitions with trans versions approaches, which confirmed that CR evolved 2.6 to 5.7 fold quicker than the other mtDNA genes. After removing CR VNTRs, phylogenetic trees were created. The findings of the VNTR study verified the *Mauremys* clade's extension to include species that were formerly part of the *Mauremys*, *Chinemys Ocadia* and *Annamemys* clades.

The three complete mtDNA control region (CRs) of *Podocnemis unifilis, Chelus fimbriata* and *Chelodina rugosa* was studied using Long-PCR method which comprised of 985 bp, 1,149 bp, and 1,016 bp length, respectively (Wang *et al.*, 2011). They found three functional domains and conservation sequences, and an interrupted poly-C stretch was found within TAS domain of *P. unifilis*, *C. fimbriata* and *C.* rugosa, which was also present in *Chrysemys picta, Trachemys scripta*, and *Trionyx triunguis*. The CSB domain, CSB2 and CSB3 determined in *Cryptodira* and CB2and CB3 were absent in *Pleurodira*, was proposed a diagnostic character between *Cryptodira and Pleurodira* at molecular level and provided some possible data for phylogenetic inference. After amplified on 2354 bp of mtDNA and 2573 bp of nuclear DNA of *Nilssoniafermosa* and the remaining *Nilssonia* species of Bangladesh and China in order to resolve their phylogenetic relationship was studied (Liebing *et al.*, 2012). The results showed that *N. fermosa* is the sister taxon of *N. leithii, N. gangetica* and *N. nigricans, N. hurum*. The complete mtDNA comprised of 16,582 and 16,661 bp length of two turtles *Chelodina rugosa* and *Chelus fimbriata*, repectively sequenced for the first time (Wang *et al.*, 2012). Their results showed that there was absence of mitogenomic initiation site for light-strand replication (OL), which was only found in *Pleurodira*, so phylogenetic relationship of these turtles and others were reconstructed.

For effective conservation measures to be developed, the genetic diversity of freshwater turtles must be identified. In the current study, inter simple sequence repeat (ISSR) markers were used to analyse the phenetic connection of the genus Pangshura and establish whether genetic information and spatial distribution patterns coincided with previously published morphological data (Baruah *et al.,* 2013). Thirty-six individuals from the four Indian turtle species *Pangshura tecta, P. tentoria, P. simithii* and *P. sylhetensis* were collected from specific locations in Uttar Pradesh, Assam and West Bengal. Among these individuals, ten microsatellite-based primers amplified 156 ISSR markers. According to estimates of Nei's genetic diversity (h), Total genetic

diversity (Ht) and Shannon's Index (I), *P. sylhetensis* and *P. tentoria* and have more genetic diversity than the other two species. Inbreeding is prevalent in these species groups, nevertheless, as indicated by the low values of gene flow (Nm) and within-sample diversity (Hs). The values of the coefficients of differentiation show that the *Pangshura* species was where the divergence first occurred. The distinctive genetic identities of these four species are projected on the principal components analysis plot. In agreement Neighbors *P. tecta, P. tentoria* and *P. sylhetensis* appear to be genetically more related to one another than to *P. smithii* in the joining dendrogram (Baruah *et al.*, 2013).

2. Future Prospects:

It is seen an increase in molecular research on freshwater turtles in past years, and this trend doesn't appear to be slowing down. There are still unanswered questions regarding genetic structure of turtle population (Bowen and Karl, 2007), reproductive behavior (Section 4) and conservation of genetics (Avise, 2007). The researches will be successful as a result of using new emerging technology. The sharing of DNA materials among laboratories might be made easier by whole amplification of genome. The novel genetic markers development is necessary for genetics of turtle population to advance above its present level. The discovery of new mtDNA, microsatellite and SNP markers may be amplified by the use of next-generation sequencing technologies. The microarrays development is facilitated by whole genome sequencing, which open up new study fields like "phylogenomics". An ecological context can be studied when using microarray technology to study function and expression of gene. There is now a new revolution to describe female polyandry as a result of current studies on many paternities. It is challenging to analyze the adult male's population genetics; although, reconstruction of male genotype from paternity data provides a new method. Multi - locus genetic information will also make it possible to test for male-mediated gene flow. In place of more conventional F-statistics, there are new alternative techniques for data analysis. For the research of freshwater turtles, the future seems very promising (Komoroske, 2017).

3. Conclusion

The ecology, habitat, and diversity of freshwater turtles were investigated at the molecular level in this study. The major part of phylogenetic trees for different turtle species have been generated using deoxyribonucleic acid (DNA) methods and procedures using morphological and molecular data. The complete mitochondrial DNA (mtDNA), dehydrogenase subunit 4 (ND4), cytochrome b (Cyt b), carapacial ridge (CR), and cytochrome c oxidase subunit I (CO I) genes of freshwater turtles were sequenced using universal PCR and long-PCR methods. The results of this study also established the foundation for future molecular research, which is important for examining the genetic diversity of different turtle species.

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