Population genetic structure of freshwater mud eel, *Monopterus cuchia* (Hamilton, 1822) in Bangladesh

Dissertation submitted to the University of Dhaka, Bangladesh for the degree of Doctor of Philosophy in Zoology

Submitted by

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Author's Declaration

I, Md. Faruque Miah, hereby declare that the dissertation entitled **"Population genetic structure of freshwater mud eel, *Monopterus cuchia* (Hamilton, 1822) in Bangladesh"** submitted for the partial fulfillment of the requirements of the award of Doctor of Philosophy, in the Faculty of Biological Sciences, University of Dhaka, is my own work unless otherwise referenced or acknowledged. I also declare that the subject matter of this thesis is the record of the work done by me and this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research in any other University/Institute. Any collaborative work has been specifically acknowledged as have all sources of information.

Dhaka, May 2016

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Certification

The dissertation entitled "Population genetic structure of freshwater mud eel, *Monopterus cuchia* (Hamilton, 1822) in Bangladesh" submitted to the Department of Zoology, University of Dhaka, Bangladesh in partial fulfillment of the requirements for the Degree of Doctor of philosophy. I certified that the candidate, Md. Faruque Miah has been completed his research under my supervision and suggestions. I have read this dissertation and that, in my opinion, it is fully adequate in scope and quality as a dissertation for the degree of Doctor of Philosophy. It is further certified that to the best of my knowledge the thesis contains original research. This has been accepted as satisfactory for the partial fulfillment of the requirements for the Degree of Doctor of Philosophy and approved as to its style and contents.

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To

My beloved parents Late Sayeda Begum and Late Md. Mir Hossain and My respected teacher Late Dr. Miah Muhammed Abdul Quddus, Professor, Dept. of Zoology, University of Dhaka, Bangladesh

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Md. Faruque Miah
Ph.D. Research Fellow

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LIST OF ABBREVIATION

DU	University of Dhaka
GEB	Genetic Engineering and Biotechnology
SUST	Shahjalal University of Science and Technology
USDA	United State Department of Agriculture
%	Percent
spp.	Species
° c	Degree Celsius
L	Liter
mm	Millimeter
cm	Centimeter
µg	Micro gram
e.g.	Exempli gratia = For example
et al.	Et alia = And others
etc.	et. cet. era = And the others
g	Gram
i.e.	id est = That is
mg	Milligram
mg/ml	Milligram per milliliter
ml	Milliliter
µl	Micro liter
sec	Second
R_f	Retention factor
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
pH	Negative logarithm of hydrogen ion (H^+) concentration
IUCN	International Union for Conservation of Nature and Natural Resources

ABSTRACT

In this study, population genetic structure of freshwater mud eel, *Monopterus cuchia* in Bangladesh was observed by RAPD and RFLP assay for finding a superior population with high genetic diversity. Some associated observations including rearing, breeding biology and artificial breeding has also been performed. First experiment was conducted to study the effect of different environments such as House tanks, Earthen ditches, Plastic tank and Back yard tanks on rearing and production performance of freshwater mud eel, *M. cuchia* where different types of biological food in different amounts were used, except the earthen ditch-2 (control). 40 fishes (approximately 1kg in weight) were stocked into each of the environment and the highest mean of final length (32.89±2.65423) and final weight (109.69±2.92777) were obtained in earthen ditch-1. The weight gain in length 17.78 ± 0.24 and in weight 85.07 ± 0.15 with 92.5% survival and production (4.07kg) were also highest in earthen ditch-1. On the other hand, the lowest mean growth considering final length (28.45±2.80092) and weight (67.24±3.79328) were recorded in house tank-2 while the weight gain in length (11.86 ± 0.26) and in weight (41.84 ± 0.59) were also observed in house tank-2 with the production of 2.02kg by 75% survival. Based on Pearson correlation coefficient no significant correlations were found between lengths and weights of fish in different culture regimes. In second study a successful protocol was developed to identify male and female *M. cuchia* through external morphology, internal anatomy and histological analysis. Furthermore, breeding biology of freshwater mud eel, *M. cuchia* was considered in terms of gonadosomatic index, length-weight relationship of gonad, ova diameter and fecundity. The ova diameter was recorded from 0.3 mm to 4.30 mm and the individual fecundity was recorded from 155 to 1495 while relative fecundity was found from 2.64 to 12.45. A peak of GSI was observed 2.14±0.2 in male and 5.1 ±1.09 in female. Artificial breeding of fish was practiced but unsuccessful spawning was recorded through different inducing agents in captive conditions. In this study, 16S rRNA and glutamine synthetase gene was used for identification of this fish and molecular standard was recorded 250bp length by 16S rRNA gene and 541bp length by the partial sequence of glutamine synthetage gene. In this study, genetic diversity of freshwater mud eel was studied by RAPD assay and a total of 735 bands with 228 polymorphic loci were detected while polymorphisms were revealed by all eight arbitrary primers. The highest and lowest number of bands was recorded 120 and 49. Thirteen different groups of inter individual pair wise similarity and 36 groups of genetic diversity were measured whereas the lowest and highest genetic distance was found 0.58 and 0.97 respectively while the average genetic diversity was found 0.8173716. The highest and lowest Nei's genetic similarity values were recorded 0.60 and 0.06 respectively and linkage distance was computed between the ranges of 3.6 to 6.24 which were generated six clusters by 11 clades. Intra and inter population genetic diversity was analyzed by the same eight RAPD primers considering 2 populations (Tanguar Haor=P1 and Hakaluky Haor =P2). A total of 353 bands with 147 polymorphic loci were detected in P1 whereas a total 371 bands with 153 polymorphic loci were found in P2. Polymorphisms were revealed by all the primers in P1 and P2 while a single monomorphic locus were seen in P1 resulting 95% and 100% polymorphism showed in P 1 and P2 respectively. 9 and 13 groups of inter individual pair wise similarity were found in P1 and P2 respectively considering averages 6.46 and 6.08. The 27 and 29 different genetic distance values were recorded in P1 and P2 while the average genetic distances were calculated 0.80 in P1 and 0.816 in P2 respectively. Nei's genetic similarity in P1 was ranged 0.11 - 0.58 with 34 diverse values and in P2 it was ranged between 0.06 – 0.54 with 36 different values, and in an average it were originated 0.323 in P1 and 0.301 in P2 respectively. A total 35 and 18 different linkage distances were estimated in P1 and P2

respectively whereas the average linkage distances were found 5.115 and 5.141 respectively in P1 and P2. A genetic relationship was made by using linkage distance where five clusters were created from 5 clades in both P1 and in P2. Based on allelic information, the frequency of q alleles were recorded highest in both the populations than the frequency of p alleles. Based on the intra-locus gene diversity the average highest gene diversity was observed in P2 in most of the primers. However, the average gene diversity was found highest 0.164635264 in P2 and lowest 0.150829501 in P1.

Genetic diversity of glutamine synthetage gene was analyzed in this freshwater fish with two restriction enzymes of +CfrI and +Hpy178III while both the enzymes were digested the size of 541 bp and polymorphism was detected in terms of wild type homogygotes, polymorphic homozygote and heterozygosity. The genetic diversity was observed by using the RFLP band analysis and four different groups of individuals were identified with the p-values of 0, 0.033, 0.05 and 1 respectively whereas different distances were found among the groups. Intra and inter population genetic diversity of *M. cuchia* was analyzed by using same fragment of glutamine synthetage gene by the same enzymes considering the ecological habitats of Tanguar Haor (P1) and Hakaluki Haor (P2) resulting less polymorphism was observed in P1 than P2. Considering different genetic distances two groups of individuals were identified very close with 0.02 P-value in P1 and five groups were revealed in P2 from very close to large distances. The frequency of q alleles were recorded highest in both the populations than the frequency of p alleles except the enzyme +Hpy178III in P2 (0.433). However, intra-locus gene diversity was found highest in P2 by both the enzymes than P1. Inter population differentiation for one locus (gst = 0.5299) shows significant delineation between populations in allele frequencies and it can say that a high percentage of genetic distance is distributed among two populations. F-statistics was analyzed for observing the genetic structure of populations where degree of allele frequency (F_{IT} = 0.7606) and differentiation of allele frequencies between two populations seems greater (F_{ST} = 0.1439), with only a moderate effect of random mating were observed within the populations (F_{IS} = 0.7204). From the above records, polymorphism was found in glutamine synthetage gene of this fish and generally higher polymorphism was observed in P2 as well.

Overall finding of this research is concluded that the earthen ditch was found one of the suitable culture environments of *M. cuchia* and live feed was recorded suitable for obtaining better growth. Breeding biology of this fish was considered with development of a successful protocol to identify male and female as prior knowledge for breeding program, and unsuccessful spawning was recorded through inducing agents. A molecular standard was recorded for species identification of this fish. Final observation in both cases of RAPD and RFLP study higher genetic diversity were observed in general as well as at population level though P2 was recorded stronger genetic status than P1. The research is indicating the good genetic status of this fish in Bangladesh particularly in the ecological habitats of Sylhet.

Introductory description

Monopterus cuchia (Hamilton 1822) is one of the common freshwater eel of Bangladesh, known as mud eel, Cuchia, or Kuichya (Figure 1.1), not commonly eaten by Bangladeshi but has high export value. It is a fish with good taste and high market value. It commonly occurs in the freshwater of Bangladesh, Pakistan and throughout India (Talwar and Jingran 1991). However, various freshwater eels are found in the different countries such as China, Japan, Korea, United States, Europe, New Zealand, Sweden, Denmark, Germany, Italy and many other countries (Ahmed *et al*. 2009). The freshwater mud eel (*M. cuchia*) trade, a high-value export fishery involving15 countries in the world, with high demand in China, Malaysia, Singapore, Japan and Taiwan (Hasan *et al.* 2012). Mud eel belongs to the family synbranchidae of the order synbranchiformes (Rosen and Greenwood 1976, Shafi and Quddus 1982). It is a slender shaped streamlined fish with a tapering tail (Day 1878). They do not have pelvic fins and most of the species lack pectoral fins. The dorsal and anal fins are fused with caudal or tail fins forming a single ribbon along the whole length of the fish. They often spend their daytime hiding under stones and mud or having a burrowing habit (Nasar 1997). The gills of *M. cuchia* are reduced and the fish has a pair of air breathing organ in the form of a sac on two sides of the head (Talwar and Jhingran 1991). The species has dual systems of oxygen uptake for respiration: aquatic respiration by uptaking dissolved oxygen through the gills and aerial respiration by uptaking air directly through the skin (Liem 1967). These mechanisms enable it to survive in oxygen-depleted conditions or in areas where water is scarce. For this special modification, this fish can survive 90 to 132 days without food and can be stored large number in a small sample container, as well as it is transported in

live through a small earthenware or plastic jar to distant places (Pullin and Shehadan 1980).

Figure 1.1. The freshwater mud eel, *Monopterus cuchia*

This freshwater mud eel is found in open water bodies such as rivers, haors, baors, beels, canals, floodplains and rice fields in Bangladesh (Rahman 1989). It spawns during monsoon in the barrow-pits of paddy fields, but does breed in enclosed ponds. The mud eels lay their eggs in the low depth ditches and in the swamps in March and June. These fish thrive on the small fishes, prawns, molluscs, aquatic insects, small frogs, etc. (Ponniah 2000).

This fish can play a unique role for socio-economic welfare of the area and thus social fishery could be developed (Quddus *et al*. 2000). Besides, the freshwater mud eel has medicinal value. In Bangladesh, the tribal people and a few other castes eat this fish only where almost all the harvested fish are exported. This fish has tremendous demand in foreign countries. Although, this fish have quite a large economic demand, the populations of the freshwater eel are declining at an alarming rate from the natural water bodies due to water obstruction, habitat destruction, pollution, over exploitation

and destructive fishing (Ponniah 2000). The freshwater eels are recorded as rare species from floodplains and beels due to disease outbreak (Hossain and Mazid 1995).

Now-a-days the freshwater mud eel is equally commercial important fish in Bangladesh and has been observed that if this fish can be cultured commercially to meet the increasing demand of animal protein in Bangladesh as well as to generate foreign currencies (Zaher and Mazid 1993). In a few grassroots level studies it has been observed that, this fish is being sold commercially in the local market at BDT. 250 to BDT 400 (3.20 to 5.13$) per kg according to grade in the recent years. In Bangladesh, it has revealed in a report that this fish economically important in various groups of people, especially the Hindu and Christian communities and only a few people of some ethnic tribal group use the flesh of eel as their meal. The annual landing of the freshwater mud eel is about 1.85 Metric tons (Ahmed *et al*. 2009). This freshwater eel comprise only 1.5% of the total fish landing centre at different parts of the country (BFFEA 1989). Like other eels, freshwater mud eel provide various values for the ecosystem. The unique adaptation that allows mud eel to be successful in their environments, primarily for wedging through small openings, while some are adapted to burrowing into soft substrates or living a pelagic existence (Nelson 1994). Furthermore, many species, particularly freshwater varieties, are also popular as a food, mainly in Asia and Europe, where the meat is considered a delicacy (Herbst 2001). Foreign consumers consider this mud eel is a delicious and has a high nutritional content. Ecologically, these eels are important in food chains, acting as predators of harmful animals and different invertebrates such as crustaceans and mollusks and being consumed in its various stages by mammals, birds and small vertebrates.

M. cuchia is presumed for a low-cost enterprise to the farmers. Raising this species is easy to do and achieves a more profit than some other small size fish culture activities (IIRR *et al*. 2001, LU *et al*. 2005). This fish can easily be cultured in a small tank, aquarium and other vessels. Most species adjust well to life in captivity and are very hardy inhabitants. A larger aquarium with adequate hiding places and a well- sealed lid is essential for maintaining this fish. Eels including *M. cuchia* contain very good nutritional value and the food value of this fish is very high. The average protein content per 100g of eel flesh is 14g and the caloric value of eel flesh is as high as 303 Kcal/100g compared to 110 Kcal/100g in other average fishes (Nasar 1997). Although different body shape, unique taste and the meat nutrient content and high protein is needed, eels contain acids saturated fat by 20%. Types of fat contained in the eel, including type of omega-3 that has many benefits including preventing coronary heart disease, increasing the development of the human brain, helps reduce to high blood pressure (hypertension), helping cancer and kidney diseases, increase vitality and stamina and improve the function of the eye. Over time and increased public knowledge about the importance of animal protein, as well as an easy way of processing eels became popular and favored by rural and urban communities. Catching a large scale to meet consumer demand caused eel populations increasingly less, especially if it means catching the highly forbidden by the government, as a surprise given the electric current using batteries. Looking at these conditions, it must find some way to preserve it.

It is good time to think about expanding freshwater eel fisheries and to develop new technologies to increase its production for capture the world market. By improving cultural techniques we can earn lot of foreign currency and this will help to develop

national economy of the country. Bangladesh government has undertaken a project on the breeding and culture of the fish through DoF and BFRI recently, though, different capture techniques are established to collect this fish, however, no culture practice of this fish is established whereas few works have so far been done on this fish in different aspects (Huges *et al*. 1974, Mishra *et al*. 1977, Nasar 1997, Narejo *et al*. 2002, Narejo *et al*. 2003a, Narejo *et al*. 2003b, Ahmed *et al*. 2009, Hasan *et al*. 2012). The mud eel has been identified for polyculture in seasonal and perennial ponds as well as in paddy fields. But no technology for commercial culture and production of this fish is developed in Bangladesh. Since there is no culture system for this freshwater eel, it is necessary to develop a scientific eel culture system, however, the preliminary observation in cemented cistern on growth, survival and diet condition of this mud eel has been observed (Narejo *et al*. 2003a, Narejo *et al*. 2003b) but it was not scientifically developed. Therefore, the freshwater mud eel, *M. cuchia* was observed considering suitable environments for survival, growth and production of this fish in Bangladesh. Considering high export demand and high nutritional elements of *M. cuchia*, this research work may be an alternative livelihood for people involved in fisheries sector. In addition, to develop a scientific mud eel culture system, it is necessary to observe their growth, foods, survival rate as well as production and habitat ecology. Besides this, breeding biology and artificial seed production of this fish is very important for their rearing, production and conservation. Though limited research has been conducted on this mud eel in Bangladesh (Huges *et al*. 1974, Mishra *et al*. 1977, Nasar 1997, Narejo *et al*. 2002, Narejo *et al*. 2003a, Narejo *et al*. 2003b, Ahmed *et al*. 2009, Hasan *et al*. 2012), however, so far no data available on breeding biology and induced breeding of this fish is available, while unsuccessful induced breeding was also observed without

spawning responds (Shuvra 2011). Therefore, to maintain the natural mud eel resources and to obtain reliable seed of this mud eel for aquaculture, examine the breeding biology, which mainly involved the study of reproduction system and sex organs of freshwater mud eel and development of induced breeding technique for this fish have been eagerly desired. Thus, the prior objectives of this research were also observed considering the breeding biology during natural conditions, captive conditions as well as through inducing agents.

The fresh water mud eel is now a red listed species in Bangladesh (IUCN 2000) because of commercial fishing, excessive use of pesticides, diseases and destroying their natural habitat by different environmental stresses. Due to the risk of this fish in Bangladesh it is more necessary to conserve. In recent years, the species has been declining over much of its range; so careful monitoring and research will be vital for its existence. Although some works have so far been done on this fish, however, no researches were carried out on genetic biotechnology or population genetic structure of this fish, while only DNA fingerprinting were observed in narrow sense using RAPD marker in China (He *et al.* 2004) and in Bangladesh (Alam *et al*. 2010). Different eels were identified by RFLP using mitochondrial DNA (Lin *et al.* 2002) but molecular identification of this fish is still unknown. Since, this fish contains high nutritional elements and has a great demand in foreign countries and artificial spawning responds was unsuccessfully recorded hence this research was taken with interest of this fish particularly in genetic biodiversity based population studies as genetic diversity is a key role for breeding programme. Thus, now-a-days the study of the population genetic structure of this fish is most important for conservation and higher production.

Genetic diversity which deals with the living organisms genetically i.e. variation in the genes of the species and the genetic makeup of species differ from each other to produce a new generation is categorized as genetic diversity. Biodiversity is important because it provides the support systems that sustain species existence. It is also crucial for our quality of life and our standard of living. The maintenance of high level of biodiversity is important for the stability of ecosystems. Besides, biodiversity is also scientifically important for human survival. Due to endanger condition of this fish in Bangladesh (IUCN 2000) it is highly important to know the genetic characteristics of this fish. Thus, this experiment was highlighted genetic diversity for saving this fish from natural and artificial disasters, to develop natural and artificial breeding and to increase high production. Genetic diversity represents the existence of variants (alleles) of individual genes resulting from alterations of the DNA sequence. The alleles of a particular gene may occur in different frequencies in different groups of interbreeding individuals (populations) and the genetic variation of a particular species is therefore distributed both within populations (expressed as different allele combinations between individuals) and between populations (differences in occurrence and frequency between populations), while fishing and aquaculture activities pose potential threats to genetic diversity of wild fish populations in different ways. For instance, over-exploitation may reduce population sizes to levels where inbreeding and loss of genetic diversity through random events (by the process of genetic drift) become serious problems, or may result in extinction of local populations or population segments. However, no data is available about molecular characteristics and population genetic structure of this freshwater mud eel in Bangladesh as well as in other countries. As this fish is red listed in Bangladesh (IUCN 2000), it is required to maintain high level of biodiversity to develop natural

and artificial breeding, and to increase high production. Due to the key role of Glutamine synthetase (GS) in detoxifying ammonia, particularly in the brain, and in ammonia excretion in the kidney in fish, it is also necessary to study genetic characteristics of GS in fish and considering the genetic diversity of GS here in the experimental fish as well. GS is an enzyme that plays an important role in the metabolism of nitrogen by catalyzing the condensation of glutamate and ammonia to form glutamine (Eisenberg *et al*. 1987). Glutamine synthetase uses ammonia produced by nitrate reduction, amino acid degradation and photorespiration (Liaw *et al*. 1995). GS (Figure 1.2) can be composed of 8, 10 or 12 identical subunits separated into two face to face rings (Eisenberg *et al*. 2000, Stryer *et al*. 2007). GS catalyzes ATP dependent condensation of glutamate with ammonia to yield glutamine (Liaw *et al*. 1995).

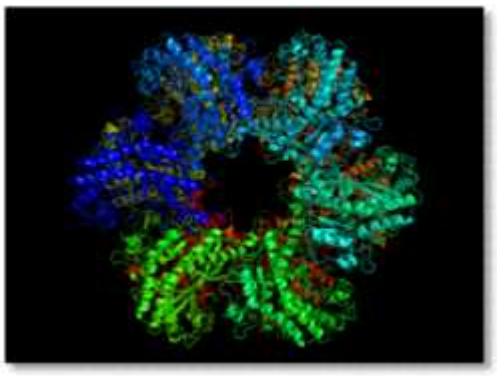

Figure 1.2. Molecular structure of glutamine synthetase

Glutamine synthetase is a key enzyme for nitrogen metabolism. It occurs in all organisms and is one of the oldest functioning genes. Many vertebrates have only one functional copy of this gene, while many plants have been shown to be multicopy for this gene. Until recently only a single copy of glutamine synthetase had been described in fish (Figure 1.2). However, six copies of this gene are expressed in

rainbow trout *Oncorhynchus myklss* and two copies of this gene are expressed in the gulf toad fish *Opsanus beta* (Walsh *et al*. 2003). In this experiment the GS gene was first time used for molecular characteristics as well as molecular identification of this freshwater mud eel in Bangladesh.

In addition, The use of mtDNA has become increasingly popular in phylogenetic and population genetic studies, first with the developments of methodology for mtDNA isolation and use of restriction enzymes to detect nucleotide differences (Lansman *et al.* 1981), and further with the development of PCR methodology and applicability of 'universal' primers (Kocher *et al.*1989) for amplification of mtDNA. Much of the interest is related to the fast rate of substitutions in mtDNA. The control region is the main regulatory region and the only major non-coding area in animal mtDNA. It contains the heavy-strand origin of replication (Desjardins & Morais, 1990) and the promoters for heavy and light strand transcription (L'Abbé *et al.* 1991). Despite its functional importance, the control region is suggested to be the most variable part of the mtDNA. In the human control region, the estimates of the rate of substitution were found to range between 2.8 (Cann *et al.* 1984) to 5 times (Aquadro and Greenberg 1983) the rate of the rest of the mtDNA. Most of the studies in which control region sequences have been used have focused on intra-specific patterns of variability and phylogenetic relationships of closely related species, a prominent example being the study of human population history (Cavalli-Sforza *et al.* 1994). A high mutation rate also means that the phylogenetic utility of the control region sequences diminishes in deep divergences due to saturation and ambiguities in homology determination. In this study, one set of universal primer was used which designed by Kitano *et al*. (2007) based on the conserved regions of the 16S mitochondrial genomes. It was designed as

vertebrate universal primer which was also first time applied for studying the identification of this freshwater mud eel.

According to the high market demand the production of this fish is very potential by culture practice which is needed good quality seed supply while artificially seed production was recorded unsuccessfully, therefore, it is necessary to study genetic research and the main target of this research was genetic diversity based population studies for finding a better population with high genetic diversity that can be used in future for artificial breeding.

Objectives

Population genetic structure is a fairly latest practice related to fish breeding, increase fish production and conservation of biodiversity. Overall aims of the research entitled "Population genetic structure of freshwater mud eel, *Monopterus cuchia* (Hamilton 1822) in Bangladesh" were considered to assess the environmental habitats and the breeding biology in-situ and ex-situ conditions and compare the genetic diversity among eel populations from different geographical area.

The specific objectives of the project were:

1. to observe the suitable environments for survival, growth and production of the fish in Bangladesh
2. to observe the breeding biology of mud eel, *Monopterus cuchia* in natural conditions as well as under hormonal treatments
3. to observe breeding performance through inducing agents
4. to perform molecular based species identification for resolving morphological ambiguity
5. to investigate genetic diversity among individuals of *Monopterus cuchia*
6. to observe the genetic population of *Monopterus cuchia* collected from different ecological habitats
7. to make relationships among populations as well as individuals
8. to identify better population for breeding program from different ecosystems in Bangladesh

The freshwater mud eel, *Monopterus cuchia* is leading amphibious life and found in plenty commonly in stagnant waters in mud-holes in shallow beels and boro-paddy fields with low oxygen content throughout the country (Rahman 1964). According to the Talwar and Jhingran (1991) the freshwater mud eel is distributed in Bangladesh, India (Northern and Northeastern), Pakistan, Nepal and Myanmar etc. The mud eel *(M. cuchia)* is a carnivorous and nocturnal prefers animal based food like small fishes, mollusks and worms etc. This fish is very tasty, nutritionally rich with medicinal value and highly price in foreign markets. This fish thrive on the small fishes, prawns, molluscs, aquatic insects, small frogs, etc. (Ponniah 2000). This fish can play a unique role for socio-economic welfare of the area and thus social fishery could be developed (Quddus *et al.* 2000). Although, this fish have quite a large economic demand, the populations of this freshwater eel are declining at an alarming rate from the natural water bodies due to several reasons specially for overfishing while population increased completely depends on natural reproduction (Ponniah 2000, Khan 2008) and thus this fish are recorded as rare species in Bangladeshi nature (IUCN 2000). In addition, no culture system is developed in Bangladesh (Quddus *et al*. 2000) and unsuccessful artificial breeding was observed through inducing agents (Shuvra 2011). Due to the risk of this fish in Bangladesh it is more necessary to conserve. Thus, this review is important and can consider the genetic research of this fish in details to find out the specific reason which will be contributed for artificial breeding, higher production, diseases resistance and conservation strategy etc.

The freshwater mud eel, *M. cuchia* (Hamilton 1822), belongs to the family synbranchidae of the order synbranchiformes (Rosen and Greenwood 1976, Shafi and Quddus 1982). It has some synonyms such as *Amphipnous cuchia* (Hamilton 1822),

Pneumabranchus albinus (McClelland 1844), *Pneumabranchus leprosus* (McClelland 1844), *Pneumabranchus striatus* (McClelland 1844) and *Unibranchapertura cuchia* (Hamilton 1822), etc. It is commonly known as Cuchia, Rice eel or Swamp eel etc. Though former researchers identifies this fish morphologically, however, recently molecular based identification is mostly used for authentication and this fish is already identified through molecular basis by using mtDNA and Glutamine Synthetage gene (Miah *et al*. 2013a) which is a part of this research.

Taxonomically this fish is identified by the taxonomic formula which was designed as B= VI, D=very rudimentary, P1. P2. A. and C= absent (Bhuiyan 1964, Shafi and Quddus 1982, Rahman 1989 and 2005, IUCN 2000). Maximum Length of this fish was recorded 20cm, (Bhuiyan 1964), 66cm (Rahman 1989 and 2005), 60cm (Talwar and Jhingran 1991, IUCN 2000) and 82cm (Galib *et al.* 2009).

The habitat of this fish is freshwater and brackish water and found in shallow, well vegetated water and mud (IUCN 2000). They are inhabits plenty in mud holes in shallow beels and boro paddy field throughout the Bangladesh (especially Sylhet, Mymensing, and Tangail districts) (Bhuiyan 1964, Rahman 1989 and 2005). However, Galib *et al*. (2009) also recorded from Chalan beel. Shafi and Quddus (1982) stated that this fish are living in ponds, canals, rivers, beel, baor shallow water comparatively rich with aquatic plants. Also found in flooded rice fields. They can live in holes without water by the help of respiratory organs. Some fishery scientist says that they pass entire summer in hole, but sometimes coming out from the hole to take oxygen. Most of the time in hole of water their mouth position is kept straight upper position and return into the hole completely when found any enemy.

The fish *M. cuchia* is common in freshwater and likes muddy environments. They often spend their daytime hiding under stones and mud or having a burrowing habit (Nasar 1997). This fish resides in the mud holes in the shallow beels along with the dykes of paddy field, pits and swamps during the winter season. The freshwater mud eel, *M. cuchia* is also an evasive nocturnal animal. This fish has versatile motility and is even capable of moving over dry land for short distances. This behavior is used for relocation according to resource availability. In the absence of water and food, this Asian swamp eel is able to survive long periods of drought by burrowing in moist earth (Campbel and Reece 2005). If its home becomes unsuitable, *M. cuchia* simply crawls ashore and make its way to a more suitable home by slithering over the land in a snake-like fashion. These characteristics enhance the ability of *M. cuchia* to disperse widely (Mc Pherson 2000). Like other eels, freshwater mud eel provide various values for the ecosystem and for the human beings. The unique adaptation that allows mud eel to be successful in their environments, primarily for wedging through small openings, while some are adapted to burrowing into soft substrates or living a pelagic existence (Nelson 1994).

The body of the freshwater mud eel, *M. cuchia* is a slender shaped streamline with a tapering tail (Day 1878). They do not have pectoral, pelvic and dorsal fin. The dorsal and anal fins are fused with caudal or tail fins forming a single ribbon along the whole length of the fish. The gills of *M. cuchia* are reduced and the fish has a pair of air breathing organ in the form of a sac on two sides of the head (Talwar and Jhingran 1991). A pair of supra branchial chambers are present and each contain a complicated labyrinthine organ (Mittal and Agarwal 1977). The pharyngeal pouches starts developing at an early stage but become functional quite late in its life history (Mittal

et al. 1980). The main feature of the body is slimy. According to the Shafi and Quddus (1982), elongated and cylindrical body form caudal at the posterior part with sequentially narrow. Ventral fins are reduced, sometimes skin folds are seen. Only gill openings are situated antero-lateral part of the body. Anus is posterior situated than normal position. Barble is absent however rib and one row of palatine teeth are present. Gill filaments are distributed up to isthmus. Wet and slippery skin is found. Very small, round and indistinct scales longitudinally arranged. Body color is deep brown whereas abdominal part is comparatively opaque. Eyes are small, head not conspicuous, gill-opening crescentic of which gills greatly reduces. A pair of suprabranchial pouch (respiratory organ) is large. Munshi *et al*. (1989) described the structure of the air-breathing organs by the examination with light, scanning and transmission electron microscopy and the morphological basis for buccopharyngeal, aerial as well as aquatic respiration while respiratory islets were found well distributed over the surface of the buccopharynx, hypopharynx and branchial arches extending deep into the gill clefts but occupy only the anterior two-thirds of the air sacs, the remaining posterior one-third part seems to be non-respiratory in function and may serve as a reservoir for residual air. Munshi *et al*. (1989) also found that arterioles penetrate deep into the epithelial region of air sacs and buccopharynx in spiral-like fashion to form the characteristic vascular papillae of the respiratory islets.

Some exceptional physiological activities particularly respiratory adaptations were observed in this fish (Shing *et al*. 1989). Hughes *et al*. (1974) indicated the areas of the air-breathing surface of *Amphipnous cuchia*. The gills of *M. cuchia* are reduced and the fish has a pair of air breathing organ in the form of a sac on two sides of the head (Talwar and Jhingran 1991). The species has dual systems of oxygen uptake for

respiration: aquatic respiration by uptaking dissolved oxygen through the gills and aerial respiration by uptaking air directly through the skin (Liem 1967). These mechanisms enable it to survive in oxygen-depleted conditions or in areas where water is scarce. Aerial respiration performed by the fish with the aid of a pair of supra branchial chambers each containing a complicated labyrinthine organ (Mittal and Agarwal 1977). The pharyngeal pouches starts developing at an early stage but become functional quite late in its life history (Mittal *et al.* 1980). For this special modification, this fish can survive 90 to 132 days without food and can be stored large number in a small sample container as well as transported in live in a small earthenware or plastic jar to distant places (Pullin and Shehadan 1980). This fish release excessive body slime for escaping from the predators. The freshwater mud eel, *M. cuchia* has versatile motility and is even capable of moving over dry land for short distances. This behavior is used for relocation according to resource availability. In the absence of water and food, this eel is able to survive long periods of drought by burrowing in moist earth (Campbel and Reece 2005). If its home becomes unsuitable, *M. cuchia* simply crawls ashore and make its way to a more suitable home by slithering over the land in a snake-like fashion. These characteristics enhance the ability of *M. cuchia* to disperse widely (Mc Pherson 2000).

Biology especially population biology of the freshwater mud eel**,** *M. cuchia* was studied (Sultana 2008). The freshwater mud eel *M. cuchia* is a voracious general predator that feed during the night on small fish, amphibians, crustaceans, echinoderms, insect larvae, aquatic invertebrates etc. (Mc Pherson 2000). The natural foods of this fish are fish fingerlings, earthworms, tubifex, snails, aquatic insects, insect pupae, slaughter house waste (liver, intestine, viscera, skin of livestock

animals) etc. The fish *M. cuchia* showed significantly highest growth rate in terms of increase in weight with receiving dead small fish as feed and lowest growth was recorded in fed with pellet feed (Khan 2008). The growth rate might be associated with the size of fingerlings stocked, quality of feed supplied, culture period and season. The survival rate of fish with different food items ranged from 80 ± 10.0 to 94 ± 6.0 (Khan 2008). A composition of typical eel growing diet is mentioned in table 2.1. Narejo *et al*. (2002) was studied the length-weight relationship and relative condition factor (Kn) of *M. cuchia* and the relationships were found significantly at 0.1% level indicating isometric growth pattern. Relative condition factor was found to be high during the monsoon months (spawning period) from April -June reflecting the maturity of the fish. First maturity was found to attain at 25.2 cm in total length. The length-weight relationship and condition factor showed that the growth of *M. cuchia* is quite satisfactory. Narejo *et al*. (2003a and 2003b) was also observed the effect of different feeds and shelters on growth, survival and production of freshwater mud eel, *M. cuchia* in the cemented cisterns considering different feeds where they found better growth, survival rate and production through the supply of dead small fish. On the basis of survival rate and production, they were also suggested that the water hyacinth is suitable shelter for the culture of *M. cuchia* in the cemented cisterns. However, it was found the best growth and survival of this fish in a cemented tank with live food (Fish fry and earthworms) as well as in earthen ditches with supplement food than other tanks where dead fish was supplied (part of my PhD research 2013).

Table 2.1. Composition of typical eel grower diet (46% protein diet)

Ingredients (air dried)	Percentage
White fish meal (65% protein)	62–69
Alfa-potato starch	20–24
Yeast and liver meal	4–6
Vitamin mixture	1–2
Mineral mixture	2
Others	1–3

Taxonomically the experimental fish was identified by the study of some important morphometric characteristics. Identification of male or female fish is so difficult but some external characteristics are helpful to observe sexual differentiation during breeding season. It was recorded that the mature female was larger than male fish and the abdomen of female fish is swollen and brownish in colour with rough abdominal skin (Miah *et al*. 2013). Anus and genital pore was observed as tubular in male round shape in female.

Internally, single gonad of both sexes comprised of a white, smooth, ribbon-like structure extending longitudinally below the gut and above the kidney for the entire length of the abdominal cavity (Miah *et al*. 2013a). In male two equal, very thin, narrow and long sperm ducts were observed which is extended from anus to liver. However, single tubular oviduct was found in female with eggs from urinogenital opening to anterior part of the gall bladder. In an average 600 round eggs were found

in the experimental females and the egg size was ranged between 0.1mm to 0.7 mm in diameter. Histologically, the testis and ovary was also studied and easily separated from male to female (Miah *et al*. 2013a).

M. cuchia is a rare species of air breathing teleostean fish in Bangladesh and India with spawning period confined down to peak summer and this fish lays its eggs in especially prepared nest-holes and keeps a guard on the developing young ones (Banerji *et al*. 1981). The yolk reserve persists till about 22 to 24 days of development and larvae respond to direct feeding even before yolk-absorption. Banerji *et al*. (1981) also mentioned that the young ones feed actively on chironomus larvae. Surface breaking habit for aerial respiration apparently commences around 15th–16th day of larval life. Adult characters are almost attained in about a month's time (Banerji *et al*. 1981). In the case of *M. cuchia*, its obligate sedentary nature because of the persisting heavy yolk mass would not permit an easy drift during the course of heavy floods. It is, therefore, a safe ecological adjustment for *M. cuchia* to spawn in the peak of summer with its nest-hole built just near the sub-surface level of the water (Das 1946). Low dissolved oxygen in the water, a possible disadvantage of the summer breeding, is adequately met in this species by the relatively large yolk mass, rich in carotene as indicated by its deep orange-yellow hue, yolk with such characteristics is known to be involved in respiratory functions. High vascularization of the body surface of the young ones of *M. cuchia*, particularly on its finfolds and yolk sac, indicates well developed skin respiration, as has also been referred to by Hughes *et al*. (1974) who accounted for the low air-sac area of smaller *Ampliphnous cuchia* to be compensated by skin respiration. This explains the tendency of the parent fish to keep the brood in an amphibian environment.

Artificial propagation of eels has long been attempted not only to address fundamental questions on the reproductive biology of this fish, but also from a fisheries management and eel aquaculture perspective. Increasing fishing pressures on this eel has led to reduced recruitment of juveniles, limiting the industry and potentially leading to ecological impacts. However, captive breeding of this fish has not yet been achieved. Sexual development in freshwater eels is halted when these fish are held in captivity (Dufour *et al.* 1988), but this arrest can be overridden by hormonal treatment. Though reproductive biology this fish has been described in terms of sex-dimorphism, gonadosomatic index, ova diameter and fecundity while peaks of Gonadosomatic Index (GSI) recorded during April to June for female (7.52 ± 1.15) and male (5.50 ± 1.25) indicated that the fish has only one breeding season during summer (Narejo *et al*. 2003c) where the ova diameter from 0.30 mm to 4.0 mm and the number of ova per gram body weight were found 4.61 and the number of ova present per gram of ovary weight was recorded 74.27. Recently the reproductive biology and gonad histology of freshwater mud eel *M. cuchia* was also performed by Jahan *et al.* (2014) considering gonadosomatic Index (GSI) and ova diameter, fecundity, vitellogenesis and this study may contribute to have successful breeding program and seed production technology of this fish species in captivity. Mass seed production and conservation of the available populations through proper management of the populations is recommended to save this threatened species from extinction, though practically no work either in private level or government or institutional level so far been done in Bangladesh on the artificial breeding of this fish (Quddus *et al*. 2000).

The freshwater habitat of Bangladesh is very suitable for living this fish and the population of this fish is very high. But due to this fish have quite a large economic demand the populations of the freshwater eel are declining at an alarming rate from the natural water bodies due to water obstruction, habitat destruction, pollution, excessive use of pestisides, industrial flow, over exploitation and destructive fishing (Ponniah 2000). The freshwater mud eel is recorded as rare species from floodplains and beels due to attack by several diseases (Hossain and Mazid 1995).

Very limited research regarding genetics and molecular biology has been performed of this freshwater mud eel in Bangladesh as well as in other countries. Although some works have so far been done on this fish, however, no researches were carried out on genetic biotechnology or population genetic structure of this fish, while only DNA fingerprinting were observed in narrow sense using RAPD marker in China (He *et al.* 2004) and Bangladesh (Alam *et al*. 2010). Different eels were identified by RFLP using mitochondrial DNA (Lin *et al.* 2002), but molecular identification and genetic characterization were not found of this species. As this fish is vulnerable in Bangladesh, it is required to maintain high level of biodiversity to develop natural and artificial breeding, and to increase high production. Glutamine synthetase (GS) is an enzyme that plays an important role in the metabolism of nitrogen by catalyzing the condensation of glutamate and ammonia to form glutamine (Eisenberg *et al*. 1987). Due to the key role of Glutamine Synthetage (GS) in detoxifying ammonia, particularly in the brain, and in ammonia excretion in the kidney in fish, therefore, this gene of freshwater mud eel was sequenced (Saha *et al*. 2010). On the other hand, comparative mitochondrial DNA sequence and amino acid analysis of the cytochrome C Oxidase Subunit I (COI) from two eel species, *M. cuchia* and *M. albus* were also

studied (Devi *et al*. 2014). Fishing and aquaculture activities pose potential threats to genetic diversity of wild fish populations in different ways. For instance, over-exploitation may reduce population sizes to levels where inbreeding and loss of genetic diversity through random events (by the process of genetic drift) become serious problems, or may result in extinction of local populations or population segments. In recent years, this species has been declining over much of its range; so careful monitoring and molecular genetic research will be vital to its survival and conservation.

Though biochemical and nutritional components of the freshwater mud eel were not recorded strongly however, it was recorded that different eels including *M. cuchia* contain high nutritional compounds (Table 2.2). The average protein content per 100g of eel flesh is 14g and the caloric value of eel flesh is as high as 303 Kcal/100g compared to 110 Kcal/100g in other average fishes (Nasar 1997). Besides, the freshwater mud eel *M. cuchia* has medicinal value and some ethnic tribal group use the fish for few diseases. Some haematological parameters of this freshwater mud eel including haemoglobin concentrations, haematocrit values, red blood cell counts, red blood cell diameter, erythrocyte sedimentation rate and plasma haemoglobin concentration have been measured by Mishra *et al*. (1977).

Table 2.2. Nutritional elements in different eels

Nutrients	Amounts	Nutrients	Amounts
Minerals		Vitamins	
Selenium	13.3mcg	Choline	133 mg
Manganese	0.1mg	Pantothenic Acid	0.1 mg
Zinc	3.3mg	VitaminB12	6.1 mcg
Sodium	104mg	Folate	30.6 mcg
Potassium	555mg	Vitamin B6	0.1 mg
Phosphorus	441mg	Niacin	7.1mg
Magnesium	40.8mg	Riboflavin	0.1mg
Iron	1.0mg	Vitamin E	8.2mg
Calcium	40.8mg	Vitamin C	3.7mg
Fatty acids		Other components	
Total omega -6 fatty acids	400mg	Ash	2.9mg
Total omega -3 fatty acids	1332mg	Water	139mg
Poly unsaturated Fat	1.9g	Proteins	38mg
Monounsaturated Fat	14.7g	Sodium	104mg
Saturated Fat	4.8g	Cholesterol	257mg
Total Fat	23.8g		
Calories			
Calories from Protein		161	
Calories from Carbohydrates		0.2	
Total calories		375	

The adaptive strategies against ammonia toxicity in the amphibious mud eel, *Amphipnous cuchia* were observed (Khongsngi 2010) whereas the freshwater mud eel, *A. cuchia* faces the problem of ammonia toxicity in its natural habitats due to build up of high ammonia during exposure to high external ammonia (HEA) and also during desiccation stress. The mud eel is primarily ammoniotelic while living in water excreting ammonia as the major nitrogenous excretory end product. The glutamine enzyme (Glutamine Synthetage=GS) is a very important enzyme for detoxification of toxic ammonia and the key enzyme in nitrogen metabolism and this enzyme was found to be widely distributed at relatively high levels in different tissues of the mud eel, with maximum activity in brain, followed by stomach, intestine, kidney, liver and muscle. Interestingly, a high level of GS enzyme activity was found in stomach and intestine of this fish, where the enzyme may have a role unrelated to that in the liver and kidney. Exposure to HEA and terrestrial habitat causes upregulation of GS enzyme activity and also the GS enzyme protein concentrations in different tissues of the mud eel. The mud eel, *A. cuchia* is uniquely adapted to nitrogen metabolism using multiple strategies to tackle the problem of ammonia toxicity, thus enabling them to survive in extreme habitats that are totally unsuitable for any typical teleost (Khongsngi 2010).

Toxicity was also examined by Mech and Rai (2014) as acute toxicity study of retinoic acid in the freshwater eel, *M. cuchia*. All-trans retinoic acid (RA) is an active metabolite of vitamin A and its administration may prevent most of the defects generated by vitamin A deficient. It was also found out the LD50 value of RA in *M. cuchia* through intramuscular injection resulting the impact of RA may be used in

managing various fish farms and to increase the immune function of the fish for better yield.

The health conditions of freshwater mud eel, *M. cuchia* was investigated through clinical and histopathological observations (Ahmed *et al*. 2009). It was found that water temperature and total hardness were at reduced level for fish during the months of November to January. Clinically weak body, rough skin, deep ulcer and grey brownish colour of the body were noticed. Major pathology in the skin and muscle such as loss of epidermis and dermis, necrosis of myotoms, huge vacuum spaces, marked melanomacrophage and fungal granuloma were found (Ahmed *et al*. 2009). Ahmed *et al*. (2009) also marked melanomacrophages, severe haemorrhage, vaccums, hepatic necrosis, fat droplets in the liver and kidney. Reduced temperature and total hardness might have played role in the occurrence of clinical and pathological changes in fish. The fishes were affected by Epizootic Ulcerative Syndrome (EUS), with the evidence of development of fungal granuloma in skin, muscle and kidney (Figure 2.1). It was found that health condition was detoriated during the winter season generally.

Very low parasitic affect was observed in the freshwater mud eel *M. cuchia.* The overall gill parasites and stomach parasites of *M. cuchia* were poor (Khan 2008). A gill parasite (*Argulus* sp.) was found in freshwater mud eel, *M. cuchia* while no stomach or visceral parasites were available. Two species of parasites were found in this fish, *viz*. *Argulus* sp. (Family -Argulidae) and *Ergasilus* sp. (Family - Ergasilidae) (Khan 2008). Shafi and Quddus (1982) is mentioned that only unicellular protozoan parasite, *Chloromyxum amphlovi* are seen in the gall bladder of this fish. Das *et al.*

(2012) was examine thirty specimens of *M. cuchia* for parasitic cestode fauna and out of these fourteen specimens were found to be infected with cestodes and they were restricted to the intestine of the fish. It also found that the female fish show higher prevalence than the male fish whereas the fish specimen with intermediate weight group shows highest prevalence of infestation.

Figure 2.1. Epizootic Ulcerative Syndrome (EUS) affected *M. cuchia* (Ahmed *et al*. 2009)

The freshwater mud eel, *M. cuchia* is used as food fish in Bangladesh but many people do not take it as food where only some tribal people accept this flesh. Some people are believed to have medicinal value.

The freshwater mud eel culture is a low-cost system compared to other small-scale fish culture projects and it is presumed a low-cost enterprise to the farmers. This fish culture does not necessarily require large bodies of water and specific expensive formulated feed. The mud eel, *M. cuchia* is quite hardy and pollution resistant. It can be profitably raised with aquatic crops like swamp cabbage (Nasar 1997). Raising this species is easy to do and achieves a more profit than some other small size fish culture activities (LU *et al*. 2005, IIRR *et al*. 2001). This fish can be easily cultured in a small tank, aquarium and other vessels while the species adjust well to life in captivity and

are very hardy inhabitants. A larger aquarium with adequate hiding places and a well-sealed lid is essential for maintaining this fish. Though the effect of different feeds and shelters on growth, survival and production of freshwater mud eel, *M. cuchia* in Bangladesh was studied by Narejo *et al*. (2003a, 2003b) whereas they found a potentiality of this fish culture in Bangladesh but no productive culture system is developed for commercial purposes. In addition, Chakraborty *et al.* (2010) was observe production potential of this mud eel in participatory semi-intensive culture systems considering rice field and ponds. Rice field and pond habitat was improved by installing mud-compost hips, bamboo roots; plastic and bamboo-made hollow pipes, and aquatic vegetations. The feed was supplied with dead fish, dry fish, live carp fry and flesh of snails and bivalves. Though, lower growth performance of *M cuchia* was observed in rice field than pond however, it was said that semi-intensive culture both in rice fields and ponds are a good proposition as an aquaculture technology to save the mud eel from decline and enhance the nutritional status and socio-economic improvement of the Adivasi (Ethnic) people.

As no commercial culture practice is available in Bangladesh, the freshwater mud eel, *M. cuchia* business is completely depends on natural capture only (Figure 2.2). Fish catchers are capturing this fish throughout the year but it is easy to capture during summer and the fish is found abundantly at this period. Due to the complexities of its habitat, behavior and life history, makes it difficult to catch the fish, therefore, the fishing methods for catching the mud eel is scantly and different techniques employed traditionally by the ethnic communities where they use woundering gears including spear, knives and sickle, ichthyotoxic plants such as *Derris elliptica* and *Milletia pachycarpa* etc., handlines, spindle shaped and cubical traps etc. (Figure 2.3) (Barman

et al. 2013). Other methods including bunding and digging, and light fishing are also documented.

Figure 2.2. Captured mud eels

Figure 2.3. The fishing traps

The freshwater mud eel from the business side is promising because the demand from within and outside the country until the current unmet demand and the growing number of day let alone there is increasing recognition by some countries that Indonesia, especially the eels came from Java island has the highest quality. Its demand for export is increasing day by day. Though, in Bangladesh very few amounts of people consume it, by exporting it, a lot of foreign currency would be earned and it will help to improve the national economy of Bangladesh.

This eel fish is popular across the world and are consumed and prepared in many different ways, in different countries. This fish has tremendous demand in foreign countries like Japan, Korea, China, Thailand, Taiwan, Honkong, New Zealand, Australia, Europe etc. (Herbst 2001). The freshwater eel, *M. cuchia* trade, a high-value export fishery involving nearly 15 countries in the world, with high demand in China, Malaysia, Singapore, Japan and Taiwan (Hasan *et al*. 2012). This mud eel is exported from the Laos, Cambodia and Vietnam and Indian part of Assam and Meghalaya. But in recent years, a scope has been established to export this fish from Bangladesh. Now-a-days the freshwater mud eel is a commercially important fish in Bangladesh (Zaher and Mazid 1993). In a few grassroots level studies it has been observed that, this fish is being sold commercially in the local market worthing TK. 250 to TK. 400 (3.20 to 5.13$) per kg according to grade in the recent years (Khan 2008). The annual landing of the freshwater mud eel is about 1.85 Metric tons (Ahmed *et al*. 2009). This freshwater eel comprise only 1.5% of the total fish landing centre at different parts of the country (BFFEA 1989). In Bangladesh this fish is only consumed by the tribal people, however it is commercially important due to its high demand for export (Narejo *et al.* 2003a).

The marketing system passes through a number of channels from catcher to exporter. A different grading system is involved for marketing of this freshwater eel in Bangladesh. It is varied according to the sex and size of the eel in the international market. Freshwater eel marketing was considered as a profitable business by most of the marketing operators. The social and religious restriction on consumption of freshwater eel was also reported as a problem that hinders the business and prevents obtaining realistic prices in the domestic market (Hasan *et al*. 2012). In marketing

systems of this fish, there are a number of people involved in Bangladesh. The market chain from collector to consumers passes through a number of intermediaries like local agents, wholesalers, suppliers, exporters and buyers (Hossain *et al*. 2007, Hasan *et al*. 2012). The demand of freshwater eel is high for export (Figure 2. 4) but supply is not enough because freshwater eel is now in vulnerable condition. Farmers never can directly communicate with consumers, market communication normally being made through supplier or local agent. The supplier usually buys the freshwater eel from the local agent but do not seem to have formal agreements with particular producers. Collectors directly sell their fish to wholesalers or through local agents. Local agents are normally based in local markets near to fish farming communities. Local agents or sellers usually sell the freshwater eel to the wholesale markets. Local agents have informal agreements with wholesalers obliging them to supply certain quantities in spite of the lower profit margins. Local agents used to carry freshwater eel from remote village where the rate is low. Sometimes seller or supplier take small amounts of *dadon* (credit) from wholesalers to ensure the supply of fish from farmers. *Dadon* is a system of tied credit through which the wholesalers advance money to the suppliers in exchange for the assured sale of fish. Wholesaler commonly use mechanical vehicles (trucks, pickups and microbus) to transport fish from local area to Dhaka packing center which takes 7-10 hours depending on the communication system (Figure 2. 5). Aluminum containers, bamboo baskets with polythene covers are commonly used for keeping the freshwater eel during the transport. Then suppliers use plastic basket and foam box for the preparation of export, they use ice and ginger during packaging to decrease mortality. Finally exporter export by cargo plane. The cargo plane can carry as much as 40-50 tons of live fish.

Bangladesh began exporting freshwater eel around 1987-88 and since 1992 the value of export earnings has been steadily increasing. In 2002, freshwater eel ranked 4 in terms of frozen food export items (Hasan *et al*. 2012). Price fluctuation, lack of buyers and market information, credit problems, high mortality and poor transportation systems in the marketing of freshwater eel have been reported by some researchers in Bangladesh. All of these problems have negative effects on the international markets. To achieve an efficient marketing system in a competitive manner it is necessary to identify the existing problems and to correct/improve these wherever they occur in the chain. It will also be useful for the policy makers to identify the existing weaknesses, by providing them with the necessary information for formulating strategies towards improvement of the eel fishery.

Two types of domestic market so far have been reported by Ahmed (1997), one is nearer to local markets in the vicinity of the fishing village and another one is the consumer markets away from fishing areas. The market price varies with the size and with the season and the selling price is normally fixed through bargaining between the seller and consumers. Besides live eel, many East Asian and South East Asian countries export different forms of eel and eel products throughout the world.

Figure 2.4. Suppliers stocked the fish in tanks for transport to exporters

Figure 2.5. Transport mud eel from Biswnath, Sylhet to Dhaka by truck through drums

According to Hasnat (2014), the freshwater mud eel is an exported item in Bangladesh and its export is increasing yearly. According to the information of fisheries department, 7157 tons of freshwater mud eel is exported from Bangladesh in 2013-2014 fiscal year, 6817 Tons in 2012-2013, 5057 tons in 2011-2012, 3295 tons in 2010-2011 and 1782 tons by 2009-2010. According to the information of Export

Development Burue (EPB), Bangladesh has been earned 1, 49, 78, 000 USD by exporting this fish in 2013-2014 and 1, 09, 21, 000 USD in 2012-2013. That means the scope of exporting this fish is increased in both amount and foreign currency earning. According to the exporter, a large amount of this fish is exporting in China, Honkong, Taiwan, USA, South Korea, Singapore, UAE, Canana, Japan and some other countries.

According to the EPB, 95% of of freshwater eel is exported in China last year and it is around 1,41,62,000 USD, 3,25,000 USD in USA, 2,71,000 USD in Hong kong, 69,000 USD in South Korea and 64,000 USD in Taiwan. Mr. Rabiul Alam, Managing Director of Seba Aqua Resources is a first eel exporter of the country in year 1995, says that the freshwater mud eel and crabs are most potential export item in Bangladesh. He told that if Government is giving a good concern regarding this fish and crabs like other frozen fish it would be a very possibility to earn foreign currency like shrimps. It was known that this freshwater mud eel is started to export since 1980s and every year it is increasing to export this fish resulting now the demand of this fish in increasing across the country. Exporters said that, they collect this fish from Laksham, Chandpur, Sylhet, Moulvi Bazar, Habiganj and Sunamganj in summer season, and in winter season they collect this fish in large amount from Gopalganj, Faridpur, RaajBari, Narail and North Bengal of Bangladesh. It is known that market value of this is fluctuated. According to the size, the price of the freshwater mud eel is tanged from150 taka to 280 taka per kg in the field level from wholesaler stake holders. Stake holder sells this fish to the exporters as rate 250 taka to 450 taka per kg. Exporters export this fish as 500 taka to 800 taka per kg. Three unknown eel export company said that every week 150 to 200 tons of this eel is exported.

However, out of this amount lot of this fish is exporting by some awful businessmen while Government is unknown (Hasnat 2014). Considering the total export earnings from freshwater eel the fishery shows future potential.

Socio-economic potential of the freshwater mud eel is immense in Bangladesh. This fish can play a unique role for socio-economic welfare of the area which will be developed social fishery in Bangladesh (Quddus *et al*. 2000). It has been observed that this fish can meet the increasing demand of animal protein in Bangladesh as well as to collect foreign currencies that will help to improve the national economy of Bangladesh (Zaher and Mazid 1993). In Bangladesh, it has revealed in a report that this fish economically important in various groups of people, especially the Hindu and Christian communities and some ethnic tribal group.

The fishery of freshwater mud eel can generate employment directly and indirectly in terms of people employed in the marketing and other associated business (Figure 2.6). More than 8,000 fishers, collector, depot owner, supplier/agent, traders, transporters and exporters were found to be involved in this sector (Hasan *et al.* 2012). Most marginalized segment of population especially land less people, widow and children are involved in eel collection for their livelihoods earning. Domestic demand needs to increase through increasing social awareness and promoting awareness of the nutritive value of this export oriented species.

Capturing this fish play a unique of some poor people for their livelihood. Though, it has no demand in local markets and majority of the people do not eat this fish in Bangladesh some fishermen release this fish in nature, and rarely people eat this fish

as medicine. Tea labours and some Hindus eat this fish as spiny eels. Along fishermen sometimes tea labours are engaged in capturing this fish. Day by day the business of this fish is promising. Therefore, this freshwater mud eel is economically benefitted whereas the interest of this fish is completely depends on natural breeding, however, no steps is taken by Government or NGOs for production and development of this fish in Bangladesh. If this fish are cultured commercially it must be play a vital role to our national economy. It will make a work facility and hope for jobless people as well. Therefore, to develop and conserve this valuable fish in the nature of Bangladesh support from government and different nongovernmental organizations needs to be greater and perceptions regarding the activity need to show attention for the culture system of freshwater mud eel and marketing in Bangladesh.

Figure 2.6. Stakeholder of freshwater mud eel, Biswnath, Sylhet

3.1. Fish

The freshwater mud eel, *Monopterus cuchia* was considered for this experiment. The experiments of this fish were conducted in different approaches such as culture, reproductive biology, artificial breeding, molecular based species identification and genetic diversity based population study. Fish were collected several times for conducting different experiments. Fish were collected from different sources such as open waters by the help of fisherman and from several Motso Arots for studying culture, biology, breeding and molecular identification. Fish were also collected by the help of professional fish catcher from "Tanguar Haour", Sunamganj and Hakaluki Haor, Moulvi Bazar for studying population genetics. Collected fish samples were brought to the fish breeding house as well as in the laboratory of Genetic Engineering and Biotechnology (GEB) at Shahjalal University of Science and Technology (SUST), Sylhet, Bangladesh. Fish were identified as mud eel by observing the characteristics of external morphology and morphometry (Bhuiyan 1964, Jayaram 1981, Shafi and Quddus 1982, Rahman 1989, Talwar and Jhingran 1991) and also by the help of local fisherman.

3.2. Experimental site

The research works were conducted in both laboratory and field level. Fish culture was conducted in cemented tanks in the fish breeding house of GEB, backyard tanks and earthen ditches in village environments, Chatak, Sunamgonj as well as a plastic tank in a hatchery pond, Kamal Bazar, Sylhet. Breeding biology was studied in the fish breeding house of GEB, SUST. Histological analysis of gonads was studied using microtomy in the laboratory of animal biotechnology in the Department of GEB,

SUST. Artificial breeding was performed in the tanks of fish breeding house of GEB, SUST, in the aquariums of the laboratory of animal biotechnology, GEB, SUST, in the hapa of the GEB experimental fish pond, in a cemented tank of a hatchery which was situated outside the Department of GEB, SUST. Molecular analyses were conducted in the general laboratory, USDA laboratory and animal biotechnology laboratory in the Department of GEB at SUST, Sylhet, Bangladesh. Water quality parameters were studied in the Water Supply Management Laboratory of the Department of Civil and Environmental Engineering (CEE), Shahjalal University of Science and Technology (SUST), Sylhet

3.3. Rearing and production study

3.3.1. Experimental set up and sample collection

The experiment was conducted in three tanks in the Fish Breeding House under the Department of Genetic Engineering and Biotechnology (GEB), Shahjalal University of Science and Technology (SUST), Sylhet (Figure 3.1). Two backyard tanks and two earthen ditches were selected in a rural house, Chatak, Sunamgonj (Figure 3.2 and Figure 3.3). Experiment with a plastic tank was designed in a pond of tilapia hatchery at Kamal Bazar, Sylhet (Figure 3.4). The fingerlings of this freshwater mud eel were collected from the "Seven Star Motso Arot", Rashidpur Bazar, about 15 kilometers away from Sylhet. Fish were identified as mud eel by external morphology (Shafi and Quddus 1982, Talwar and Jhingran 1991) and also by the help of local fisherman. The fish fries were put into a bamboo cage and brought to the fish breeding house of the Department of GEB, SUST carefully. Collected fish fries were divided into six categories and placed in different environmental conditions such as house tanks,

earthen ditches, backyard tanks and plastic tank. In each environment 40 individuals in number approximately equal 1kg fish was released.

Figure 3.1. Breeding house and breeding tanks of GEB, SUST

Figure 3.2. Two backyard tanks

Figure 3.3. Two earthen ditches

Figure 3.4. Plastic tank

3.3.2. Shelter management and stocking density

Three cemented tanks in breeding house were selected for part of this experiment. The length, width and height of each tank were 1 x 1.5 x 1 meter. Tanks were connected with regular water supply and maintained water depth in each tank with 13-15 inches. First of all, three tanks were filled with water and kept as same for two days without fish. Then, after two days fish were placed in each tank. Two earthen ditches were selected near of a rural house, Chatak, Sunamgonj which was already made. The length, width and depth were around 4^2 meter with 18-24 inches depth of water. The earthen ditches were filled with water and maintained with 24 inches for two days without fish. One tank was constructed with bamboo and lined with plastic and measure with 1 x 1.5 x 1.5 meter which was settled down in the experimental pond of tilapia hatchery at Kamal Bazar, Sylhet. In this case pond water was used for rearing fish considering 15 inches of water depth. There were water-hyacinth placed to cover the fish. Two backyard tanks were selected in the back place of a house and the length, width and height of each tank was 1 x 1.5 x 0.5 meter respectively filled with 10-12 inch of water. An amount of 1kg (40 in number) of fish was placed in each shelter. But before releasing the fingerlings, initially fishes were measured for all environments considering length and weight of fish (Table 3.1).

Table 3.1. Stocking rates of fish in different environments

Culture environments	Fingerlings size (Average in cm)	Fingerlings weight (Average in g)	Number of fish	Av. weight of fish (kg)	Rearing period (month)
House tank 1	15	24.6	40	1	6
House tank 2	15.5	25.4	40	1	6
House tank 3	14	23.5	40	1	6
Backyard tank 1	14.5	23.8	40	1	6
Backyard tank 2	15.7	25.4	40	1	6
Plastic tank	13.9	23.8	40	1	6
Earthen ditch 1	14.5	25	40	1	6
Earthen ditch 2	15.7	25.5	40	1	6

3.3.3. Water management for all the culture systems

Before construction of any culture environment, it is wiser to think about water management system including source, discharge and quality of water (Akankali *et al*. 2011). For culturing fish it is necessary to supply water regularly and without water supply it is quite difficult to maintain fish in alive. In this experiment different captive environments were used. Different sources of water were used for the different culture systems. Water temperature, pH and dissolved oxygen level were recorded through the experiment. For breeding house tanks, water was supplied through pipes from the submerge system. Each tank was well designed and water level was maintained about 13-15 inches. Water has been exchanged within three days interval. Natural water was used for earthen ditches considering 18-24 inches deep, however, when needed water

was supplied from outside. In plastic tank, water was maintained around 15 inches and water was changed weekly by bucket from pond. In case of backyard tanks, water was supplied from the pond by plastic bucket considering 10-12 inches of depth and water was exchanged biweekly. Sometimes algae and tiny mosses were used in backyard tanks and plastic tank.

3.3.4. Feed supplements

In this experiment different types of feed were given to the different environments for culture and production of *M. cuchia*. Different types of feed such as small dead fish, earth worms, small live fish (Tilapia fry), snails, small frog and chicken viscera were given. Feed were supplied to each of the different environment in different amounts (Table 3.2). Two people were appointed for looking after fishes that were placed outside the SUST campus. Some instructions were given them to supply food in different amounts. Every time feed were given at the morning at 9 am and alternate food was supplied. Tilapia fry was used as feed given weekly at the morning.

Table 3.2. Different types of feed given in different culture conditions

Name of supplied feed	Amount of different feed (g)							
	HT 1	HT 2	HT 3	BT 1	BT 2	PT	ED1	ED2
Small live fish (Tilapia fry)	100	-	50	-	-	50	-	-
Earth worms	100	-	100	150	150	50	150	-
Small dead fish	100	100	-	-	-	-	100	-
Snails	50	50	-	50	50	-	50	-
Small frog	100	100	-	100	100	100	100	-
Chicken viscera	-	-	-	50	50	-	-	-

HT=house tank, BT=Backyard tank, PT= Plastic tank, ED= Earthen ditch

3.4. Breeding biology and artificial breeding

3.4.1. Study of sexual dimorphism

Fish samples were collected by fisherman from flood plains, Sylhet and then identified through morphological characteristics (Rahman 1989, Talwar and Jhingran 1991). Collected fish samples were brought to the laboratory of Genetic Engineering and Biotechnology at Shahjalal University of Science and Technology, Sylhet, Bangladesh and were kept into glass aquariums until tissue isolation for histological analysis as well as for studying external and internal anatomy. Male and female fish were identified by morphological, internal anatomy and histological analysis. Note that, Morphological study was observed by naked eye with important characteristics (Bhuiyan 1964, Jayaram 1981). Specifically, some measuring parameters were analyzed using measuring scale and lifting balance. Different internal organs especially gonads, liver, kidney and intestine were observed through dissection process. Also, gonad shape, size, length etc. and sperm duct or oviduct was analyzed. Moreover, the number of mature eggs in the oviduct was also counted. At the same time, the histological analysis of gonads was studied using microtomy.

3.4.2. Observation of breeding biology of freshwater mud eel

The breeding biology of freshwater mud eel, *M. cuchia* was observed by analyzing twenty fish specimens (ten males and ten females) where fishes were collected randomly during the period of February to June 2013 and collected fish was identified through observing taxonomic characteristics (Jayaram 1981, Shafi and Quddus 1982, Talwar and Jhingran 1991). Total length and weight of fish, length and weight of

gonads, egg diameter, fecundity, etc. were measured monthly by using balance (Figure 3.5), measuring scale, measuring tape and forceps, etc. The individual gonad weight was divided by the individual body weight to give the gonadosomatic index (GSI). The GSI of male and female was estimated separately by using the formula of GSI is [Gonad weight (g) / Fish weight (g)] × 100, and the relative fecundity was calculated by the formula of relative fecundity is Individual Fecundity / Body Length (cm). Mean of egg diameter was calculated by $\sum XiFi/N$. Beside these periods of natural observations, on the other hand, breeding biology was also observed with 20 fish specimens after one week of hormonal treatments as the same process where different doses of Human chorionic gonadotrophin (HCG), Pituitary Gland (PG), Gonadotrophin releasing hormone (GnRH) and Ovulin- a synthetic hormone were induced and fish specimens were selected randomly.

Figure 3.5. Weight measurement of gonad by lifting and digital balance

3.4.3. Observation of artificial breeding in different conditions

3.4.3.1. Experimental fish

Matured freshwater mud eel were collected from flood plains by helping local fishermen and identified the fish through observing morphological characteristics (Jayaram 1981, Shafi and Quddus 1982, Talwar and Jhingran 1991). Male fish was considered with 60-65cm of lengths and 450-550g of weights whereas the lengths and weights of female fish were 70-75cm and 750-850g respectively. Due to difficulty to determine the sex of this fish some external characteristics was also examined according to the Narejo *et al*. (2003c). Male and female fish were identified based on the section 3.4.1 of this thesis.

3.4.3.2. Observation of breeding in cemented tanks without hormonal treatment

Brood fishes were collected from nature by the fisherman and stocked in a cemented tank as 1:1 ratios with recirculatory water in a hatchery for 20 days (Figure 3.6). During this observation lives tilapia fry, small dead fish, prawns and pellet feed were supplied up to 15 days by three day a time in the evening. No feed was given for last week of the experiment. Water temperature and pH were maintained with 26-30°C and 7 respectively. Aerator was used in the tank to maintain dissolved oxygen.

Figure 3.6. Fish in cemented tank for observing breeding performance without hormonal treatment

3.4.3.3. Observation of breeding through IVF technique

In this study female fish was dissected to removal of oviduct membrane carefully for taking out of eggs and kept them in a petridish after harvesting from the oviduct. Male gonad/testis with sperm duct was taken out carefully and kept it in a petridish. Cut it in small pieces and kept them in 2.5% saline water. Eggs were poured immediately in petridish where sperms were available and mixed very well with a spoon. After five minutes some water was given and again mixed them very well. Finally mixture gamates was transferred into a small glass aquarium with filter water and aerator where temperature was maintained from 27-30^{o}C and kept it for three days, and it was checked daily.

3.4.3.4. Observation of breeding through inducing agent

3.4.3.4.1. Trial with PG

First trial of this experiment with PG was conducted in cemented tanks. The tanks were filled with water and water hyacinth. Three doses of PG such as 100mg/kg, 120mg/kg and 150mg/kg respectively were used for this first trial (Table 3.3). First dose was conducted at 2: 3 ratio of male and female where second dose was 3: 3 ratios and third dose was 3: 2 ratio of male and female. PG was prepared by mortar pestle and injected to the female and male fish (Figure 3.7 and Figure 3.8). Dorsal muscle was used as a suitable place for the injection (Figure 3.9). After treating the injected fish were kept in a glass aquarium with aerator for few minutes then transferred to three cemented tanks separately (Figure 3.10). During this observation of induced breeding in case of all doses small live fish, earth worms and dead fish were supplied as food (Table 3.2).

Table 3.3. Trial with different doses of PG in cemented tanks

No	Sex	1st Dose (100 mg/kg)		2nd Dose (120mg/kg)		3rd Dose (150mg/kg)	
		Weight of Fish (kg)	Amount of PG (mg)	Weight of Fish (kg)	Amount of PG (mg)	Weight of Fish (kg)	Amount of PG (mg)
1	Female	0.6	60	0.35	42	0.5	75
2	Female	0.4	40	0.4	48	0.45	67.5
3	Female	0.45	45	0.5	60	-	-
4	Male	0.3	34	0.3	36	0.42	63
5	Male	0.4	40	0.4	48	0.4	60
6	Male	0.4	40	0.45	54	o.5	75

Figure 3.7. PG, Ovulin, GnRH, HCG and syringe

Figure 3.8. Preparation of PG extract

Figure 3.9. Injecting the hormones

Figure 3.10. Hormonal injected fish in a tank

3.4.3.4.2. Trial with HCG

Trial with HCG (Figure 3.7) was conducted in three cemented tanks with three doses such as 2500 IU/kg, 2000 IU/kg and 1500 IU/kg. However, three doses were conducted 2: 3 ratio, 2:2 ratio and 2:2 ratios of male and female fishes respectively (Table 3.4.). All of the injections were given in dorsal side and injected at morning and all injected fish were transferred into the cemented tanks. During this experiment earth worms and live fish fry were given as feed (Table 3.2).

Table 3.4. Different doses of HCG

No	Sex	1st Dose (2500 IU/kg)		2nd Dose (2000 IU/kg)		3rd Dose (1500 IU/kg)	
		Weight of Fish (kg)	Amount of HCG (IU)	Weight of Fish (kg)	Amount of HCG (IU)	Weight of Fish (kg)	Amount of HCG (IU)
1	Male	0.3	750	0.32	640	0.25	375
2	Male	0.45	1125	0.3	600	0.3	450
3	Female	0.47	1175	0.45	900	0.4	600
4	Female	0.51	1275	0.43	860	0.5	750
5	Female	0.4	1000	-	-	-	-

3.4.3.4.3. Trial with GnRH

Induced breeding of *M. cuchia* was trialed with GnRH (commercial name: Flash) (Figure 3.7) in three cemented tanks with three different doses. 12 inches of water dept was maintained in each tank. 1st dose was injected with 1ml/kg fish with 3 : 3 ratio of male and female, and 2nd dose was given with 1.2/kg fish with 2:2 ratio of male and female while 3rd dose was injected 1.5/kg fish with 2 : 2 ratio of the male and female fishes (Table 3.5). Small live fish and earth worms were supplied as feed during this experiment (Table 3.2).

Table 3.5. Doses of GnRH for cemented tanks

No	sex	1st Dose (1 ml/kg)		2nd Dose (1.2 ml/kg)		3rd Dose (1.5 ml/kg)	
		Weight of Fish (g)	Amount of GnRH (ml)	Weight of Fish (kg)	Amount of GnRH (ml)	Weight of Fish (kg)	Amount of GnRH (ml)
1	Male	0.35	0.35	0.45	0.54	0.35	0.525
2	Male	0.42	0.42	0.3	0.36	0.4	0.6
3	Male	0.4	0.4	--	-	-	-
4	Female	0.45	0.45	0.4	0.48	0.51	0.77
5	Female	0.35	0.35	0.52	0.62	0.5	0.75
6	Female	0.5	0.5	-	-	-	-

3.4.3.4.4. Trial with Ovulin

Trial with ovulin (Figure 3.7) was also conducted in three cemented tanks and in hapa. One group of fish was taken for injection with 2:2 ratio of male and female (Table 3.6). The 1st dose with 0.6 ml ovulin/kg of fish was used and this dose was injected to the both male and female at morning. Again the dorsal muscle was used for the injection. 2nd dose (0.8 ml/kg) and 3rd dose (1 ml/kg) was injected at 2:3 and 3:2 ratio respectively. After treating by ovulin both male and female fish were observed in the environments of cemented tank with sufficient water and in hapa (Figure 3.11).

Table 3.6. Doses of Ovulin for cemented tanks and Hapa

No	Sex	1st dose (0.6ml/kg)		2nd dose (0.8ml/kg)		3rd dose (1ml/kg)	
		Fish weight (kg)	Amount of Ovulin (ml)	Fish Weight (kg)	Amount of Ovulin (ml)	Fish Weight (kg)	Amount of Ovulin (kg)
1	Male	0.40	0.24	0.45	0.36	0.35	0.35
2	Male	0.35	0.21	0.30	0.24	0.40	0.40
3	Male	-	-	-	-	0.25	0.25
4	Female	0.52	0.312	0.45	0.36	0.52	0.52
5	Female	0.45	0.27	0.60	0.48	0.45	0.45
6	Female	-	-	0.50	0.4	-	-

Figure 3.11. Injected fish in breeding hapa

3.5. Molecular species identification

3.5.1. Tissue isolation

In this experiment, fish samples were collected from a Motso Arot, Sylhet and collected fish were brought to the General Laboratory of the Department of Genetic Engineering and Biotechnology at Shahajalal University of Science and Technology, Sylhet, Bangladesh. 15 fishes were dissected and different tissue samples such as liver, kidney, etc. was isolated from each individual (Figure 3.12). Isolated tissues were washed by using distilled water and 70% alcohol (Figure 3.13) and preserved separately in 100% alcohol at -20°C until DNA extraction.

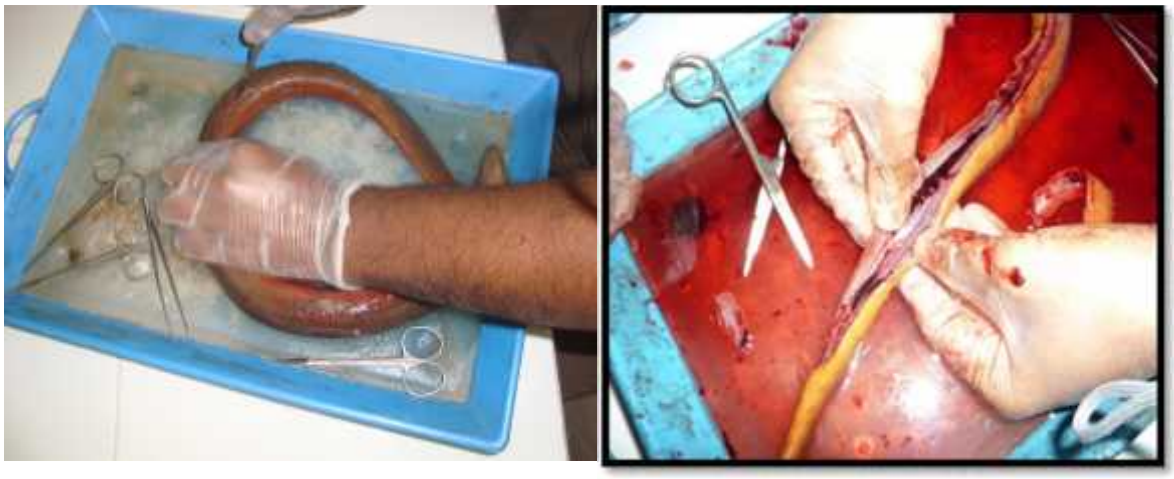

Figure 3.12. Dissecting the experimental fish for tissue isolation

Fig. 3.13. Isolated tissues in petridish with water

3.5.2. DNA extraction

DNA extraction was carried out by using a commercially available kit, Bioserve, CAT.NO.2025. A total of 15 fish samples were used for DNA extraction with good bands of DNA (Figure 3.14).

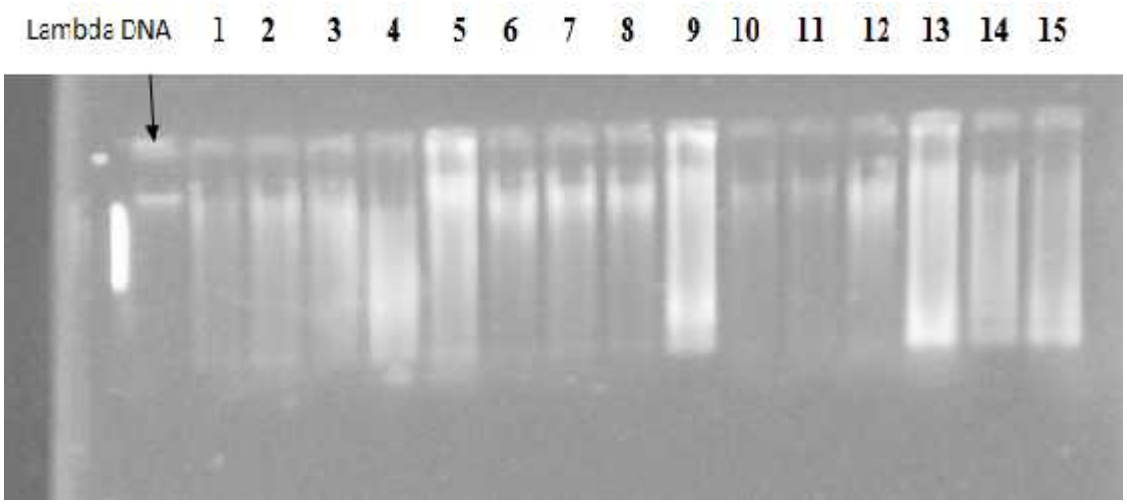

Figure 3.14. Extracted DNA of 15 individuals for species identification (Lamda DNA=Marker)

The quality of DNA was checked by electrophoresis on 0.8% agarose gel (Figure 3.15) comparing with 30bp long lamda DNA.

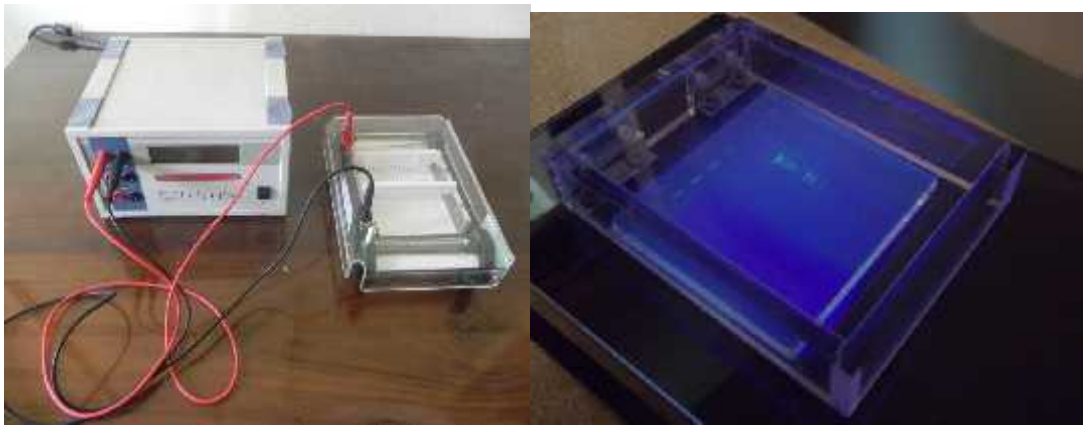

Figure 3.15. Gel electrophoresis chamber with its comb and tray (HU 10.MINI-PLUS HORIZONTAL).

The gel was run at 70 V for 40 minutes dying with ethedium bromide solution. Finally, photographs were taken by digital camera using gel documentation system (Figure 3.16).

Figure 3.16. Gel documentation system with computer

3.5.3. PCR amplification

Vertebrate universal primer, accession no. 16SrRNA L2513 (5' GCCTGTTTACCAAAAACATCAC 3') and accession no. 16SrRNA H2714 (5'CTCCATAGGGTCTTCTCGTCTT 3') (Kitano *et al*. 2007) as well as gene specific primer of glutamine synthetase, accession no. GSase 152041 (5' GAGGGCTCCAACAGCGATATGTA 3') and accession no. GSase 152042 (5'CTGAAGTTTGTATGGCAGCCAGC 3') (Saha *et al*. 2010) were used to identify the species. PCR reaction was done for both the primers with 25µl of master mix for each sample (Table 3.7).

Table 3.7. Different concentrations of PCR mixture

PCR compositions	Reaction mixture
25mM Mgcl2	2.5
10xReaction buffer	2.5
dNTP	0.5
Primer(F)	1
Primer(R)	1
Taq DNA polymerase	0.25
Template	1
dDw(ddi)	16.25
Total	=25µl

In this experiment, PCR reaction of 16s mitochondrial DNA was conducted by 40 cycles with preheated at 94ºC for 3 minutes followed by denaturation at 94ºC for 1 minute, annealing at 58ºc for 1 minute and 2 minutes for extension at 72ºC. A final step of 7 min for 72ºC was added to allow complete extension of the amplified fragments. PCR amplification of glutamine synthetage gene was maintained like 16s mtDNA, where denaturation at 94°C for 1 min. and annealing temperature at 64^0C for 1 min. and run with 35 cycles. Amplified products were stored at -20ºC. The amplified DNA fragments were separated on 1.2% agarose gel for 40 minutes at 70V comparing with 10,000 bp ladder (Figure 3.17). Next, the photographs were taken by a digital camera using gel documentation system.

Figure 3.17. Two types of PCR apparatus

3.6. Genetic diversity based population studies

3.6.1. Experimental design

The experiment was designed using the PCR-RAPD and PCR-RFLP methodologies for observing the population genetic structure of freshwater mud eel, *Monopterus cuchia* in Bangladesh.

3.6.2. Ecological selection of experimental fish

The research works were conducted in both laboratory and field level. Two aquatic habitats such as Tanguar Haor, Sunamganj (population 1= P1) and Hakaluki Haor, Molvibazar (population 2 = P2) were considered for sample collection. 30 individuals of mud eel were considered from two ecological locations where 15 individuals were taken from each habitat (Figure 3.18).

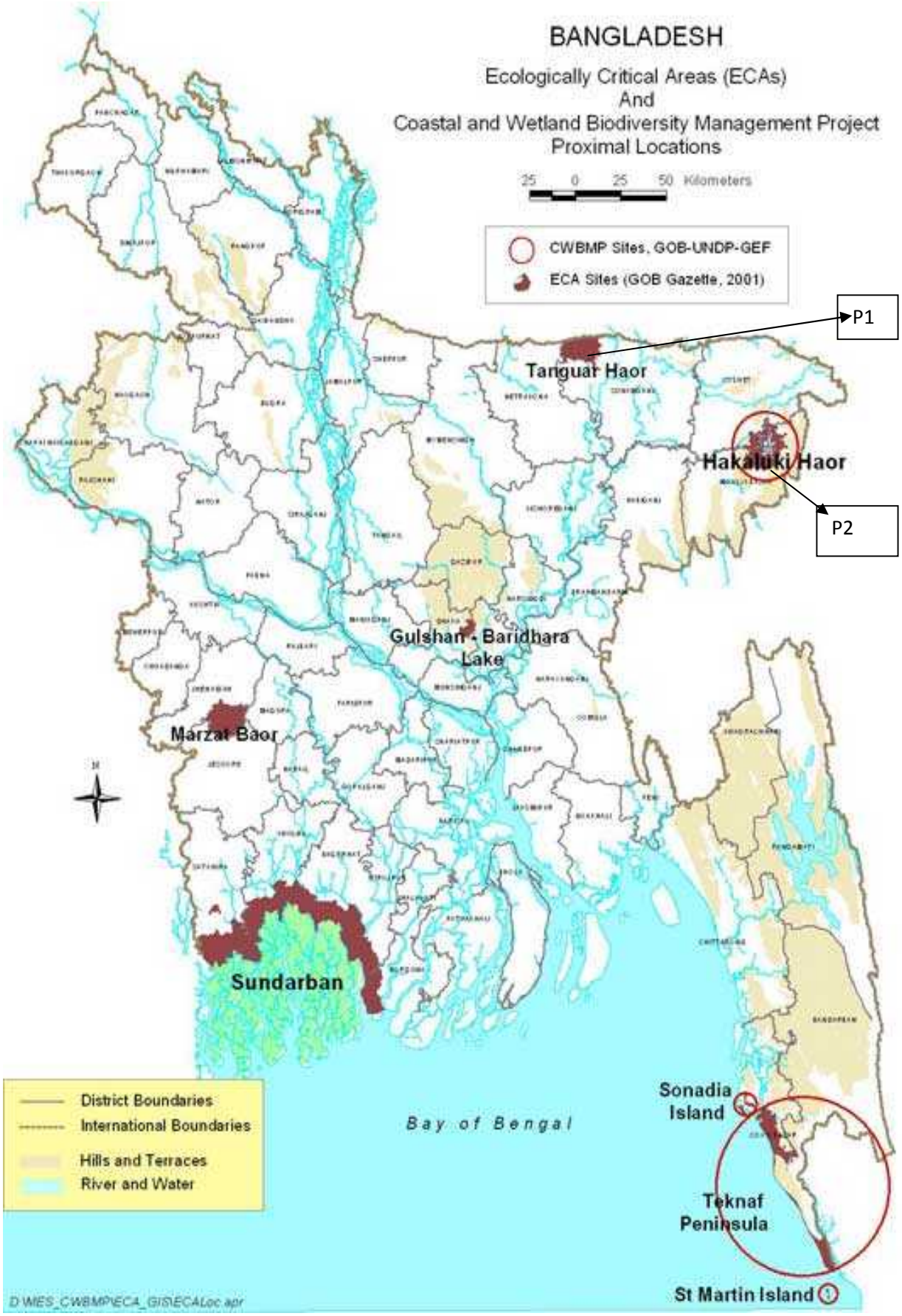

Figure 3.18. Location of two ecological habitats as P1 and P2

3.6.3. Ecological conditions of the experimental locations

3.6.3.1. Parameters and equipments

The water samples were collected from upper surface of each water source and brought to the laboratory by plastic water bottles. Water quality of two experimental ecosystems was monitored in order to check environmental status related to molecular characterization of *M. cuchia*. Different physico-chemical parameters of the water of those experimental locations were analyzed and values were recorded in table 3.8. Different sophisticated equipments and essential chemicals were used for the measurement of different water parameters such as pH, temperature, CO_2, COD, DO, BOD, Alkalinity, NO_2-N, NO_3-N were measured in the Water Supply Management Laboratory of the Department of Civil and Environmental Engineering (CEE), Shahjalal University of Science and Technology (SUST), Sylhet (Table 3.8). Different equipments such as digital pH meter, thermometer, beaker, measuring cylinder, dropper, conical flask, nessler tube, BOD bottle, etc. were used to measure different parameters of the water, and different chemicals like phenolphthalein indicator, standard 0.02 N sulfuric acid, methyl orange indicator, standard N/44 sodium hydroxide, phenol-disulfonic acid, standard nitrate solution, manganous sulfate solution, alkaline potassium iodide solution, sodium thiosulfate, starch solution indicator, concentrated sulfuric acid, standard potassium permanganate, dilute sulfuric acid, standard ammonium oxalate were also used.

Table 3.8. Physico-chemical parameters (mg/l) of the water sample of two ecological habitats

Parameters	Tanguar Haour, Sunamganj	Hakaluki Haor, Molvibazar
pH	6.7	8
Temperature	25.3	28.1
CO_2	5	4.3
COD	4.5	4.2
DO	4.4	3.8
BOD	4.2	3.6
Alkalinity	168	196
NO_2-N	0.018	0.021
NO_3-N	0.085	0.061

3.6.3.2. Methodology for the measurements of parameters

Temperature of the experimental waters was measured by dipping the thermometer and water pH was measured by a bench top electrometric pH meter. The water samples were fixed up by using alkaline iodide and manganous sulfate and after few times, concentrated H_2SO_4 was added into the BOD bottle for measurements of DO.

Two drops methyl orange indicator was added into 10 ml of water sample and H_2SO_4 solution was taken in the burette for the measurement of alkalinity. In the titration of alkalinity, light pink color was observed at the end point. 0.1N NaOH was taken in the burette and phenolphthalein indicator (5 drops) was added to the solution. Permanent

pink color was observed at the end point. Two drops starch solution was added to the fixed up sample (20 ml) and Na_2SO_3 was taken in burette. The titration was continued to first disappearance of blue color. The following formula was used to calculate alkalinity of the waters:

Alkalinity=T×10 ppm (Here T=Total Sulfuric acid used).

CO_2 of water was determined by titration method where take 100 ml of water sample into a beaker and same quantity of distilled water into another beaker. Add 10 drops of phenolphthalein indicator into each sample. If pink colour develops in the samples that means no CO_2 is present in the water. On the other hand, if no pink colour is seen, add N/44 Solution hydroxide from a burette to the sample and stir gently until a slight permanent pink colour appears as compared with the distilled water. Record the ml of sodium hydroxide used. The following formula was used to calculate CO_2 of the waters:

$$CO_{2mg/l} = \text{ML OF N/44} \times 10$$

For the determination of Biochemical Oxygen Demand (BOD) of the water, water sample was taken in an 8 –oz glass coppered bottle carefully to avoid contact of the sample with air. The bottle should be completely filled. Immediately after collection, 1 ml of manganous solution was added by means of pipette, dipping the end of the pipette just below the surface of the water. Then 1 ml of alkaline potassium iodide solution is added in a similar manner. A stopper was inserted and mixed by inverting the bottle several times. The precipitate was allowed to the settle halfway and mixed

again. Further, the precipitate was allowed to the settle halfway. 1 ml of concentrated sulfuric acid was added and the stopper was inserted again at once after the addition of acid and mixed as before. The solution was allowed to stand at least 5 minutes. 100 ml of the solution was withdrawal and transferred it into a conical flask and immediately 0.025N sodium thiosulfate was added drop by drop from a burette until the yellow colour was almost disappeared. About 1 ml of starch solution was added and continues the additions of the sodium thiosulfate until the blue colour was just disappeared. Recorded the ml of sodium thiosulfate used. BOD was calculated by the following formula:

DO present in sample in mg/l=ML of 0.025N sodium thiosulfate used × 2

BOD in mg/l=(Di-df)/P, where P=decimal volumetric fraction of sample used.

100 ml of the water sample was taken by pipette into a 250 ml conical flask. 10 ml of diluted sulfuric acid and 10 ml of standard potassium permanganate were added for determination COD. The sample was heat to boil in a water bath for exactly 30 minutes. The water was kept in the bath above the level of the solution in the flask. Considered the solution when appeared faintly coloured, the above procedure was repeated using a smaller sample diluted to 100 ml with distilled water. After 30 minutes in the water bath 10 ml of standard ammonium oxalate was add. The mixture was titrated and heated the sample with standard potassium permanganate to observe first pink coloration. Recorded the ml of potassium permanganate was used. The calculation for COD measurement as bellow:

Oxygen consumed (COD) in mg/l=ML of $KMnO_4$ used in step no. 6 × 100/ML of sample used

Nitrate nitrogen (NO_3-N) of water was determind by filter 30 to 35 ml of the water sample through a filter paper. Evaporate 25 ml of the filtrate to dryness on a water bath. Moisten the residue with 1ml of phenol-di-sulfonic acid and the sample was diluted with about 20 ml with distilled water. A 50% solution of sodium hydroxide was added until the maximum yellow color is developed. The mixture was filtered into a 100ml Nessler tube, rise the dish and paper with distilled water. The filtered rinsing was added to the filtrate and made up to the mark with distilled water. When permanent standards were found available preceded the step no.8, otherwise, made up temporary standards by placing 0.2, 0.4, 0.6, 0.8, 1, 1.5, 2, 3, 4 and 5 ml of standard sodium nitrate solution in 100 ml Nessler tubes and then 2ml of 50% sodium hydroxide was added. The sample was diluted to the mark with distilled water. The colors were compared and recorded the standard having color nearest to that of the sample. The calculation for Nitrate Nitrogen is as under:

Nitrate Nitrogen, (NO_3-N)=ML of standard $NaNO_3$/ML of sample in step no.2

Measurement of nitrite nitrogen (NO_2-N) is similar as measurements of NO_3-N. Only $NaNO_2$ is used instead of $NaNO_3$.

3.6.4. Collection and identification of fish

Fish was collected by the help of professional fish catcher from two different ecosystems such as Tanguar Haor and Hakaluki Haor. A total of 30 individuals were selected for genetic diversity as population level where 15 individuals from each of the two different ecosystems were considered for tissue isolation and DNA extraction.

Collected fishes were transferred to the Animal Keeping Laboratory of the Department of Genetic Engineering and Biotechnology at Shahajalal University of Science and Technology, Sylhet, Bangladesh and kept the samples in plastic tank and plastic bucket with until tissues were isolated (Figure 3.19). Collected fishes were identified through morphometric characteristics by Shafi and Quddus (1982), Rahman (1989), Talwar and Jhingran (1991) etc.

Figure 3.19. Collected fish from two ecological habitats for DNA extraction

3.6.5. Tissue isolation

Collected fish were brought to the General Laboratory of the Department of Genetic Engineering and Biotechnology at Shahajalal University of Science and Technology, Sylhet, Bangladesh. Each fish was dissected and different tissue samples such as liver, kidney, etc. were isolated from each individual. Isolated tissues were washed by using distilled water and 70% alcohol and preserved separately in appendorf tube in 100% alcohol at -20°C.

3.6.6. DNA extraction

DNA extraction was carried out by using a commercially available kit, commercial name Bioserve, CAT.NO.2025. Liver tissues of fish samples were carefully homogenized by using a mortar and pestle from individual fish, of course, separately. During homogenization 400µl of Buffer TA and 20µl proteinase K were added to the micro centrifuge tube for each sample. Then the lysed samples were incubated at 60°c for 30 minute in a water bath. The homogenate were centrifuged for 10 minutes at 8000 rpm. Followed centrifugation, supernatant was transferred into a new fresh micro centrifuge tube. Equal volume of Buffer TB was added to the each homogenate, and it was mixed gently. Samples were loaded to the column matrix individually. The centrifugation was done at 6000 rpm for 1 minute. Each bioserve column was placed on each collection tube and 500µl Wash Buffer-1 was added. Then each collection tube was centrifuged at 10,000 rpm for 1 minute. Again each bioserve column was placed back into the 2ml collection tube and 600µl of Wash Buffer-2 were added. The samples were centrifuged for 1 minute at 10,000rpm. Flow through was discarded. Each column was spun by opening the caps at 11,000 rpm for 3 minutes. The caps were left open for 2 minutes before going to the next step. At last each of the biosreve columns was placed in a fresh 1.5ml micro centrifuge tube. 100µl of Buffer TE were added to each of the micro centrifuge tube. It was left at room temperature for 10 minutes. Last centrifugation was done at 11,000 rpm for 2 minutes resulting eluate was containing the desired genomic DNA. Then the DNA samples were stored at-20ºc.

3.6.7. Checking DNA by gel electrophoresis

DNA quality was checked by electrophoresis on 0.8% agarose gel. First, 50 ml TBE buffer measured by using a measuring cylinder and poured into a conical flask. (0.4gm) agarose powder was added to the conical flask. The mixture was mixed gently and it was place in stirrer heating machine about 15-20 minutes until agarose powder was fully dissolved in the buffer. Then agarose gel was poured into the gel tray about 30 minutes for cooling and appears as thick gel. DNA sample were taken out from the freezer and thawed. In several eppendorf tubes were used for 3µl loading dye and 3 µl DNA samples. Then DNA mixture was placed into each hole separately that created by the comb. 1kb plus DNA was placed in a hole aside near the samples so that migration of DNA can be compared. The TBE buffer was poured into the gel chamber until it was fully submerged. The gel was run at 70 Volt for 40 minutes. Then, electrophoreses gel was poured in ethidium bromide solution for dyeing for 30 minutes. This gel was then placed on UV apparatus (gel documentation system) and photograph was taken by digital camera (Panasonic"DMC-fs20 which in 10 mega pixels). Quality of DNA was found from each of the individual was good which was compared with 1kb plus DNA ladder (Figure 3.20).

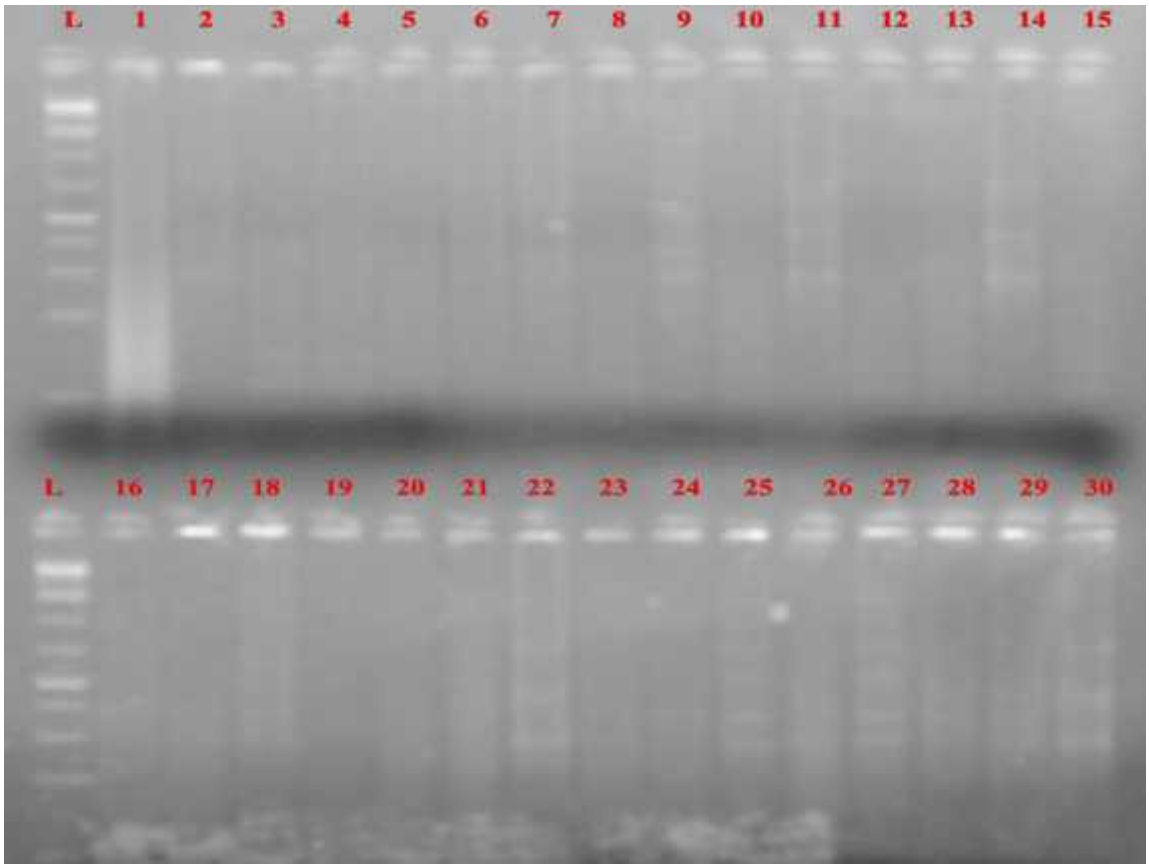

Figure 3.20. Extracted DNA of 30 individuals for studying genetic diversity (L= Ladder)

3.6.8. DNA selection for population studies

Genetic diversity based population study of freshwater mud eel was conducted considering two aquatic habitats such as Tanguar Haor, Sunamganj which was considered as Population 1 (P1) and Hakaluki Haor, Molvibazar which was considered as population 2 (P2) (Table 3.9 and Figure 3.18).

From the above figure (Figure 3.18), 15 individuals of each of the two population were considered from two experimental ecological habitats where the Tanguar Haor (P1) marked as T1 to T15 and Hakaluki Haor (P2) marked as H1 to H15 (Table 3.9).

Table 3.9. Locations and number of fish for three populations

Populations	Locations	Individual Identity
P 1	Tanguar Haor, Sunamganj	T1-T15
P 2	Hakaluki Haor, Molvibazar	H1-H15

A good quality DNA was observed from each of the individual of population 1 where 1 kb plus DNA ladder was used to compare the quality of extracted DNA (Figure 3.21).

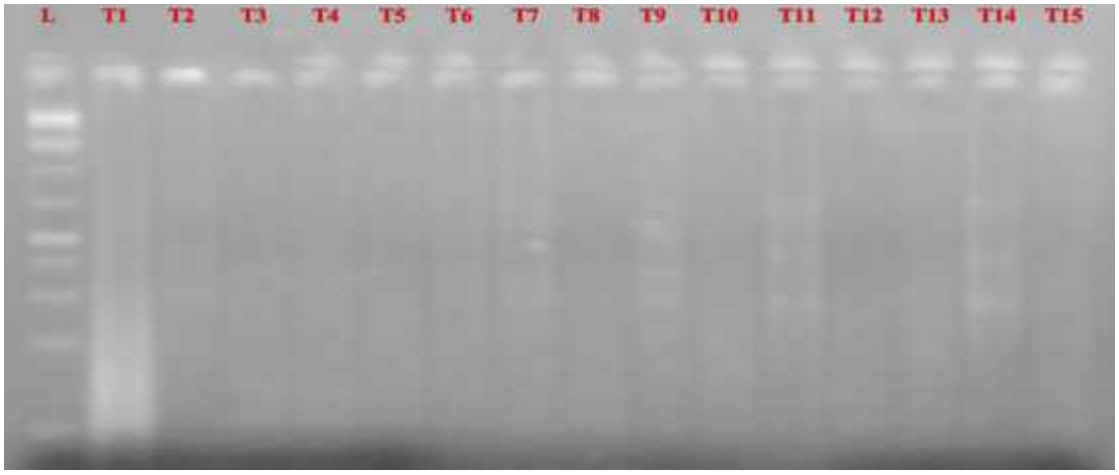

Figure 3.21. Extracted DNA of 15 individuals of population 1/Tanguar Haor (from the figure 3.20; L = Ladder)

High-quality DNA was distinguished from each of the individual of population 2 where again 1 kb plus DNA ladder was also applied to compare the quality of extracted DNA (Figure 3.22).

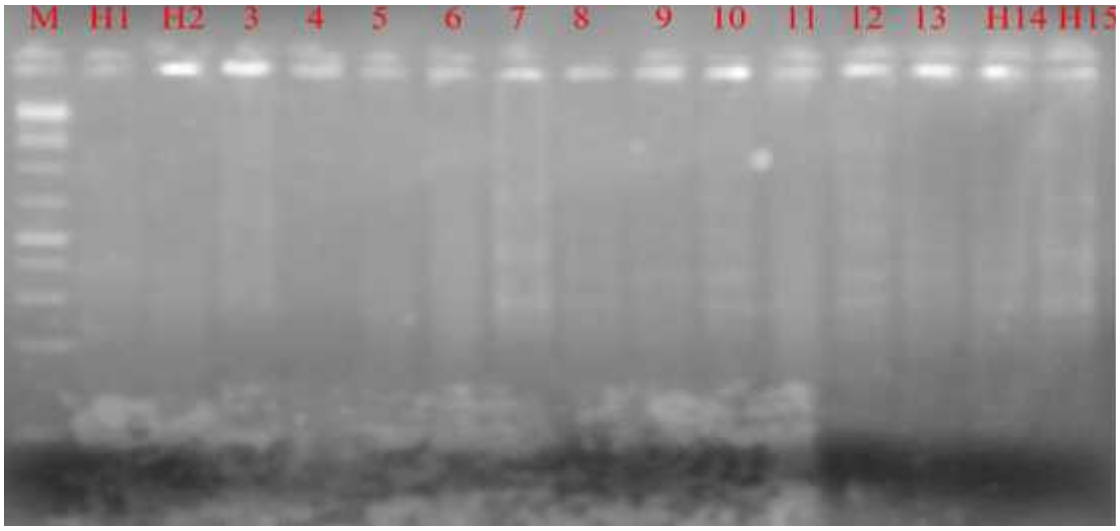

Figure 3.22. Extracted DNA of 15 individuals of population 2/Hakaluki Haor (from the figure 3.22; L = Ladder)

3.6.9. RAPD Analysis

3.6.9.1. RAPD Primers

Initially, 12 decamer primers (Operon Technologies, Inc., Alameda, CA, USA) were selected and amplified for accurate scoring. Finally, eight primers were adopted and produced quality bands for detecting polymorphisms of this experimental fish. The selected eight primers were listed in the following table (Table 3.10).

Table 3.10. Eight decamer primers used for diversity based population studies

Primers	Sequence(5'→3')	Length (bp)	Tm (°C)	References
B-03	5'- CAT CCC CCT G-3'	10	34	Schwenk *et al.* 1998
OPF 14	5'-TGCTGCAGGT-3'	10	35	Operon Technologies Inc. USA
C-04	5'- CCG CAT CTA C-3'	10	35	Schwenk *et al*. 1998
OPB 05	5'-TGCGCCCTTC-3'	10	36	Alam *et. al*. 2010
OPB 08	5'-GTCCACACGG-3'	10	36	Alam *et. al*. 2010
OPB 19	5'-ACCCCCGAAG-3'	10	36	Alam *et. al*. 2010
OPB-12	5'- CCT TGA CGC A-3'	10	36	Alam *et. al*. 2010
UBC122	5'-GTAGACGAGC-3'	10	32	Thaewnon-ngiw *et al*. 2003

3.6.9.2. PCR amplification of RAPD assay

Eight decamer RAPD primers were considered for studying genetic diversity and population study of freshwater mud eel, *M. cuchia*. PCR reactions were performed in a 15µl reaction mixture for each sample with 8µl of master mix (Promega Hot Start), 1µl of primer, 2µl of template DNA and 4µl deionized distilled water (Table 3.11).

Table 3.11. Concentration of PCR reaction mixture for RAPD

Components	Volume (µl)
PCR mixture	8µl
Primer	1µl
DNA template	2µl
ddH_2O	4µl
Total	15

Three types of PCR were used in different times for this research (Figure 3.23). Different PCR steps such as preheating, denaturation, annealing, elongation or extension, and complete extension temperature of the amplified fragments were conducted with different cycles for different primers (Table 3.12).

a **b** **c**

Figure 3.23. Three different types of thermal cycle (a) NYX TECHNIK A6 Serial 00101; 85-264 V AC, (b) Multigene Gradient. Catalog #: TC 9600-G-230V and (c) Biorad My Cycler T^m thermal cycler

Table 3.12. PCR conditions for eight different RAPD primers

Primers	Preheating	Denaturation	Annealing	Elongation	Extension	Cycles
B-03	94˚c for 3 min	94ºc for 1 min	32˚c for 1 min	72˚c for 2 min	72ºc for 7 min	35
OPF 14	94˚c for 3 min	94ºc for 1 min	33ºc for 1 min	72˚c for 2 min	72ºc for 7 min	40
C-04	94˚c for 3 min	94ºc for 1 min	33ºc for 1 min	72˚c for 2 min	72ºc for 7 min	35
OPB 05	94˚c for 3 min	94ºc for 1 min	34ºc for 1 min	72˚c for 2 min	72ºc for 7 min	40
OPB 08	94˚c for 3 min	94ºc for 1 min	34ºc for 1 min	72˚c for 2 min	72ºc for 7 min	40
OPB 19	940C for 3 min	94^0c for 1 min	34^{0c} for 1min	72˚c in 2 min	72ºc for 7 min	40
OPB-12	94˚c for 3 min	94ºc for 1 min	34˚c 1 min	72˚c in 2 min	72ºc for 7 min	35
UBC122	94˚c for 3 min	94°c for 45sec	30°c for 1min	72°c for 1.5min	72°c for 5 min	35

3.6.9.3. RAPD analysis

Different formulae, calculations and software were used for analyzing genetic diversity and population genetics of this experiment which was mentioned below:

3.6.9.3.1. Molecular weight of bands: For measuring molecular weight of bands, the software AlphaEaseFC 4.0 was used.

3.6.9.3.2. Proportion of polymorphic loci:

$P\text{-}n_{pj}/n_{total}$

Where, P= Proportion of poly morphic loci

N_{pj}= Number of polymorphic loci

N_{total}= Total number of loci

3.6.9.3.3. Average number of alleles per locus

$$N=(1/K)\sum_{i=1}^{k} ni$$

Where, K-the number of loci

ni= the number of alleles detected per locus

3.6.9.3.4. Genetic distance analysis

Using pair wise similarity D = 1- Nxy / Nx+Ny- Nxy

Where,

D = the genetic distance between sample x and y

Nxy = number of band shared by sample x and y

Nx =the number of bends in sample x

Ny =the number of bends in sample y

3.6.9.3.5. Nei's genetic similarity (Nei's 1978)

Using pair wise comparison F= 2Nxy/ Nx+Ny

Where,

F= Nei‟s genetic similarity,

Nxy= Number of shared Band between X and Y,

Nx= Number of bend in X,

Ny = Number of band in Y

3.6.9.3.6. Linkage distance: Calculating with Squared Euclidean distances using new.sta.

3.6.9.3.7. Genetic relationships: Based on linkgae distance using a softwere "Statistica".

3.6.9.3.8. Hardy-Weinberg equilibrium: $(p + q)2 = p2 + 2pq + q2 = 1$

3.6.9.3.9. Intra locus gene diversity

hj= (1-p2-q2)

3.6.9.3.10. Average heterozygosity (Hi): Average of intra locus gene diversity

3.6.10. RFLP analysis

3.6.10.1. RFLP primers

Population genetic structure of this freshwater mud eel, *M. chchia* was also analyzed by RFPL pattern. A 541 bp length partial sequence of glutamine synthetase gene was considered with restriction enzymes. Gene specific primers of glutamine synthetase was accession number GSase 152041 (5' GAGGGCTCCAACAGCGATATGTA 3') and accession no GSase 152042 (5' CTGAAGTTTGTATGGCAGCC AGC 3') which was designed by Saha *et al.,* (2010). The following 541 bp sequences of glutamine synthetase gene were considered as a part of this experiment for studying genetic diversity and population genetic study.

Glutamine synthetase gene (541bp)

CACAGCGATATGTATCTGATTCCTGCTGCTATGTTCCGTGATCCATTCCG
CAAAGACCCCAACAAGCTGGTCCTGTGTGAAGTGACTAAGTACAACCGCA
AACCTGCAGAAACAAACCTTCGAATCACATGTAAGAAGGTGATGGAGAT
GGTGAAGGACCAGCATCCCTGGTTTGGTATGGAGCAGGAGTACACTATCC
TGGGCACAGACGGACACCCATTTGGCTGGCCATCTAATGGTTTCCCTGGA
CCACAA**GGGCCA**TACTACTGCGGTGTGGGAGCTGACAAAGCCTATGGCA
GAGATATAGTGGAGGCACATTACAGAGCATGTCTGTATGCTGGAG**TCCAG**
ATTGGCGGCACAAATGCGGAAGTGATGCCTGCTCAGTGGGAGTTCCAGGT
TGGACCTTGTGAAGGTATCAACATGGGCGATCACCTCTGGGTGGCGCGCT
TCATCCTGCACCGTGTCTGTGAAGACTTCGGTGTTGTTGCTTCATTTGACC
CCAAACCAATCCCGGGTAACTGGAACGGT**GCTGGCTGCCAAC**

Selected primers

fwd_seq:	5’ gagggctccaacagcgatatgta 3’
rev_seq:	5’ ctgaagtttgtatggcagccagc 3’
Complementary	3’ gctggctgccatacaaacttcag 5’

Selected enzymes

+ Hpy178III*	TCNNGA=TCN(C)N(A)GA
+ CfrI	YGGCCR =Y(G)GGCCR(A)

3.6.10.2. PCR amplification for extracted DNA with GS primers

The PCR reaction was used to amplify the 541 bp of the glutamine synthetase gene. PCR reactions were performed each sample in a 15µl reaction mixture containing 8µl of master mix (Promega Hot Start), 2µl of COI primer, 2µl of template DNA and 3µl deionized distilled water. PCR reaction was done for both the primers with 25µl of master mix for each sample. PCR amplification of glutamine synthetage gene was maintained by 35 cycles with preheated at 94ºC for 3 minutes followed by denaturation at 94ºC for 1 minute, annealing at 64ºc for 1 minute and 2 minutes for extension at 72ºC. A final step of 7 min for 72ºC was added to allow complete extension of the amplified fragments. Denaturation was maintained at 94°C for 1 min. and annealing temperature at 64^0C for 1 min. and each PCR was run with 35 cycles. Amplified products were stored at -20ºC.

3.6.10.3. PCR products check by agarose-gel electrophoresis

The efficiency of the PCR products of glutamine synthetase gene of *M. cuchia* was checked by agarose-gel electrophoresis with 1.2% agarose. Every time the same ladder mix (10,000 bp long-Promega) was used for checking the length of the DNA fragments at 541 bp length. The electrophoresis was run at 75 V for 40 minutes. The gels was then placed in gel documentation system and photograph was taken by digital camera (Panasonic"DMC-fs20 which in 10 mega pixels). After getting good bands of GS gene compare with 10,000 bp ladders (Figure 3.24) and the PCR DNA products of two populations were kept in freezer at -20°C for further analysis by restriction enzymes.

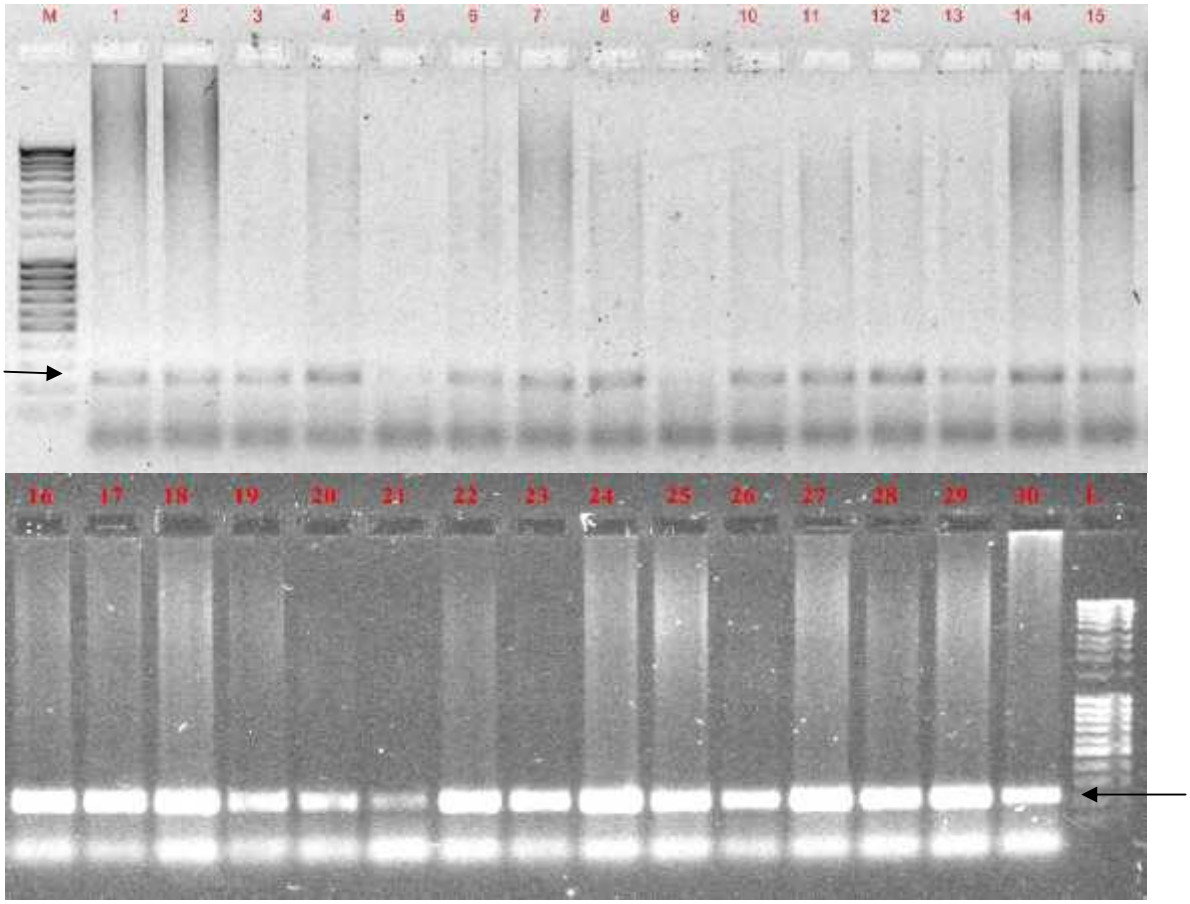

Figure 3.24. PCR products of GS gene of 30 individuals for observing genetic diversity (Arrow mark indicating 541bp length, Marker (M)/Ladder (L)=>250bp)

3.6.10.4. GS product selection for population studies

Genetic diversity based population study of freshwater mud eel was also conducted by PCR-RFLP considering same as RAPD assay. Two aquatic habitats such as Tanguar Haor, Sunamganj which was considered as Population 1 (P1) and Hakaluki Haor, Molvibazar which was considered as population 2 (P2) (Table 3.9 and Figure 3.18). From the above figure (Figure 3.24), 15 individuals of each of the two population were considered from two experimental ecological habitats where the Tanguar Haor (P1) marked as T1 to T15 and Hakaluki Haor (P2) marked as H1 to H15 (Table 3.9).

A quality DNA of GS was observed at 541 bp length from each of the individual of population 1 comparing with 1 kb plus DNA ladder which is started as 250 bp length (Figure 3.25).

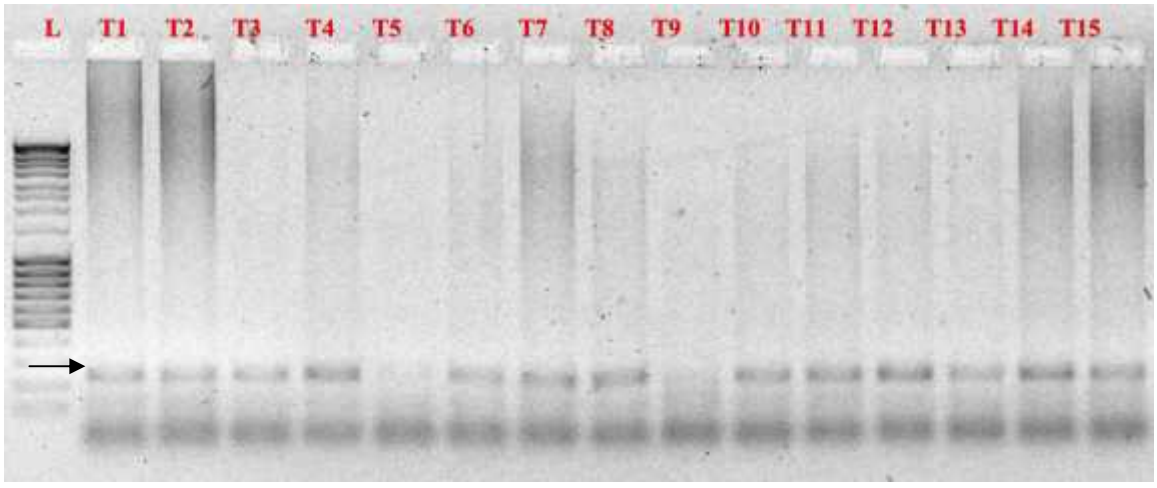

Figure 3.25. PCR products of GS gene of 15 individuals of population 1/Tanguar Haor (from the figure 3.24) (Arrow mark indicating 541bp length, Ladder (L) =>250bp)

Very good DNA bands were found in each of the individuals of population 2 (Hakaluki Haor) indicating arrow mark for 541 bp length of GS gene and again 1 kb plus DNA ladder was used to compare GS gene where DNA ladder is initiated from 250 bp length (Figure 3.26).

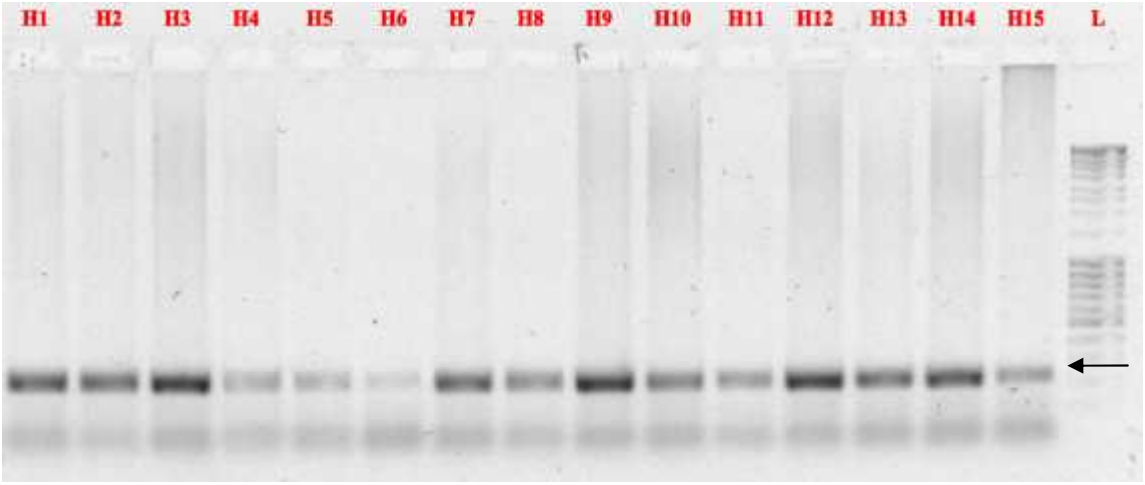

Figure 3.26. PCR products of GS gene of 15 individuals of population 2/Hakaluki Haor (from the figure 3.24) (Arrow mark indicating 541bp length, Ladder (L) =>250bp)

3.6.10.5. Restriction Fragment Length Polymorphism (RFLP) analysis

In RFLP analysis the glutamine synthetase gene had an approximate size of 541 bp. Two restriction enzymes such as + Hpy178III* [TCN(C)N(A)GA] with one cleavage site (344-349) and +CfrI [Y(G)GGCCR(A)] with also a single cleavage site (226-231) were used to detect polymorphism in the different two populations of freshwater mud eel in Bangladesh. Enzymes were selected using glutamine synthetase gene base pairs through "Blast" and the "restriction enzyme site" of the EBI nucleotide data base. These two enzymes commonly have a suitable restriction site to digest in case of *M. cuchia*. The RFLP mix was prepared with milli-Q water, enzyme

and enzyme's buffer. For the enzyme + Hpy178III*, the RFLP mixture was prepared by mixing 12.5 µl of milli-Q water, 2 µl of buffer G and 0.5 µl + Hpy178III* enzyme for each sample. Finally 5 µl of PCR-DNA sample was added and the mixture was run through PCR at 37°C for two hours. On the other hand, the RFLP mixture of +CfrI was prepared by mixing 12.5 µl of milli-Q water, 2 µl of buffer Tongu and 0.5 µl +CfrI enzyme for each sample, and then 5 µl of PCR-DNA sample was added. For this restriction enzyme the mixture was run through PCR at 55°C for two hours and 80°C for 20 minutes.

3.6.10.6. Checking the migration patterns of RFLP products on agarose gel

The effectiveness of the PCR-RFLP products of freshwater mud eel was checked by agarose-gel electrophoresis again. The gel was made by 0.75 g agarose (1.5% agarose) and 7 µl of digested DNA was used for each sample. In this circumstance, the electrophoresis was at 100 V for 60 minutes. A picture of the DNA fragments was taken by camera through gel documentation system and the length of the restriction fragments was checked by comparing with the 10,000bp long DNA ladder mix (Promega / MassRuler^{TM} DNA Ladder Mix with 10X dilutions).

3.6.10.7. Calculating the proportion of shared fragments (F) and genetic distances (P value)

The restriction patterns of individual of freshwater mud eel were compared to detect polymorphism, using matrices based on either presence/absence or relative abundance of bands. The similarity index or proportion of shared fragments (F) was calculated by comparing banding patterns between the two individuals using the following formula:

$F=2\ N_{XY} / (Nx + Ny)$

Where,

Nx and Ny are the number of restriction fragments in individuals X and Y

Nxy is the number of shared fragments between the two (X, Y) individuals.

A matrix of genetic distances (P value) between all individuals based on dissimilarity indices were calculated by applying the given formula:

$P=1-[0.5(-F+ (F^2+8F)^{0.5})]^{1/r}$

Where, r is the average number of enzyme sequences.

Both calculations were done by using Microsoft Office Excel 2007.

3.6.10.8. Calculation the polymorphism or rate of polymorphism

$Pj=q\leq 0.95$ or $Pj= q\leq 99$

Where, Pj= rate of polymorphism and q= allele frequency

3.6.10.9. Hardy-Weinberg equilibrium

$(p + q)^2 = p^2 + 2pq + q2 = 1$

3.6.10.10. Intra locus gene diversity

$h_j= (1-p_2-q_2)$

Where, heterozygosity per locus

P and q=allele frequencies

3.6.10.11. Inter-population differentiation for one locus

$g_{ST}=1-(h_S/h_T)$

Here, h_S= population diversity

h_T= total diversity

Where, $h_S= (ñ/(ñ-1)[1-(1/s)\sum\sum x_{ij}^2 – (h_0/2ñ)]$

$hT= 1 - \sum[(1/s)\sum x_{ij}]^2 + (h_S/ ñS) – (h_0/2ñS)$

ñ= harmonic average of population sizes

S= number of populations

H_0=average observed heterozygosity

X_{ij}=estimated frequency of the i^{th} allele in the j^{th} population

3.6.10.12. F statistics

The equation for the genetic structure of populations =

$(1 – F_{IT}) = (1 – F_{IS}) (1-F_{ST})$

$F_{IT} = 1 – (H_I/H_T)$

$F_{IS} = 1 – (H_I/H_S)$

$F_{ST} = 1 – (H_S/H_T)$

Where, H_T = total gene diversity or expected heterozygosity in the total population as estimated from the pooled allele frequencies

H_I=Intrapopulation gene diversity or average observed heterozygosity in a group of populations

H_S = average expected heterozygosity estimated from each subpopulation

F_{IT}=the deficiency or excess of average heterozygotes in a group of populations

F_{IS} = the deficiency or excess of average heterozygotes in each of population

F_{ST} = the degree of differentiation among populations in terms of allele frequency

3.7. Statistical analysis

Some statistical analysis in terms of mean, standard deviation, paired sample statistics, paired sample correlations and paired sample t-test etc. were performed when necessary in different experiments.

4.1. Rearing and production performance of freshwater mud eel

4.1.1. Physico-chemical parameters of the water

During the experiment, physico-chemical parameters such as pH, dissolved oxygen and temperature for different rearing conditions were maintained and recorded monthly (Table 4.1). The recorded pH from 7.33 to 7.45, temperature from 23-30℃ and dissolved oxygen from 4.4-5.5 were observed in house tanks. The pH of earthen ditches was ranged from 7.30-7.45 and temperature range was from 22.3℃-30.5℃ as well as dissolved oxygen was recorded from 4.5-5.5. The pH, temperature and dissolved oxygen of back yard tanks were recorded from 7.30-7.60, from 22.2℃-29.1℃ and from 4.4-5.2 respectively. Finally, the pH, temperature and dissolved oxygen were recorded 7.20-7.55, 23.2℃-30.3℃ and 4.5-5.5 respectively for plastic tank (Table 4.1).

Table 4.1. Physico-chemical parameters of the experimental environments

Months	Different culture systems	Parameters		
		pH	Temperature (°C)	DO
	House tank (1,2,3)	7.45	29	5.5
	Back yard Tank (1,2)	7.41	28.2	5.1
	Plastic tank	7.20	30.3	5.2
JUNE	Earthen ditch-1	7.33	30.5	4.7
	Earthen ditch-2 (Control)	7.45	30.2	4.8
	House tank (1,2,3)	7.41	30	5.4
	Back yard tank (1,2)	7.40	29.1	4.8
JULY	Plastic tank	7.37	31.2	4.5
	Earthen ditch-1	7.50	30.1	5.5
	Earthen ditch-2 (Control)	7.65	30.4	5.4
	House tank (1,2,3)	7.33	28	4.6
	Back yard tank (1,2)	7.60	29.1	4.5
AUGUST	Plastic tank	7.35	27.5	4.5
	Earthen ditch-1	7.50	28	4.6
	Earthen ditch-2(Control)	7.33	28.2	4.5
	House tank (1,2,3)	7.45	27.5	4.6
	Back yard tank (1,2)	7.30	27.9	4.4
SEPTEMBER	Plastic tank	7.40	27	5.5
	Earthen ditch-1	7.33	26.3	5.1
	Earthen Ditch-2 (Control)	7.40	26.3	5.2
	House tank (1,2,3)	7.40	26.2	4.4
	Back Yard Tank (1,2)	7.44	26.5	4.8
OCTOBER	Plastic tank	7.35	27	4.8
	Earthen Ditch-1	7.41	27.1	5.1
	Earthen Ditch-2 (Control)	7.45	27.4	5.1
	House tank (1,2,3)	7.44	23	5.4
	Back Yard Tank (1,2)	7.40	22.2	5.2
NOVEMBER	Plastic tank	7.55	23.2	4.7
	Earthen Ditch-1	7.40	23.4	4.8
	Earthen Ditch-2(Control)	7.30	22.3	4.8

4.1.2. Observed growth performance in different culture conditions

Growth performance of this experiment was measured considering the length and weight parameters. After six months of experiment all fishes from different culture conditions were collected separately, and length and weight was measured (Table 4.2) while in house tank-1 the initial average weight and length of fish were 24.4g and 15cm respectively with 87.5% survival rate and in that case final average weight and length were found 95.15g and 30.6cm respectively. On the other hand, initial average length and weight of the 40 fishes were 15.5 cm and 25.4g respectively while survival rate was recorded 77.5% and final weight and length 67.55g and 25.05cm were recorded in the house tank 2. Furthermore, in case of house tank-3, initial length and weight were observed 14cm and 23.3g respectively with 82.5% of survival rate and final growth was recorded with average weight and length 87.75g and 29.1 cm respectively. The average growth of these three house tanks were observed 70.75g, 42.15g and 64.45g respectively.

In the another experiment, the initial average length and weight of the fishes were recorded 14.5cm and 25g in earthen ditch and 15.7 cm 1 and 25.5g in earthen ditch 2 respectively. After six months of experiment the average final weight and length of the fishes were recorded 110.15g and 32.75cm respectively in earthen ditch 1 with 92.5% of survival rate whereas with 87.5% survival the final length and weight was recorded 30.2cm and 91.65g respectively in earthen ditch 2. Finally the average growth of each of the ditch was found 85.15g and 66.15 g respectively (Table 4.2). Furthermore, the average final length and weight were found 29.1 cm and 87.15g respectively in the plastic tank whereas the initial average length and weight of fishes

were observed 13.9cm and 23.8g respectively (Table 4.2). The average growth of plastic tank was recorded 63.35 g with 82.5% survival of fish. In one more experiment, growth performance of collected fish from backyard tanks has been studied where the average initial length and weight were found 14.5cm and 23.8g respectively and the final length and weight in average were recorded 25.1cm and 67.65g respectively for the backyard tank 1. Survival rate was found 75% in backyard tank 1 where 77.5% was observed in back yard tank-2. Another observation was seen in backyard tank 2 where the average initial length and weight were observed 15.7cm and 25.4g and the average final length and weight were recorded 25.4cm and 68.55g respectively. The average growth in back yard tanks 1 and 2 were found 43.85g and 43.15g respectively.

Table 4.2. Observed growth performance *M. cuchia* in different environments

Environments	IL (Av. in cm)	FL (Av. in cm)	IW (Av. in g)	FW (Av. in g)	Survival (%)	WG
House tank-1	15	30.6	24.4	95.15	87.5	70.75
House tank-2	15.5	25.05	25.4	67.55	75	42.15
House tank-3	14	29.1	23.3	87.75	82.5	64.45
Earthen ditch-1	14.5	32.75	25	110.15	92.5	85.15
Earthen ditch-2	15.7	30.2	25.5	91.65	87.5	66.15
Plastic tank	13.9	29.1	23.8	87.15	82.5	63.35
Backyard tank-1	14.5	25.1	23.8	67.65	80	43.85
Backyard tank-2	15.7	25.4	25.4	68.55	77.5	43.15

Initial Length (IL), Final Length (FL), Initial weight (IW), Final weight (FW), Weight gain (WG)

4.1.3. Distinguish weight gain from different culture regime

At the end of the experiment, average growth performance of *M. cuchia* in different environments was presented in figure 4.1. The highest average growth (85.15g) was found in earthen ditch-1 where the different external feed was supplied with natural food and the lowest growth (42.15g) was recorded in house tank-2 where small dead fish was given. Second highest growth was found in house tank-1 (70.75g) where live feed was given. Despite of maintaining as a control, it was found that the average growth was recorded 66.15 g in earthen ditch-2 which was third position in case of growth of experimental fish with only natural food. The fourth highest growth was found from house tank-3 (64.45 g) where only live feed was given, followed by plastic tank (63.35 g), back yard tank-1 (43.85 g) and back yard tank-2 (43.15 g) respectively.

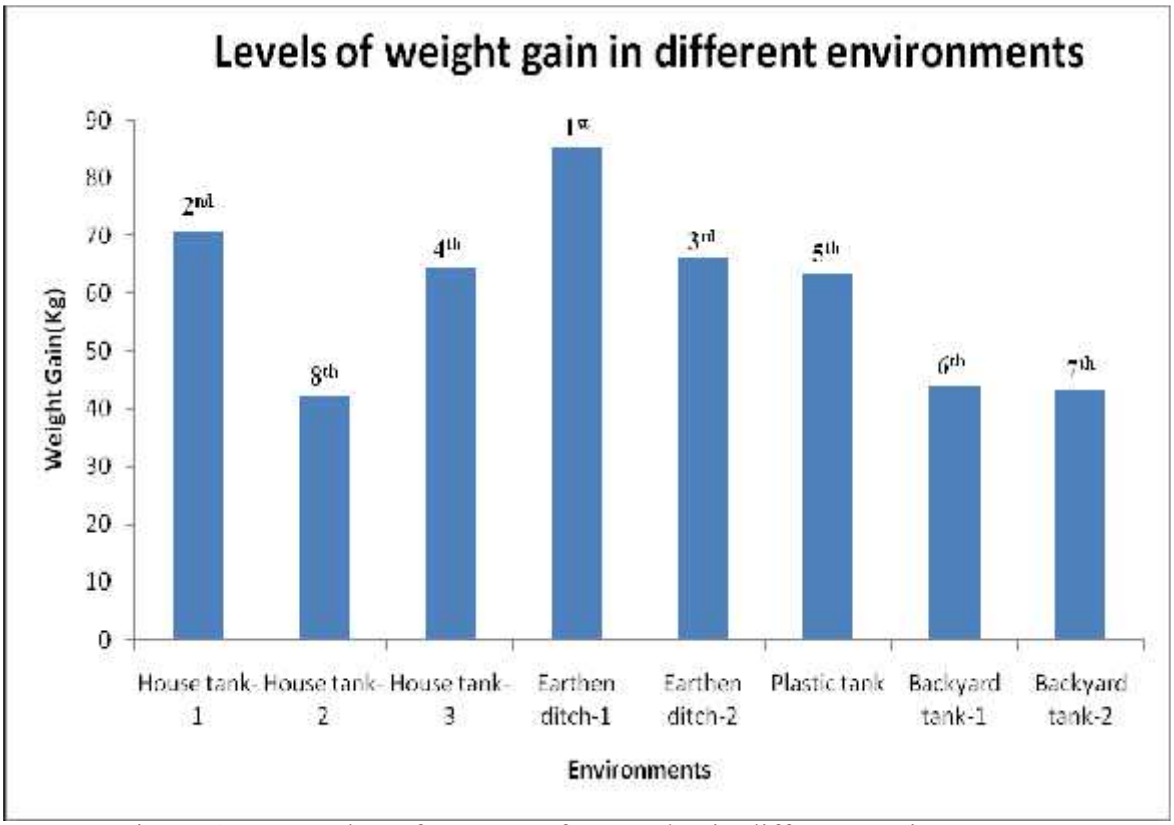

Figure 4.1. Growth performance of *M. cuchia* in different environments

4.1.4. Production performance of experimental fish in different environments

Based on Paired Sample Statistics, it has been shown that the highest mean final length (32.89±2.65423) with weight gain in length 17.78 ± 0.24 and final weight (109.69±2.92777) with weight gain in weight 85.07 ± 0.15 both was recorded in earthenditch-1 whereas lowest mean of final length (28.45±2.80092) with weight gain in length 11.86 ± 0.26 and final weight (67.24±3.79328) with weight gain in weight 41.84 ± 0.59 was in house tank-2. Pearson Correlation coefficient has been calculated for each environment for both length and weight of *M. cuchia*. Here, correlation coefficient was calculated as a pair such as initial length, final length and initial weight, final weight and it was clearly indicating that there were no significant correlations (<0.00) between these pairs. Descriptive statistics gives an indication that earthen dich-1 gives more mean value in both length and weight. Paired sample t test was done since we have the same object for measure before and after intervention. For each of the environments (P<0.000) implies that *M. cuchia* can be reared significantly except back yard tank-1 (P>0.009). But, in earthen ditch-1 it was up to 95% confidence level due to supplied live foods. In case of earthen ditch-1 mean values were highest so it may be suggested to implement with earthen ditch-1 for getting better production.

Growth performance of experimental fishes collected from all different environments showed satisfactory results. The highest production was found in earthen ditch-1, where 37 fishes were found representing an amount of 4.07kg of fish (Table 4.3.). Conversely, the lowest growth performance was found in house tank-2 showed the opposite of this study having the least figure (2.02kg) out of the eight categories

(Table 4.3). Another significant production was found 3.3kg of fish in house tank-1. The growth performance of rest of the environments such as back yard tank-1 and 2, plastic tank, house tank-3 and earthen ditch-2 were recorded 2.09kg, 2.12kg, 2.87kg, 2.89kg and 3.2 kg of fish respectively. After six months, total amount of 22.56kg fish was found from 265 harvested individuals (Table 4.3).

Table 4.3. Production performance of fish in different environments

Conditions	Final harvest (No.)	Weight gain (g)		Production (kg)
House tank-1	35	70.75	L= 15.38 ± 0.13	3.3
			W= 71.15 ± 1.86	
House tank-2	30	42.15	L= 11.86 ± 0.26	2.02
			W= 41.84 ± 0.59	
House tank-3	33	64.45	L= 15.07 ± 0.39	2.89
			W= 65.01 ± 0.38	
Earthen ditch-1	37	85.15	L= 17.78 ± 0.24	4.07
			W= 85.07 ± 0.15	
Earthen ditch-2 (Control)	35	66.15	L= 14.1 ± 0.48	3.2
			W= 66.66 ± 0.82	
Plastic tank	33	63.35	L= 16.45 ± 0.61	2.87
			W= 62.74 ± 0.65	
Backyard tank-1	31	43.85	L= 5.1 ± 1.04	2.09
			W= 44.05 ± 1.45	
Backyard tank-2	31	43.15	L= 10.46 ± 1.02	2.12
			W= 42.7 ± 0.82	
Total	265	479.00		22.56

4.2. Breeding biology and artificial breeding

4.2.1. Sexual differentiation

Taxonomically the experimental fish was identified by the study of some important morphometric characteristics. Identification of male or female fish was so difficult but some external characteristics were helpful to observe sexual differentiation during breeding season. The body of both male and female fish was found long and slender, and seems scale less. Actually, it has smooth, tiny scales that were embedded in the skin. Head was found long and tapers to a small mouth consisting two small eyes on the head. Mature age male fish length was recorded approximately 60-65cm and weight was near about 500-600g. But mature female was larger than male fish. The abdomen of female fish was observed swollen and brownish in colour with rough abdominal skin. Anus and genital pore were observed as tubular in male round shape in female (Figure 4.2).

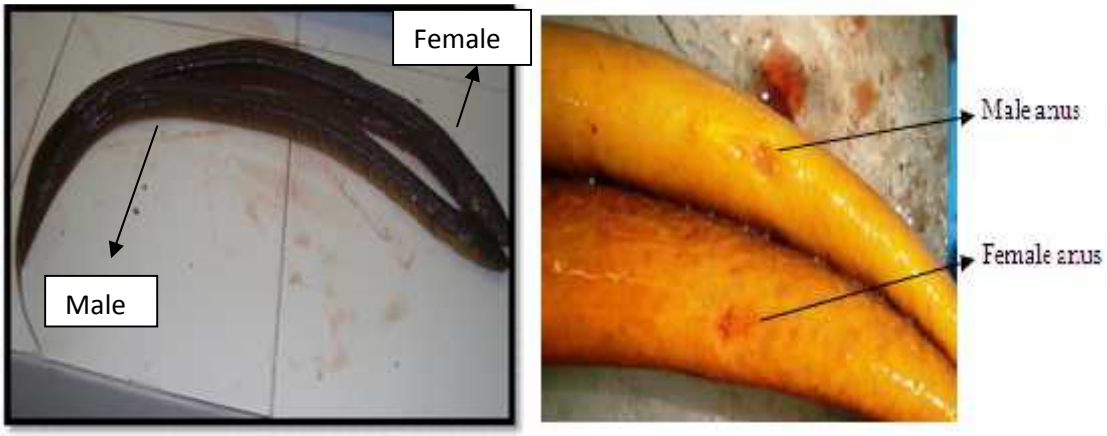

Figure 4.2. Male and female *M. cuchia*

Internally, single gonad of both sexes comprised of a white, smooth, ribbon-like structure extending longitudinally below the gut and above the kidney for the entire length of the abdominal cavity. In male two equal, very thin, narrow and long sperm ducts were observed which was extended from anus to liver (Figure 4.3). However, single tubular oviduct was found in female with eggs from urinogenital opening to anterior part of the gall bladder (Figure 4.4). The ovary length was found in 11.2cm and brownish in colour (Figure 4.4) while the sperm duct was almost 15cm long and white in colour (Figure 4.3). In an average 600 round eggs were found in the experimental females and the egg size was ranged between 0.1 mm to 0.7 mm in diameter (Figure 4.7).

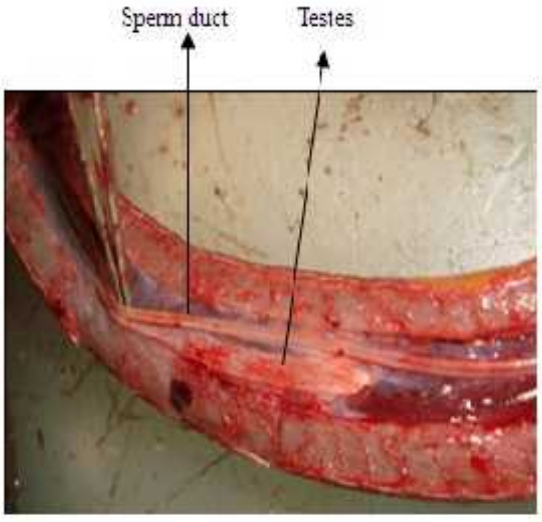

Figure 4.3. Male genital organ of *M. cuchia*

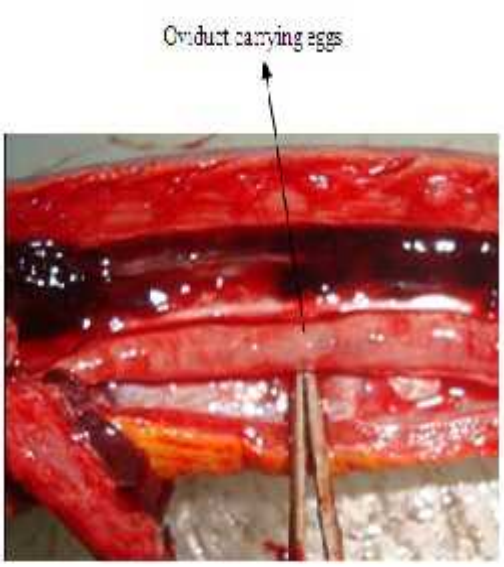

Figure 4.4. Ovary of female *M. cuchia*

Histologically, the testis was dominated by the presence of small spermatogonia and it was recognizd by the more compact within a tubular arrangement with more cells. The testis has well-defined tubules with clear lumina which have spermatocytes on their inner margin. Spermatocytes were also recognized by their smaller nuclear size and darkly staining chromatin material (Figure 4.5).

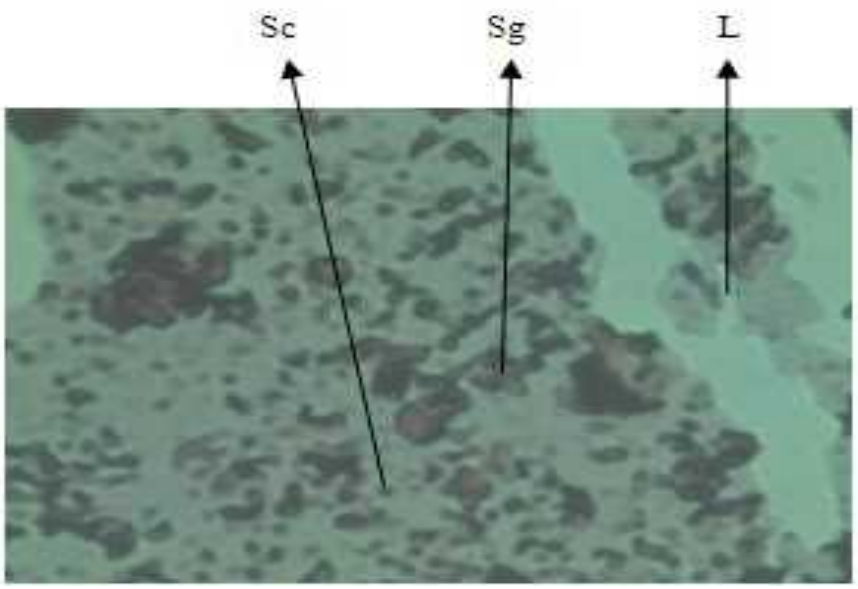

Figure 4.5. Histological sections of testis

Ovary was dominated by the presence of vacuolated oocytes and an increase in oocyte size was associated with cytoplasm that was less basophilic. Immature cells with small vacuoles were observed at the periphery of the oocyte and the vacuoles gradually move towards the nucleus of mature cells (Figure 4.6).

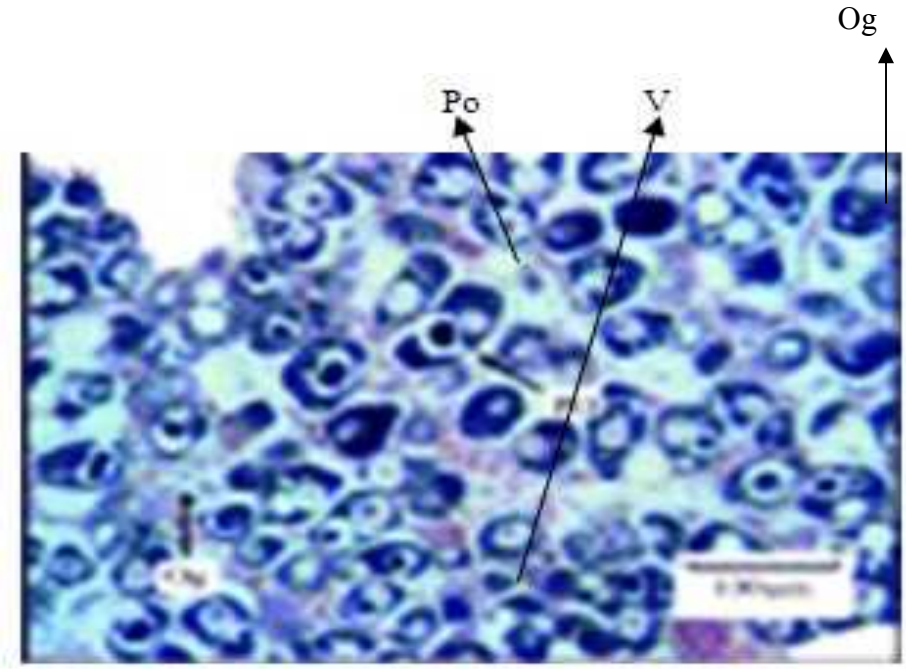

Figure 4.6. Histological sections of representative ovary (Sg, spermatogonia; Sc, spermatocytes; L, lumen), (Og, oogonia; PO, primary oocytes; V, vacuoles).

4.2.2. Breeding biology of *M. cuchia*

Breeding biology of freshwater mud eel, *M. cuchia* was observed where the length of male fish ranged from 40 cm to 82 cm and the lowest length was recorded 40 cm in male fish during February to March and higher length was found 80cm during May-June in natural fish while highest length 82 cm was considered in treated fish (Table 4.4). The body weight of experimental male fish was 90g to 330g in natural fish while lowest 90g was found during February to April and 330g was found during May to June, however, hormonal treated fish weight was observed 550g. The length of testis with sperm duct was found lowest 11cm during February to March and highest 16.5cm in natural fish during May-June while highest length was observed 20cm in treated fish. In addition, observed weight of testis with sperm duct was found lowest 0.5g in February to March and highest 6.7 in natural fish during May to June where 8.1 g was found in treated fish. On the other hand, testis was also measurement without sperm duct where the lowest length was found 4cm during March-April and highest was observed 8.75 during May-June. The weight of testis was found without sperm duct 0.35g (February-March) to 4.2g (May-June) in natural fish while highest 4.7g was recorded in treated fish.

Table 4.4. Measurement of gonad and ova diameter

Study option	Male Fish		Female Fish		Testis				Ovary		No. of eggs individually	Egg Diameter (mm)	
					With sperm duct		Without sperm duct						
	Wt (g)	Lt (cm)	Wt (g)	Lt (cm)	Wt (g)	Lt (cm)	Wt (g)	Lt (cm)	Wt (g)	Lt (cm)		Range	Mean
Feb15-Mar14	90-280	41-70	150-500	50-83	0.5-1.9	11-16	0.35-1.60	4.5-7.2	2.4-6.75	6-10.5	280-621	0.3-2.2	1.075
Mar15-Apr14	90-250	40-75	170-300	65-77	1.4-3.5	12-16	1-2.4	4-7	4.4-11	6-7.6	203-603	0.4-3.2	1.805
Apr15-May14	120-280	43-78	150-400	55-82	3-4.4	13.2-16	1.8-3.2	4.25-7.2	6.1-17.6	6-8	203-742	0.5-4	2.5
May15-June14	180-330	55-80	220-400	60-82	4.6-6.7	12.7-16.5	2.4-4.2	5.75-8.75	9.8-24.5	8-12	385-1495	0.5-4.3	2.49
Treated fish	90-550	44-82	140-520	52-90	2.1-8.1	12-20	1.45-4.7	5-8.2	6.9-20.4	5.9-11.3	155-998	0.5-4	2.195

(Wt = Weight, Lt = Length)

In case of female fish, the body length and weight varied from 50 to 90 cm and 150g to 520g respectively whereas the length and weight of female ovary were found 5.9cm to 12cm and 2.4g to 24.5g respectively (Table 4.4). Highest length and weight of ovary were found 12cm and 24.5g respectively during May-June and usually the female gonad weight was found higher than the male. The individual fecundity was recorded 203 to 1495 while lowest number of eggs was observed 203 during the month of March to May and highest was observed 1495 during May to June (Figure 4.7). However, in case of treated fish the number of eggs was found 155 to 998. The mean egg diameter 1.075mm was observed during February-March and 2.49mm during May-June, and 2.195mm was recorded in treated. Unacceptably, it was observed that the eggs of the treated fish was found less, smaller in size as well as spoiled (Figure 4.8).

Figure 4.7. Normal eggs

Figure 4.8. Ovary with spoiled eggs

4.2.3. Monthly changes of gonadosomatic index (GSI) in male and female

The gonadosomatic index (GSI) is an indicator of the seasonal development of the gonad. The monthly variation in GSI of the male was found from 0.64±0.14 to 2.14±0.2 while GSI of the female was observed from 1.6±0.36 to 5.1±1.09 (Table I4.5). In case of male fish specimens, it was found that the mean GSI of hormonal

treated fish (2.16±0.43) was higher than the untreated fish (1.9±0.25) during the same time but in case of female the mean GSI of hormone induced fish (4.72±1.65) specimens lower than the untreated fish (4.87±0.96).

Table 4.5. Monthly changes of GSI in males and females

Trials	Periods	Mean GSI (male)	Mean GSI (female)
1	15 Feb - 14 Mar	0.64±0.14	1.6±0.36
2	15 Mar - 14 Apr	1.42±0.24	2.86±0.67
3	15 Apr - 14 May	1.9±0.25	4.87±0.96
4	15 May - 14 June	2.14±0.2	5.1±1.09
5 (Induced Fish)	15 Apr - 14 May	2.16±0.43	4.72±1.65

4.2.4. Monthly variations in body length, body weight and fecundity of *M. cuchia*

Estimation of body length, body weight and fecundity are the prerequisite in successful breeding program. In the present study monthly mean body length varied from 63.9±10.28 to 71.8±7.5 and monthly mean body weight was found from 230±43.53 to 300.5±57.12, where the highest mean body length was found in April-May while the highest mean body weight was found in May-June. In the present study the individual fecundity recorded from 1495 (65 cm / 310g) to 155 (240 cm / 69g) while relative fecundity ranged from 2.64 to 12.45. The average monthly absolute fecundity for female *M. cuchia* was found 376.6±134.14 to 572±216.97 while relative fecundity varied from 5.31±2.08 to 8.41±2.61. The absolute high fecundity and relative fecundity was found in May-June. The mean body length, mean body weight, mean absolute fecundity and relative absolute fecundity of the hormonal induced fish

were found 67.5±11.44, 267±108.54, 472.7±264.58 and 7.02±3.55 respectively (Table 4.6).

Table 4.6. Monthly variations in body length, body weight and fecundity

Period	Mean body length	Mean body weight	Absolute fecundity	Relative fecundity
Feb 15-Mar 14	63.9±10.28	276.5±113.02	411.8±118.75	6.48±2.76
Mar 15-Apr 14	71.1±3.65	230±43.53	411.1±102.42	5.79±1.38
Apr 15-May 14	71.8±7.5	255±70.57	376.6±134.14	5.31±2.08
Apr 15-May 14 (Induced)	67.5±11.44	267±108.54	472.7±264.58	7.02±3.55
May 15-June 14	67±6.49	300.5±57.12	572±216.97	8.41±2.61

4.2.5. GSI in both treated and non-treated fish (control)

The GSI values showed significant difference in both males and females. In male fish pituitary gland hormone (PG) as compared to control group (2.26± 0.52) significant differences were seen in the group injected for 5 days (2.31 ± 0.71) and 10 days (2.86 ± 0.57) while in case of females as compared to control group (4.26 ± 0.65) significant differences were seen in group of injected fish for 5days (4.1 ± 0.31) and 10 days (4.74 ± 0.65) (Table 4.7).

Table 4.7. Observation of GSI in both hormonal treated and non-injected fish

Fish	Treatment	No. of fish	Maximum	Minimum	Mean ± SD
	Control	5	3.2	1.7	2.26 ± 0.52
Male	5 days	5	3.5	1.3	2.32 ± 0.71
	10 days	5	3.6	2.1	2.86 ± 0.57
	Control	5	5.1	3.3	4.26 ± 0.65
Female	5 days	5	4.8	3.3	4.1 ± 0.31
	10 days	5	5.5	3.8	4.74 ± 0.65

4.2.6. Observation of artificial breeding

Unsuccessful trial was done for artificial breeding through IVF with and without hormonal treatments. After five to seven days, in treated fish, some fishes were become weak and injected area was infected. At the end of first, second and final trials, it was seen that mature eggs in the oviduct were reduced, absorbed and some eggs were found in spoiled conditions. Therefore, it was concluded that all experiments of the induced breeding were failed and no positive results were found.

4.2.6.1. Breeding in cemented tank without hormonal treatment

In this experiment for breeding in cemented tank without hormonal treatment, no spawning respond was seen during the 20 days of observation.

4.2.6.2. Breeding through IVF technique

Unsuccessful trial was done for artificial breeding through IVF technique. Every day checked it and finally the decision was taken that no fertilization was seen there.

4.2.6.3. Induced breeding by hormonal treatment

In this experiment different types of techniques were used for induced breeding of freshwater mud eel, *M. cuchia.* After six months of experimental analysis, it was seen that fish was unable to spawn through inducing agents. Chronologically three separate experiments were done in both house tanks and in hapa for each inducing agent (PG, HCG, GnRH and Ovulin). During one to two weeks of observation for each treatment no spawning behavior were seen within the treated fish while checking regularly. After five to seven days, some fishes were become weak and injected area was infected. At the end of first, second and final trials, it was seen that mature eggs in the oviduct were reduced, absorbed and some eggs were found in spoiled conditions. Therefore, it was concluded that all the experiments of induced breeding were failed and no positive results were found.

4.3. Molecular species identification

4.3.1. Based on 16s mtDNA

PCR products of agarose gel with 15 individuals were analyzed using 16s mitochondrial DNA (universal primer) for identification of the freshwater mud eel, *M. cuchia*. Nice bands were found in all the individuals except the individual 1, 12, and 13 (Figure 4.9) and each DNA sample shows clear bands at the position of 250bp length based on the 10000 bp length ladder whereas the ranges of ladder was started at 250bp length. Unfortunately, primer-dimer mixture was seen in both gels.

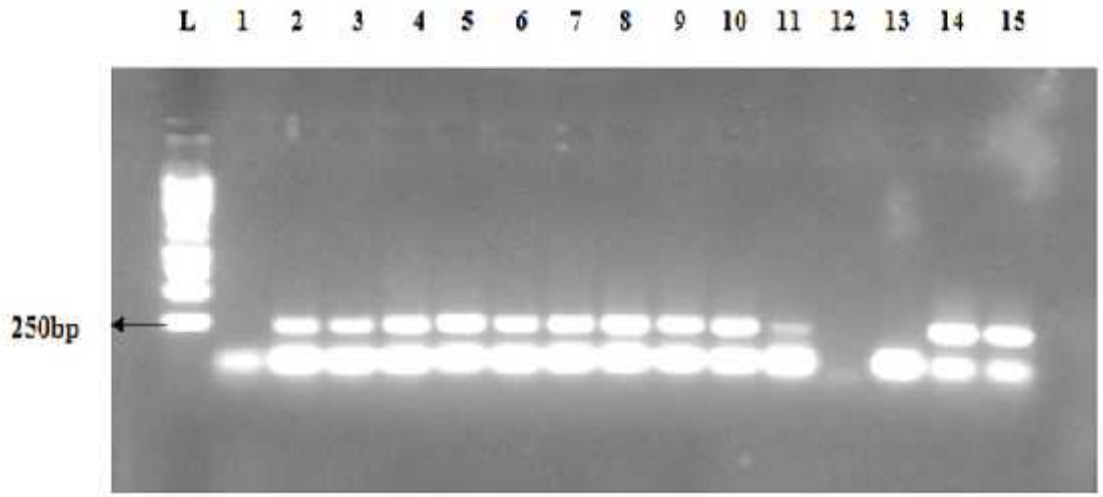

Figure 4.9. PCR products of 15 individuals using16s mitochondrial DNA

4.3.2. Based on glutamine synthetase gene

541 bp long glutamine synthetase gene sequences were analyzed for identification of this freshwater mud eel. It is an important enzyme in detoxifying ammonia for aquatic animals as well as *M. cuchia*. The agarose gels with 15 individuals were done for PCR products of this fish and found equal bands at 541bp length compared with 10,000bp ladder (Figure 4.10). In this case the ranges of the ladder were also started from 250 bp length. Unfortunately some primer-dimer mixed bands were observed.

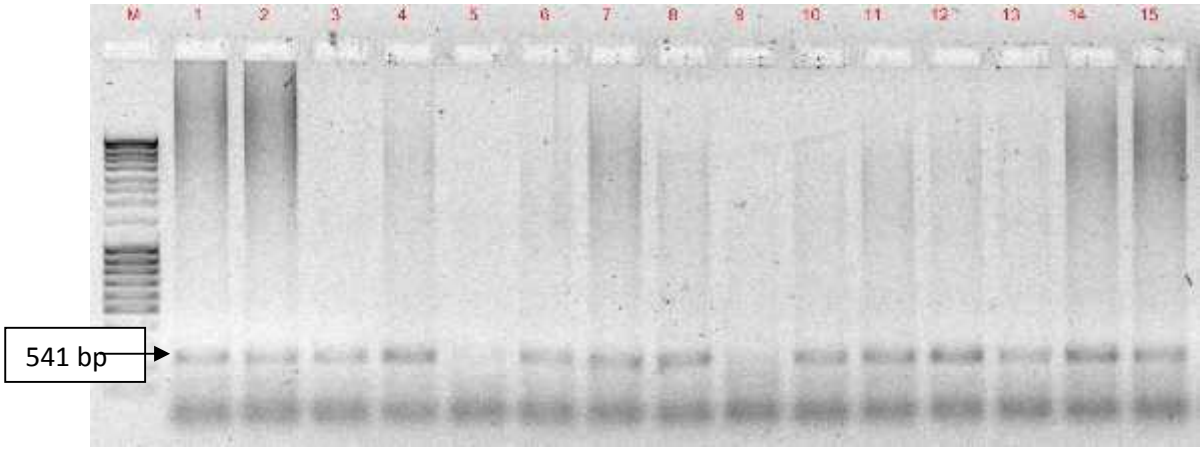

Figure 4.10. PCR amplification result of glutamine synthetase gene in 15 individuals

4.4. Genetic diversity based population studies

4.4.1. RAPD analysis

4.4.1.1. Genetic diversity by RAPD

4.4.1.1.1. RAPD based data

In this study, RAPD based markers were used to genetic study of freshwater mud eel, *M. cuchia* in Bangladesh and thirty individuals were considered for this research. Among twelve RAPD primers, only eight primers (B 03, OPF 14, C 04, OPB 05, OPB 08, OPB 19, OPB-12 and UBC122) were amplified the genomic DNA of this species to assess genetic status particularly for genetic diversity based population studies. The results of the experiments were given as under in systematic ways.

4.4.1.1.2. DNA profiling and data scoring

DNA profiling and data scoring were studied separately for each of the primers. The size of amplification products were measured in base pairing Alpha EaseFc 4.0 software. 1 kb plus DNA ladder was used as molecular weight where marker ranged from 75 bp to 20,000 bp (Generuler^{TM}) for comparison of the observed RAPD bands. Each amplified band profile was defined by the presence or absence of bands at particular positions on the gel. Fragments were scored as 1 if the presence of bands or 0 if the absence of bands, separately for each individual and each primer.

4.4.1.1.3. Banding patterns

Bands were found individually from the PCR products of eight decamer primers considering agarose gel image. Different lengths of bands were observed in different eight primers where the ranged for primer B 03 from 151 bp to 2608 bp (Appendices 1a and 1b), from 71 bp to 1566 bp for primer OPF 14 (Appendices 2a and 2b), from 40 bp to 1473 bp for primer C03 ranged (Appendices 3a and 3b), from 71 bp to 1605 bp for primer OPB 05 (Appendices 4a and 4b), from 60 bp to 1605 bp for primer OPB 08 (Appendices 5a and 5b), from 170 bp to 1920 bp for primer OPB 19 (Appendices 6a and 6b), from 280 bp to 2703 bp for primer OPB 12 (Appendices 7a and 7b) and from 310 bp to 635 bp for primer UBC 122 (Appendices 8a and 8b). The bands were seen in different levels of length of DNA.

A total of 735 bands with 228 polymorphic loci were detected among 30 individuals of this experimental fish (Table 4.8). Polymorphisms were revealed by all the primers while no monomorphic loci were seen. The highest number of bands (120) was amplified by the primer C04 and the lowest number of bands (49) was amplified by the primer UBC122. Highest polymorphism (100%) showed by all the primers among the tested individuals. The highest number of bands (4.00) per individual was amplified from the primer C-04 and the lowest number of bands (1.63) per individual was amplified by the primer UBC122.

Table 4.8. Summary of the bands revealed from eight RAPD primers

Primers	Size of DNA (bp)	Total no. of Bands	Polymorphic loci in No.	Monomorphic loci in no.	Polymorphic loci in %	No. of Bands per sample
B O3	151-2608	88	36	00	100%	2.93
OPF 14	71-1566	107	24	00	100%	3.57
C O4	40-1473	120	26	00	100%	4.00
OPB 05	71-1605	105	33	00	100%	3.50
OPB 08	60-1605	100	23	00	100%	3.33
OPB 19	170-1920	109	42	00	100%	3.63
OPB 12	280-2703	57	32	00	100%	1.90
UBC122	310-635	49	12	00	100%	1.63
Total		735	228	00		
Average		91.88				

4.4.1.1.4. Inter individual pair wise similarity indices

Inter individual pair wise similarity of freshwater mud eel, *M. cuchia* was studied and based on these similarity 13 groups of individuals such as 14, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 and 1 respectively were found (Table 4.9). Highest similarity (14) was found between individual pair 7 & 11 and 7 & 16 as well as lowest similarity (1) was found in the individual pairs of 5 & 24, 5 & 29, 6 & 24, 10 & 24, 14 & 24 and 17 & 22 respectively.

See GD

4.4.1.1.5. Genetic distances

The genetic distance among individuals of *M. cuchia* was calculated by using the data from pair wise similarity index (Table 4.10). 36 groups of genetic diversity were measured among 30 individuals of *M. cuchia* whereas the lowest genetic distance was found between the individuals 7 & 16 (0.58) followed by 7 & 11 (0.59) and 11 & 20 (0.60), and the highest genetic distance was recorded 0.97 between the individual pairs of 17 & 22, 14 & 24 and 10 & 24 and second highest was found 0.96 between the individual pairs of 6 & 24 and 5 & 24 respectively. Relatively higher genetic distance was recorded in other individuals (Table 4.10). The average genetic diversity was found 0.8173716 and this value is indicating the good genetic status of this experimental fish in Bangladeshi nature especially in Sylhet environment.

See in the file of GD

4.4.1.1.6. Nei's genetic similarity

Considering the Nei's genetic similarity analysis different values of similarities were found from these 30 individuals whereas highest and lowest values were recorded 0.60 between the individual pair of 7 & 16 and 0.06 between the individual pairs of 5 & 29, 10 & 24, 13 7 24 and 16 & 22 respectively. However, in general, Nei genetic similarity values of these individuals were found in diverse level (Table 4.11).

See in the file of GD

4.4.1.1.7. Linkage distance among individuals of freshwater mud eel

Based on Squared Euclidean Distances, the values of pair-wise comparisons of the linkage distance were computed from combined data for these experimental individuals and different levels of values were found between the range of 3.6 to 6.24 while individual 5 & 6 have shown lowest linkage distance and highest 6.24 linkage distance was observed between the individuals of 2 & 12, 8 & 21 and 8 & 23 respectively (Table 4.12). Comparatively weak linkage bonding was observed between all other individuals.

See in the file of GD

4.4.1.1.8. Genetic relationships among individuals

A cluster analysis using UPGMA based on linkage distance was done to resolve the phylogenetic relationships among experimental individuals of *M. cuchia*. According to their linkage distance the UPGMA clustering system generated six clusters by 11 clades (Figure 4.11). 22 individuals/samples were involved to form 11 clades and rest of the samples was connected to those clades with a specific linkage distance. Sample (S) 1 and S 4 were found closely related as clade 1 which was created cluster 1 with clade 2 (S5, S6). Both of these clades were situated between the linkage distances of 3.5 to 4. Sample 3 (=S3), S2, S13, S10 and S15 were individually connected to the clade 1 and clade 2. Although they were somehow distantly related but situated under the cluster 1, however, their linkage distance was observed approximately 4.9. Clade 3 (S7, S16) and clade 4 (S11, S20) were close enough between the linkage distance of 4-4.5 and formed cluster 2 which was comparatively close enough to cluster 1. Clade 5 (S17, S28), clade 7 (S24, S26) and clade 8 (S29, S30) were very close to their respective pairs and their linkage distance was observed between 4-4.5 except clade 6 (S19, S27) which was around 4.6. All these clades were located under the cluster of 3 and formed cluster 4 when connected with cluster 2. S14 and S23 were found very similar to each other as well as S2, S18 and their linkage distances were seen 4.7 and 4.9 respectively. S23 also connected to the clade 9 (S9, S14) with 5.2 linkage distance, clade 9 and S23 were placed under the cluster of 5 which was also connected to the cluster 3 and 4. Clade 10 was also linked to the cluster 5. The most distance related samples from the others were S8, S12 with a linkage distance around 5.3 and connected to the most outer cluster 6.

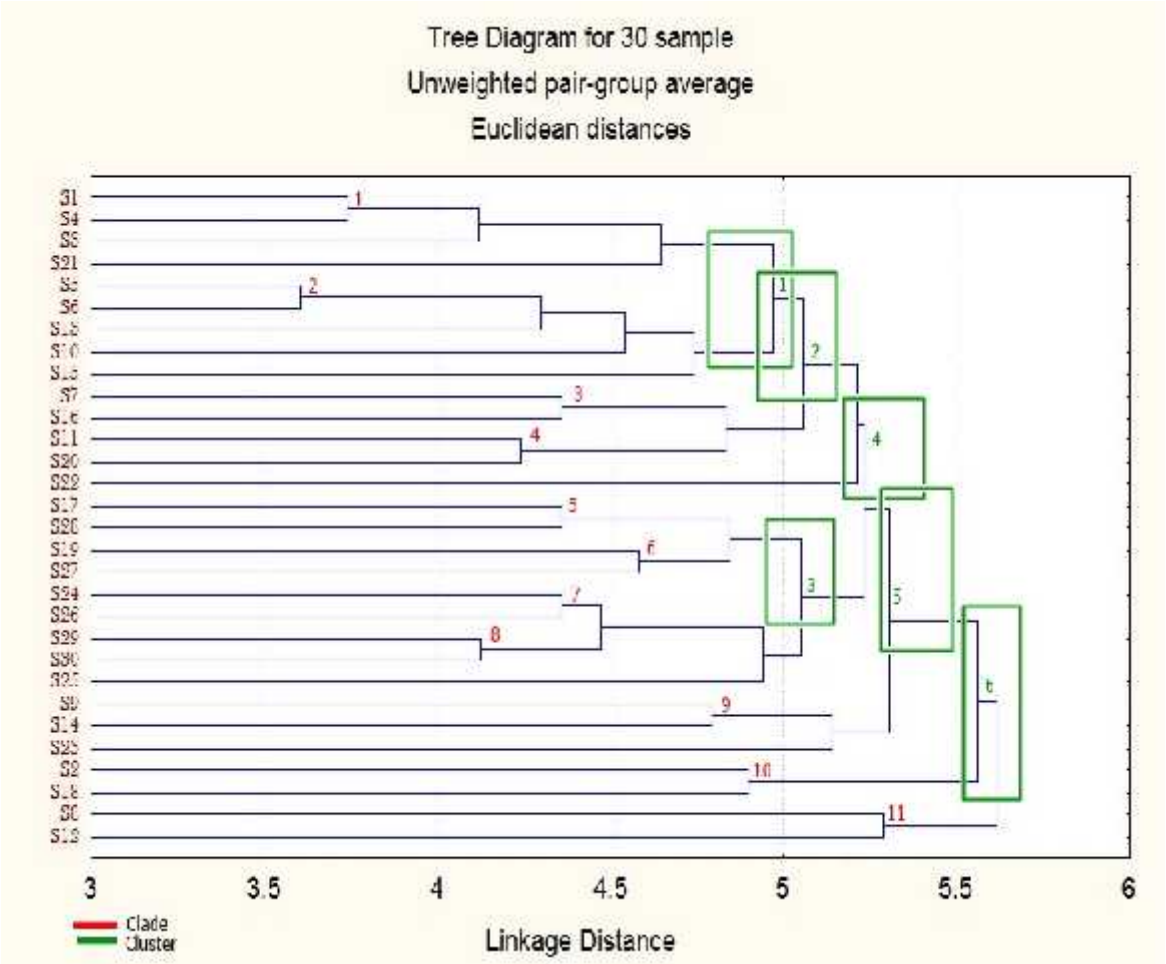

Figure 4.11. Genetic relationship among individuals by Dendrogram

4.4.1.2. Intra and inter population genetic diversity by RAPD analysis

4.4.1.2.1. RAPD data scoring and bands summary of P1 and P2

Population genetic structure of freshwater mud eel, *M. cuchia* in Bangladesh was analyzed based on RAPD assay considering 2 populations whereas 15 individuals were considered for each population. Eight arbitrary primers (B 03, OPF 14, C 04, OPB 05, OPB 08, OPB 19, OPB-12 and UBC122) were used to amplify the genomic DNA of two experimental populations which was already used in the study of basic genetic diversity. DNA profiling and data scoring were studied same as basic genetic diversity study while Alpha EaseFc 4.0 software were used to measure the size of amplified DNA products. 1 kb plus DNA ladder was used to compare the size of amplified DNA where the band size was started from 75 bp (Generuler^{TM}). Bands of each primer was distinguished by the presence or absence of bands at particular positions. Data was scored as 1 if the presence of bands or 0 if the absence of bands, separately for all the primer.

Different lengths of bands were observed in different eight primers in two populations. The ranged of primer B 03 from 372 to 2608 bp in P1 and 151 bp to 2265 bp in P2 were observed (Appendices 9a, 9b and 10a, 10b). In the primer OPF 14 the bands were ranged from 71 bp to 1566 bp in P1 and 71bp to 423 bp in P2 (Appendices 11a, 11b and 12a, 12b). Bands were ranged from 40 bp to 1473 bp in P1 and 60 bp to 1409 bp in P2 by the primer C03 (Appendices 13a, 13b and 14a, 14b), whereas in the primer OPB 05 bands were found between 71 bp to 1605 bp in P1 and 71bp to 1566bp in P2 (Appendices 15a, 15b and 16a, 16b). Furthermore, the bands were observed between 60 bp to 1605 bp in P1 and 60bp to 1517bp in P2 by the

primer OPB 08 (Appendices 17a, 17b and 18a, 18b), whereas bands ranged were observed 212 bp to 1836 bp in P1 and 179bp to 1920 in P2 by using the primer OPB 19 (Appendices 19a, 19b and 20a, 20b). In addition, in the primer OPB 12, bands were found between 280 bp to 1920 bp length in P1 and 280bp to 2703bp in P2 (Appendices 21a, 21b and 22a, 22b). Lastly in the primer UBC 122, the range of bands was recorded from 360bp to 635bp in P1 and 310 bp to 360 bp in P2 (Appendices 23a, 23b and 24a, 24b).

A total of 353 bands with 147 polymorphic loci were detected in P1 whereas a total 371 bands with 153 polymorphic loci were found in P2 (Table 4.13). The highest number of bands (62) was amplified by the primer OPB 14 and lowest number of bands (27) was observed by the primers OPB 12 and UBC 122 in P1. On the other hand, in P2, highest number of bands (60) was recorded by using the primer C04 and the lowest number of bands (22) was amplified from the primer UBC 122. Polymorphisms were revealed by all the primers in P1 and P2 while a single monomorphic locus were seen in P1 resulting highest polymorphism (100%) showed by all the primers among the tested populations, though, 95% polymorphism was observed by using the primer OPB 08 in P1. Per individual the highest number of bands was amplified 4.13 by OPF 14 in P1 and 4.00 by C 04 in P2 respectively while the lowest number of bands (1.8) per individual was amplified by the primer OPB 12 and UBC122 in P1, and 1.47 in P2 by using the primer UBC 122 (Table 4.13).

Table 4.13. Summary of the bands revealed from eight RAPD primers for P1 and P2

Primers	Size of DNA (bp)		Total no. of Bands		Polymorphic loci in No.		Monomorphic loci in no.		Polymorphic loci in %		No. of Bands per sample	
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
B O3	372-2608	151-2265	36	50	19	22	00	00	100%	100%	2.4	3.33
OPF 14	71-1566	71-423	62	47	18	18	00	00	100%	100%	4.13	3.13
C O4	40-1473	60-1409	60	60	22	15	00	00	100%	100%	4.00	4.00
OPB 05	71-1605	71-1566	52	53	21	20	00	00	100%	100%	3.47	3.53
OPB 08	60-1605	60-1517	46	54	12	19	01	00	95%	100%	3.07	3.6
OPB 19	212-1836	170-1920	53	55	29	32	00	00	100%	100%	3.53	3.67
OPB 12	280-1920	280-2703	27	30	18	22	00	00	100%	100%	1.8	2.00
UBC122	360-635	310-360	27	22	8	5	00	00	100%	100%	1.8	1.47
Total			363	371	147	153	01	00				
Average			45.38	46.38	18.38	19.13						

4.4.1.2.2. Inter individual pair wise similarity indices of P1 and P2

Inter individual pair wise similarity among the individuals of P1 of freshwater mud eel, *M. cuchia* was observed and based on these similarity 9 groups of individuals such as 3, 4, 5, 6 7, 8, 9, 10, 11 and 14 respectively were recorded (Table 4.14). Highest similarity (14) was found between individual pair 7 & 11 only whereas lowest similarity (2) was found in different individual pairs of P1, and generally, less similarity was found.

Table 4.14. Inter individual pair wise similarity indices for P1

T	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	-	6	5	7	4	4	4	3	3	3	4	6	4	4	4
2		-	9	7	5	5	7	7	7	6	8	5	8	3	4
3			-	7	5	6	7	6	4	2	5	5	5	3	5
4				-	4	6	5	6	5	4	5	7	3	5	4
5					-	8	8	5	4	8	6	6	6	5	5
6						-	9	4	6	7	7	8	8	8	6
7							-	12	8	9	14	9	9	5	8
8								-	9	6	11	11	5	5	6
9									-	8	11	8	6	9	7
10										-	11	8	9	9	9
11											-	9	7	7	10
12												-	9	9	8
13													-	4	7
14														-	6
15															-

In case of P2, inter individual pair wise similarity of freshwater mud eel, *M. cuchia* was found 13 groups of individuals such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 respectively were documented (Table 4.15). Highest similarity (11) was found between individual pair 2 & 15 and 11 & 15 as well as lowest similarity (1) was found in the individual pairs of 2 & 7 only and different other values were recorded between the values of 1 to 11 in P2, and diversified similarity was observed here in the individuals of P2.

Table 4.15. Inter individual pair wise similarity indices for P2

H	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	-	6	9	7	9	6	4	7	3	10	4	9	8	3	5
2		-	9	7	4	4	1	4	4	5	4	8	10	5	11
3			-	5	5	4	3	4	6	6	3	9	9	5	9
4				-	7	6	5	6	2	9	4	10	8	4	7
5					-	7	5	4	5	7	6	8	5	4	4
6						-	6	5	5	7	5	5	8	3	4
7							-	5	4	8	5	5	4	3	2
8								-	4	9	7	6	7	5	6
9									-	5	5	5	5	5	5
10										-	8	8	7	7	8
11											-	8	6	7	10
12												-	10	8	11
13													-	5	10
14														-	10
15															-

4.4.1.2.3. Genetic distance among individuals of P1 & P2

The genetic distance among individuals of P1 of *M. cuchia* was calculated (Table 4.16) by using the data from pair wise similarity index (Table 4.14). Genetic diversity were measured and 27 different distance values such as 0.59, 0.65, 0.67, 0.69, 0.70, 0.71, 0.72, 0.74, 0.75, 0.76, 0.77, 0.78, 0.79, 0.80, 0.81, 0.82, 0.83, 0.84, 0.85, 0.86, 0.87, 0.88, 0.89, 0.90, 0.91, 0.93 and 0.94 were recorded respectively, indicated that the population 1 has higher genetic diversity (Table 4.16).

Table 4.16. Genetic distance of the P1of *M. cuchia*

T	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0	0.83	0.80	0.67	0.85	0.84	0.88	0.91	0.90	0.90	0.88	0.82	0.86	0.87	0.86
2		0	0.74	0.79	0.87	0.86	0.84	0.84	0.83	0.85	0.81	0.90	0.79	0.93	0.90
3			0	0.70	0.81	0.76	0.79	0.82	0.88	0.94	0.86	0.86	0.83	0.91	0.83
4				0	0.85	0.74	0.85	0.81	0.83	0.87	0.85	0.79	0.90	0.83	0.86
5					0	0.65	0.75	0.86	0.88	0.71	0.82	0.83	0.79	0.84	0.83
6						0	0.70	0.89	0.79	0.75	0.78	0.76	0.69	0.71	0.79
7							0	0.67	0.78	0.74	0.59	0.78	0.74	0.88	0.77
8								0	0.74	0.84	0.70	0.72	0.87	0.88	0.84
9									0	0.75	0.67	0.79	0.82	0.72	0.78
10										0	0.67	0.79	0.70	0.72	0.70
11											0	0.78	0.81	0.82	0.70
12												0	0.74	0.75	0.77
13													0	0.89	0.77
14														0	0.82
15															0

The genetic distances were measured in P2 with 29 different distance values including 0.63, 0.66, 0.67, 0.68, 0.71, 0.72, 0.73, 0.74, 0.75, 0.76, 0.77, 0.78, 0.79, 0.80, 0.81, 0.82, 0.83, 0.84, 0.85, 0.86, 0.87, 0.88, 0.89, 0.90, 0.91, 0.92, 0.93, 0.95 and 0.97 respectively resulting higher genetic diversity was recorded (Table 4.17).

Table 4.17. Genetic distance of P2 of *M. cuchia*

H	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0	0.83	0.76	0.80	0.72	0.84	0.89	0.81	0.91	0.72	0.89	0.75	0.78	0.92	0.88
2		0	0.73	0.77	0.88	0.89	0.97	0.89	0.85	0.86	0.88	0.76	0.68	0.84	0.63
3			0	0.86	0.86	0.90	0.92	0.90	0.79	0.85	0.92	0.75	0.75	0.86	0.75
4				0	0.77	0.82	0.84	0.82	0.93	0.73	0.88	0.68	0.76	0.88	0.79
5					0	0.78	0.83	0.89	0.80	0.79	0.79	0.75	0.86	0.87	0.89
6						0	0.81	0.86	0.82	0.81	0.85	0.87	0.77	0.91	0.90
7							0	0.85	0.84	0.75	0.83	0.85	0.89	0.90	0.95
8								0	0.86	0.74	0.77	0.84	0.81	0.85	0.84
9									0	0.83	0.79	0.83	0.83	0.79	0.83
10										0	0.75	0.78	0.82	0.79	0.78
11											0	0.74	0.82	0.74	0.66
12												0	0.71	0.74	0.67
13													0	0.85	0.71
14														0	0.66
15															0

4.4.1.2.4. Nei's genetic similarity among individuals of P1

Nei's genetic similarity among individuals of P1 was analyzed and different values of similarities such as 0.11, 0.13, 0.16, 0.17, 0.18, 0.19, 0.21, 0.22, 0.23, 0.24, 0.25, 0.26, 0.27, 0.28, 0.29, 0.30, 0.31, 0.32, 0.33, 0.34, 0.35, 0.36, 0.37, 0.38, 0.39, 0.40, 0.41, 0.42, 0.44, 0.46, 0.47, 0.50, 0.52 and 0.58 were found whereas the highest and lowest similarities 0.11 and 0.58 were recorded respectively. In this study, diverse values of Nei genetic similarity were found (Table 4.18).

Table 4.18. Nei's genetic similarity among individuals of P 1

T	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0	0.29	0.33	0.50	0.27	0.28	0.21	0.16	0.18	0.18	0.21	0.30	0.24	0.23	0.24
2		0	0.42	0.34	0.23	0.24	0.27	0.27	0.30	0.26	0.31	0.19	0.35	0.13	0.17
3			0	0.47	0.31	0.39	0.35	0.30	0.22	0.11	0.25	0.24	0.29	0.16	0.29
4				0	0.27	0.41	0.26	0.32	0.29	0.24	0.26	0.35	0.18	0.29	0.24
5					0	0.52	0.40	0.25	0.22	0.44	0.30	0.29	0.34	0.27	0.29
6						0	0.46	0.21	0.34	0.40	0.36	0.39	0.47	0.44	0.35
7							0	0.50	0.36	0.41	0.58	0.36	0.42	0.22	0.37
8								0	0.41	0.27	0.46	0.44	0.23	0.22	0.28
9									0	0.40	0.50	0.35	0.31	0.44	0.36
10										0	0.50	0.35	0.46	0.44	0.46
11											0	0.36	0.33	0.31	0.47
12												0	0.40	0.38	0.36
13													0	0.37	0.30
14														0	0.18
15															0

Different values of Nei's genetic similarities such as 0.06, 0.10, 0.13, 0.15, 0.16, 0.17, 0.18, 0.19, 0.20, 0.21, 0.22, 0.23, 0.24, 0.25, 0.26, 0.27, 0.28, 0.29, 0.30, 0.31, 0.32, 0.33, 0.34, 0.35, 0.36, 0.37, 0.38, 0.39, 0.40, 0.41, 0.43, 0.45, 0.49, 0.50, 0.51 and 0.54 were recorded among the individuals of P2 whereas the diverse similarities were found between 0.06 to 0.54 (Table 4.19).

Table 4.19. Nei's genetic similarity among individuals of P2

H	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0	0.27	0.43	0.37	0.22	0.20	0.06	0.20	0.26	0.24	0.22	0.39	0.49	0.28	0.54
2		0	0.29	0.24	0.24	0.18	0.15	0.18	0.34	0.26	0.15	0.40	0.40	0.25	0.40
3			0	0.31	0.38	0.30	0.28	0.30	0.13	0.43	0.22	0.49	0.39	0.22	0.34
4				0	0.38	0.36	0.29	0.21	0.33	0.34	0.34	0.40	0.25	0.23	0.20
5					0	0.39	0.32	0.24	0.30	0.32	0.26	0.23	0.37	0.16	0.19
6						0	0.26	0.26	0.28	0.40	0.29	0.26	0.21	0.18	0.10
7							0	0.21	0.24	0.41	0.37	0.28	0.33	0.26	0.28
8								0	0.29	0.29	0.34	0.29	0.29	0.34	0.29
9									0	0.28	0.40	0.36	0.31	0.35	0.36
10										0	0.26	0.41	0.31	0.41	0.51
11											0	0.27	0.45	0.41	0.50
12												0	0.19	0.26	0.45
13													0	0.15	0.51
14														0	0.17
15															0

4.4.1.2.5. Linkage distance among populations p1 and p2

The pair-wise comparisons of the linkage distance were measured by calculating the Squared Euclidean Distances and the linkage distance 3.6, 3.7, 4.0, 4.1, 4.2, 4.24, 4.36, 4.4, 4.47, 4.5, 4.58, 4.6, 4.69, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.29, 5.3, 5.39, 5.4, 5.48, 5.5, 5.57, 5.6, 5.66, 5.7, 5.74, 5.9, 5.92, 6.0, 6.1 and 6.24 was observed respectively in P1 (Table 4.20). In this study, comparatively weak linkage bonding was observed between the individuals.

Table 4.20. Squared Euclidean distances for P1

T	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0	5.4	4.2	3.7	4.7	4.6	5.5	5.7	5.3	5.3	5.5	5.1	4.8	5.2	4.8
2		0	5	5.2	5.6	5.5	6.1	5.9	5.7	5.7	5.7	6.24	5.48	6	6
3			0	4	4.7	4.4	5.1	5.3	5.3	5.7	5.3	5.66	5	5.39	5
4				0	4.7	4.1	5.1	5.1	4.9	5.1	5.3	5.1	5	4.8	5
5					0	3.6	4.9	5.5	5.1	4.5	5.1	5.48	4.36	5.2	4.8
6						0	4.6	5.6	4.6	4.6	5	5	4.24	4.47	4.69
7							0	4.9	5.1	5.1	4.5	5.66	5.2	5.74	5.2
8								0	5.1	5.7	4.9	5.29	5.57	5.92	5.39
9									0	4.9	4.7	5.48	5.2	4.8	5
10										0	4.7	5.48	4.58	4.8	4.58
11											0	5.66	5.39	5.57	4.8
12												0	5.2	5.39	5.39
13													0	5.66	4.9
14														0	5.29
15															0

Linkage distance among individuals of P2 were calculated and different levels of values such as 4.12, 4.36, 4.47, 4.58, 4.69, 4.8, 4.9, 5.0, 5.1, 5.2, 5.29, 5.39, 5.48, 5.57, 5.66, 5.74, 5.83 and 5.92 were found between the range of 4.12 to 5.92 while the recorded linkage distances indicated that the relatively week linkage bonding was observed in P2 as well (Table 4.21).

Table 4.21. Squared Euclidean distances for P2

H	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0	5.29	5.29	4.9	4.8	5.48	5.48	5.48	5.39	5.1	5.48	5.2	5	5.83	5.92
2		0	4.9	4.9	5.39	5.48	5.83	5.66	4.8	5.48	5.1	6.8	4.36	4.9	4.36
3			0	5.66	5.57	4.48	5.66	5.83	4.8	5.66	5.66	5.2	5.2	5.29	5.39
4				0	4.8	5.1	5.1	5.29	5.2	4.9	5.1	4.58	4.8	5.29	5.39
5					0	5	4.8	5.57	4.69	5	4.58	4.9	5.29	5.2	5.48
6						0	5.1	5.29	4.58	5.48	5.29	5.74	5.2	5.66	5.92
7							0	4.29	4.58	5.1	5.29	5.57	5	5.1	5.74
8								0	5	5.1	4.69	5.57	5.2	5.29	5.39
9									0	4.8	4.36	4.9	4.9	4.36	4.69
10										0	4.69	5.39	5.57	4.9	5.39
11											0	4.8	5.2	4.47	4.36
12												0	4.9	4.8	4.69
13													0	5.39	4.9
14														0	4.12
15															0

4.4.1.2.6. Genetic relationship among 2 populations

Based on linkage distances, a genetic relationship was made among experimental individuals of *M. cuchia* in P1. Five clusters were created from 5 clades by UPGMA clustering system (Figure 4.12). Five clades were made by 10 individual samples and rest of the samples was connected to that clade with a specific linkage distance. Individual sample 1 and 4 was found closely related with linkage distance 5.4 which was marked as clade 1. Individual samples 5 and 6 were found closely related with linkage distance near about 5.3 which was indicated clade 2. Another 3rd clade was made by the individual sample 10 and 15 and these two individuals were situated as same linkage distance 6.6. The 4th clade was indicated by the linkage distance 7 which was made by the individual samples 9 and 14 where as the 5th clade was created by the individual samples 7 and 11 at the linkage distance point 6.5. Different sub clusters were formed by adding several single individual with different clades, such as individual 3 with clade 1, individual 13 with clade 2 and individual 8 with clade 5 respectively with different linkage distances. Individual 12 was directly linked with cluster 3 and formed cluster 4 at the point of linkage distance 7.9. Finally, individual 2 also directly linked with cluster 4 and created the cluster 5 at the linkage distance position 8.6.

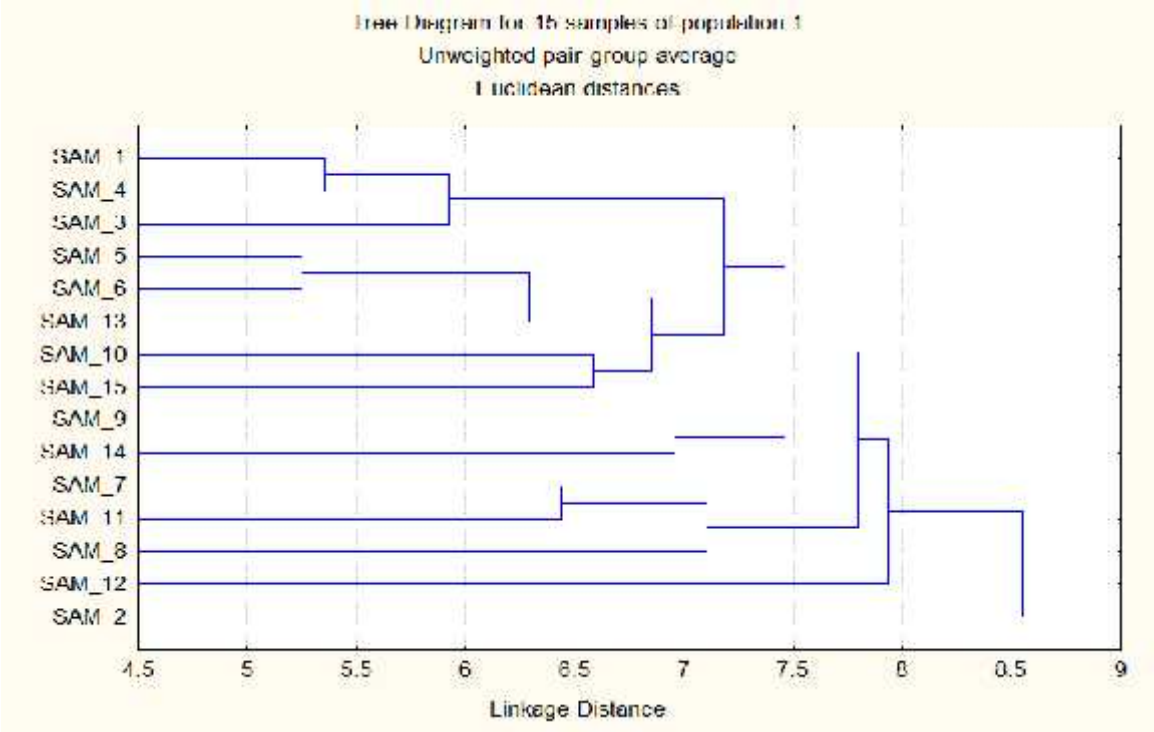

Figure 4.12. Genetic relationships among individuals for P1

A genetic relationship has been identified between the individuals of *M. cuchia* in P2 considering by the linkage distances. Five clusters and five clades were generated by studying dendrogam (Figure 4.13). Ten individual samples were involved to create 5 clades where each clade was made by 2 individual and rest of the samples was connected to different clades with a specific linkage distance. First clade was made by the individual samples 5 and 7 with linkage distance 6.9 whereas individual 10 was linked with clade 1 at 7.8 linkage distance and form a sub cluster, later on this sub cluster was linked with individual 1 and finally form cluster 1 at the linkage distance of 7.5. Individual sample 2 and 13 was found closely related with linkage distance 6.3 and formed clade number 2. Clade 3 was made by the individual sample 4 and 12 while their close relationship was found at linkage distance 6.7. The 4th clade was created by the individual 9 and 11 indicated by the linkage distance 6.4. Furthermore,

individual 14 and 15 were involved to form 5th clade and they were closely related with linkage distance 6. Individual 6 was joined with sub cluster 1 at the point of linkage distance 7.6 and created sub cluster 2, sequentially sub cluster 3 was found when the individual 8 was linked with sub cluster 2 at the linkage distances 7.7. Caldes 2 and 3 were made sub cluster 4 where sub cluster 5 was created by the clades 4 and 5 consequently cluster 4 was created by the sub clusters 4 and 5. Finally clusters 3 and 4 were formed cluster 5 along with the link of individual 3 at the point of linkage distance 7.9.

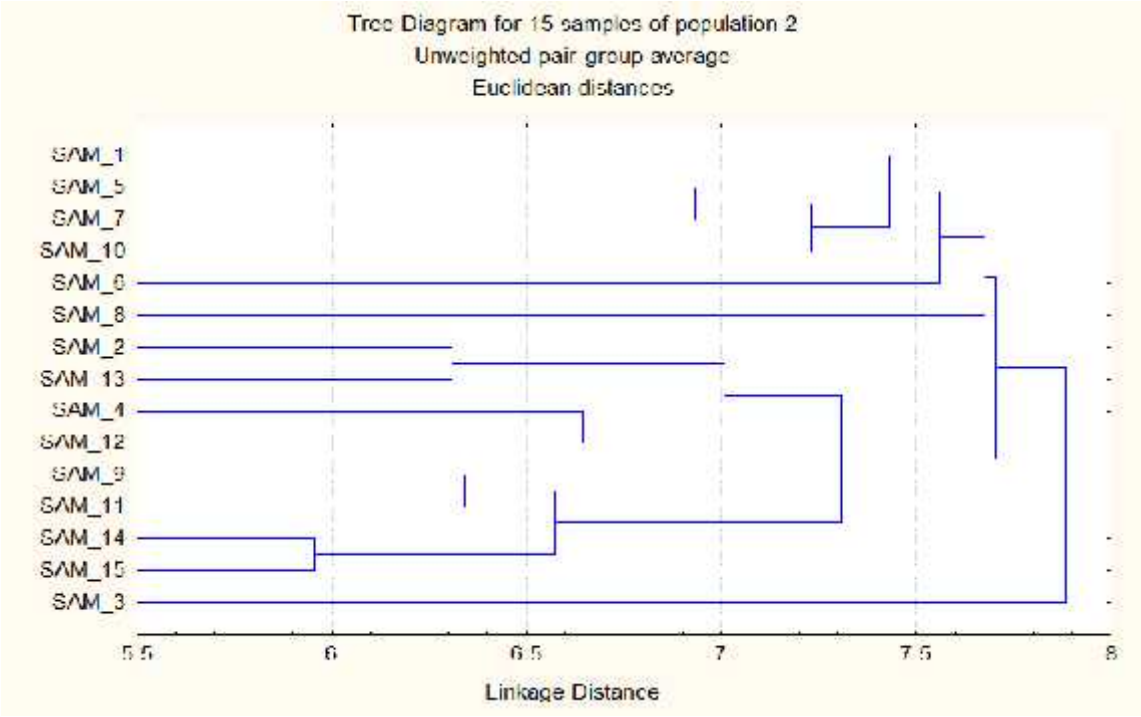

Figure 4.13. Genetic relationships among individuals for P2

4.4.1.2.7. RAPD genotyping of P1 and P2

The results were summarized from the above data of P1 and P2. The results of inter individual pair wise similarity of P1 was observed where 9 different values in number were recorded while high value was found 14 and lowest value was found 2 and average value was recorded 6.46 (Table 4.22). In P2 a total 13 various values in number was recorded where highest and lowest values were observed 11 and 1 respectively while the average value was observed 6.08.

27 and 29 different genetic distance values were found in P1 and P2 respectively whereas highest and lowest values were recorded 0.94 and 0.59 in P1 as well as 0.97 and 0.63 respectively (Table 4.22). The average genetic distances were calculated 0.80 in P1 and 0.816 in P2 respectively.

Table 4.22. Comparison of some genetic parameters of population 1 and population 2

Parameters	P1: TH				P 2: HH			
	Total values in No.	H	L	Av.	Total values in No.	H	L	Av.
Inter individual pair wise similarity indices	9	14	2	6.46	13	11	1	6.08
Genetic distance among individuals	27	0.94	0.59	0.80	29	0.97	0.63	0.816
Nei's genetic similarity among individuals	34	0.58	0.11	0.323	36	0.54	0.06	0.301
Linkage distance	35	6.24	3.6	5.115	18	5.92	4.12	5.141

In this research, average Nei's genetic similarities were originated 0.323 in P1 and 0.301 in P2 respectively from 34 different values in P1 and from 36 different values in P2 (Table 4.22). The highest Nei's genetic similarity was observed 0.58 in P1 and 0.51 in P2 respectively, whereas the lowest Nei's genetic similarity was recorded in P1 and P2 correspondingly 0.11 and 0.06.

A total 35 and 18 different linkage distances were estimated in P1 and P2 respectively whereas the average linkage distances were found 5.115 and 5.141 respectively in P1 and P2. Again the highest and lowest linkage distance was measured 6.24 and 3.6 respectively in P1 as well as 5.92 and 4.12 respectively in P2.

4.4.1.2.8. Allelic information of P 1 & P2 by RAPD assay

In this study genotype, Hardy-Weinberg Equilibrium, genotype frequency, allele frequency and average gene diversity were analyzed in all the primers (Appendix 9c, Appendix 10c, Appendix 11c, Appendix 12c, Appendix 13c, Appendix 14c, Appendix 15c, Appendix 16c, Appendix 17c, Appendix 18c, Appendix 19c, Appendix 20c, Appendix 21c, Appendix 22c, Appendix 23c, Appendix 24c). Average genetic diversity was analyzed based on different primers considering the study of number of locus, p alleles and q alleles (Table 4.23). The frequency of q alleles were recorded highest in both the populations than the frequency of p alleles. Based on the intra-locus gene diversity the average highest gene diversity was observed in P2 in most of the primers, except the primers OPF 14, OPB 19 and OPB 12. However, the average gene diversity was found highest 0.164635264 in P2 and lowest 0.150829501 in P1 (Table 4.23).

Table 4.23. Allelic information of P1 and P2 from RAPD data

Primers	Allele frequency						Average Gene Diversity (Hi)	
	P1			P2			P1	P2
	No. of Locus	p	q	No. of Locus	p	q		
B O3	19	0.066198849	0.933801151	22	0.080146921	0.919853079	0.12023389	0.14273646
OPF 14	18	0.127167783	0.872832217	18	0.090530758	0.909469242	0.20492367	0.15227182
C O4	22	0.098691242	0.901308758	15	0.151975215	0.848024785	0.16625388	0.22938291
OPB 05	21	0.089316068	0.910683932	20	0.094330830	0.905669171	0.1515266	0.16467167
OPB 08	13	0.093709709	0.906290291	19	0.103063399	0.896936601	0.14494615	0.17282057
OPB 19	29	0.063773738	0.936226262	32	0.059804852	0.940195148	0.09673452	0.08831818
OPB 12	18	0.051632741	0.948367259	22	0.046750003	0.953249997	0.11613069	0.10955696
UBC122	8	0.122056699	0.877943301	5	0.164671564	0.835328436	0.20588661	0.25732354
Average	148	0.089068354	0.910931646	153	0.098909193	0.901090807	0.150829501	0.164635264

4.4.1.2.9. Genetic status of 2 populations based on RAPD assay

It was observed that the higher genetic diversity has been recorded in this experimental fish which is indicating the good genetic status of this fish in Bangladeshi nature particularly at the ecological habitats of Sylhet region by the analysis of basic genetic diversity whereas 36 divers values of genetic distances were calculated mentioning highest genetic distance 0.97 and lowest genetic distance 0.59 while the average genetic distance was recorded 0.8173716 (Table 4.10).

According to the genetic diversity based population study, overall higher genetic diversity was recorded 0.80 and 0.816 respectively in both the populations (Table 4.22). However, considering two populations, it was observed that the genetic diversity was little bit stronger in P2 (Hakaluki Haor) where average genetic distance was recorded 0.816 (Table 4.22) and average gene diversity was found 0.164635264 (Table 4.23). On the other hand, comparatively lower genetic status was observed in population 1 (Tanguar Haor) where the genetic distance and average gene diversity was recorded 0.80 and 0.150829501 respectively (Table 4.22 and Table 4.23).

4.4.2. RFLP analysis

4.4.2.1. Genetic diversity by RFLP

4.4.2.1.1. RFLP bands analysis

In this study, PCR products of 30 individuals of freshwater mud eel, *M. cuchia* were digested with enzymes +CfrI and +Hpy178III for RFLP analysis. These two enzymes have a single suitable restriction site to digest the glutamine synthetage gene of *M. cuchia*. These products of the glutamine synthetage gene had an approximate size of 541 bp (Figure 3.24). For practical reasons, 30 individuals of *M. cuchia* were split over three gels by the digestion of enzyme +CfrI (Figure 4.14). One individual DNA was used in the first gel without enzyme as a control to check proper enzyme activities, and this DNA did not cut and was found as a strong band on the gel indicating approximately 541 bp of the glutamine synthetage gene (upper arrow). The middle arrow indicates the larger digested fragment of approximately 280 bp and the lower arrow shows the small digested fragment of around 254 bp lengths of DNA fragments. At the bottom, an unexpected mixed band was observed with primer-dimer and a small undigested fragment of DNA. Thick bands are indicating the different size of the DNA ladder mix (10,000bp) where the marker was started with 250bp. The figure 4.15 shows that enzyme +CfrI detected few of polymorphism within the individuals with two bands, indicating wild type homogygotes, except the individuals of 13, 14, 28, 29 and 30 with a single band indicating the polymorphic homogygote. Bands were calculated and number of total bands revealed from 30 individuals of freshwater mud eel, *M. cuchia* after digestion by +CfrI (Appendix 25).

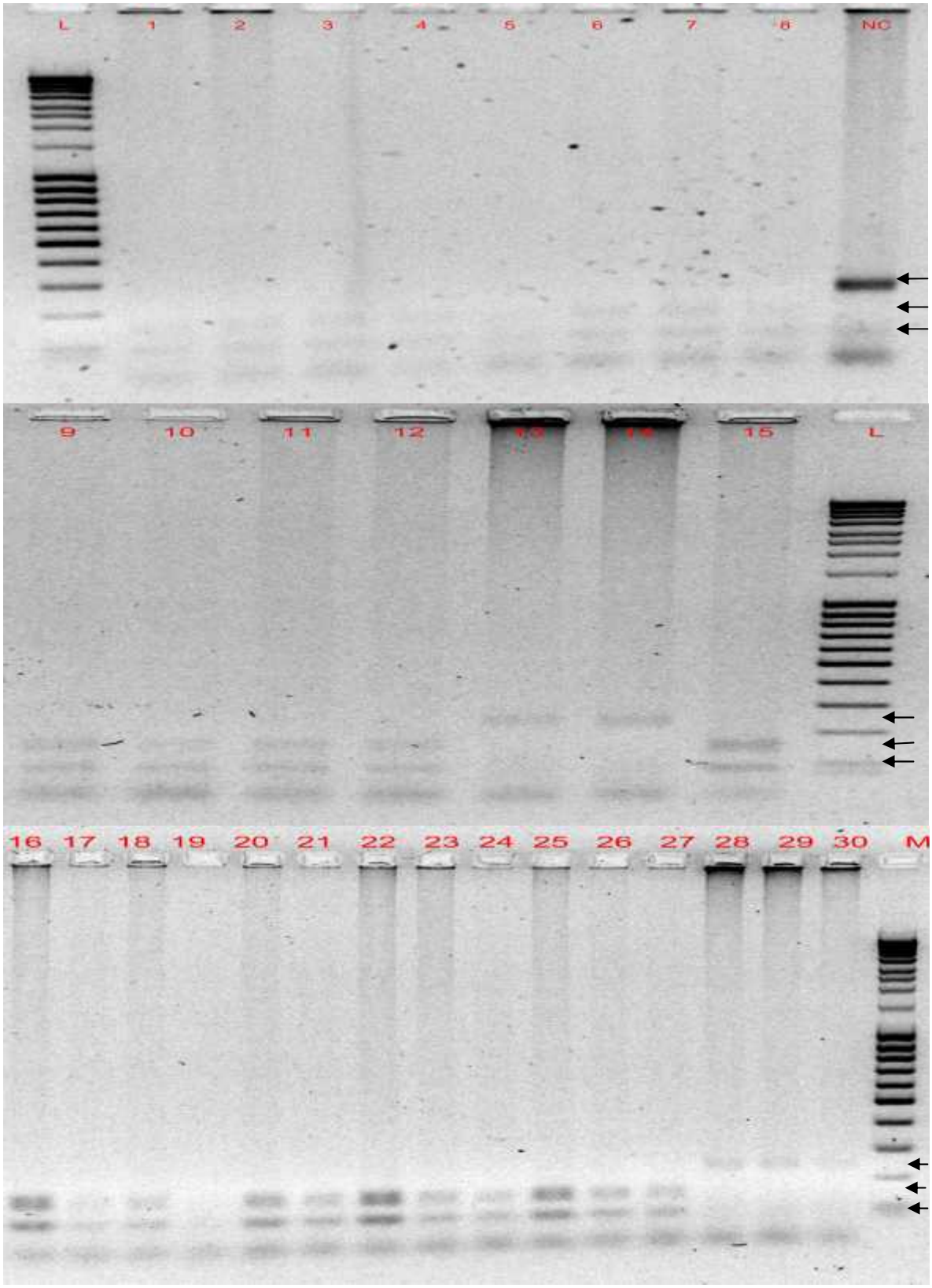

Figure 4.14. PCR-RFLP result of glutamine synthetage gene of *M. cuchia* digested by +CfrI (Upper arrow= ~541bp, Middle arrow= ~280bp, lower arrow= ~254bp length of DNA and L & M= Ladder/Marker started with 250bp)

The same PCR products of the glutamine synthetage gene of an approximately 541 bp length DNA fragment was again used to digest by +Hpy178III through two gels (Figure 4.15). The enzyme +Hpy178III cut all the individuals of first gel with two bands of 343bp and 192bp lengths approximately and it was showed that all the individuals were wild type homogygotes. The second gel show the upper arrow for uncut DNA fragments (541bp), the middle one for the large digested fragment (approximately 343 bp) and the lower one for the small digested fragment (around 192 bp). An unexpected bottom line appeared with a mixture of primer-dimer and a small undigested fragment of DNA. DNA ladder mix (10,000 bp) was used in two gels where the bands were started with 250bp lengths. Enzyme +Hpy178III detected some polymorphism among individuals (Figure 4.16). In second gel, individuals 16, 20, 23 and 26 had not been cut and these individuals might be polymorphic homozygote. Two bands were also found in the individuals of 17, 18, 21 and 28, indicating that these individuals were wild type homozygote. Rest of the individuals of second gel was found heterozygote with three bands and polymorphism was found only these five individuals by the enzyme +Hpy178III. Number of bands was revealed from 30 individuals of freshwater mud eel, after digestion by +Hpy178III (Appendix 25).

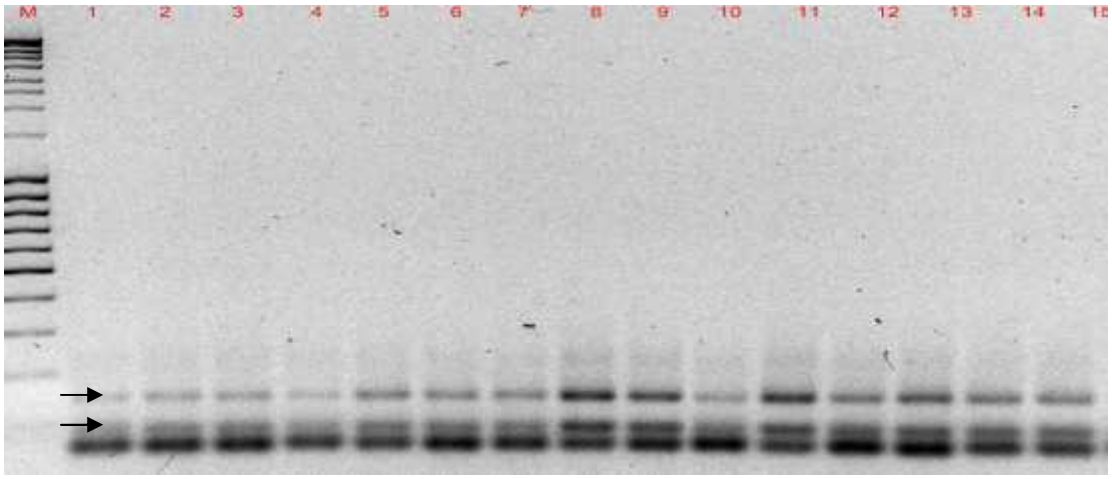

Figure 4.15. PCR-RFLP result of glutamine synthetage gene of *M. cuchia* digested by +Hpy178III (Upper arrow= ~343bp, lower arrow= ~192bp length of DNA and M= Marker, started with 250bp)

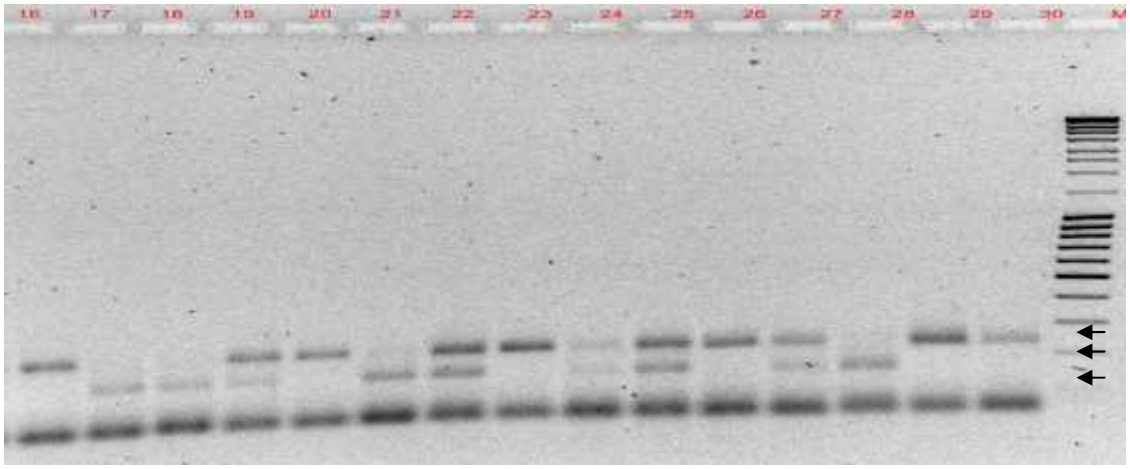

Figure 4.16. PCR-RFLP result of glutamine synthetage gene of *M. cuchia* digested by +Hpy178III (Upper arrow= ~541bp, Middle arrow= ~343bp, lower arrow= ~192bp length of DNA and M= Marker, started with 250bp)

4.4.2.1.2. Observed genetic diversity

Using the number of RFLP bands (appendix 25.), proportions of shared fragments (F-value) were calculated from 30 individuals of freshwater mud eel, and seven different F-values such as 0.00, 0.4, 0.45, 0.5, 0.57, 0.67 and 1 were found (appendix 26). Using the F-values, genetic distances (P-value) were calculated from 30 individuals and seven different P-values were found and the values was 0.00, 0.02, 0.033, 0.04, 0.05, 0.06 and 1 (Table 4.24). On the basis of genetic distances, four different groups of individuals (value=0) were identified. Individuals of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 17, 18 and 21 were found in one group with zero P-value. Another seven individuals (Value=0.033) such as 13, 14, 16, 20, 23, 26 and 28 were recorded in a second group with zero P-value. The third group with five individuals (value=0.05), 19, 22, 24, 25 and 27 were observed also with zero P-value. The final group with two individuals (value=1) such 29 and 30 were found with zero P value. The genetic distance between individuals in group one and group two was found at P-value 0.02

with closely related. Individuals in group two was also at short genetic distance (P-value is 0.04) with individuals of group three. The genetic distance between the individuals of group 3 and 4 was found 0.06 that means individuals of group 4 was far distance from the individuals of group 3 which was larger distance from each other. Individuals of group one with group 3 and 4 were relatively far from each other at a distance of P-value 0.04 and 0.06 respectively. In this study by RFLP of glutamine synthetage gene polymorphism was found.

Table 4.24. Genetic distance of 30 individuals of *Monopterus cuchia* calculated from F value. Formula: $P=1-[0.5(-F+ (F^2+8F)^{0.5})]^{1/r}$ on the diagonale: The amount of bands in each individual

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	4																													
2	0	4																												
3	0	0	4																											
4	0	0	0	4																										
5	0	0	0	0	4																									
6	0	0	0	0	0	4																								
7	0	0	0	0	0	0	4																							
8	0	0	0	0	0	0	0	4																						
9	0	0	0	0	0	0	0	0	4																					
10	0	0	0	0	0	0	0	0	0	4																				
11	0	0	0	0	0	0	0	0	0	0	4																			
12	0	0	0	0	0	0	0	0	0	0	0	4																		
13	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	3																	
14	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.02	3																
15	0	0	0	0	0	0	0	0	0	0	0	0	0.033	0.033	4															
16	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	1	1	0.033	3														
17	0	0	0	0	0	0	0	0	0	0	0	0	0.033	0.033	0	0.033	4													
18	0	0	0	0	0	0	0	0	0	0	0	0	0.033	0.033	0	0.033	0	4												
19	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	1	1	0.05	0.04	0.05	0.05	5											
20	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	1	1	0.033	0	0.033	0.033	0.04	3										
21	0	0	0	0	0	0	0	0	0	0	0	0	0.033	0.033	0	0.033	0	0	0.05	0.033	4									
22	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	1	1	0.05	0.04	0.05	0.05	0	0.04	0.05	5								
23	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	1	1	0.033	0	0.033	0.033	0.04	0.02	0.04	0.04	3							
24	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	1	1	0.05	0.04	0.05	0.05	0	0.04	0.05	0	0.04	5						
25	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	1	1	0.05	0.04	0.05	0.05	0	0.04	0.05	0	0.04	0	5					
26	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	1	1	0.033	0	0.033	0.033	0.04	0.02	0.033	0.04	0.02	0.04	0.04	3				
27	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	1	1	0.05	0.04	0.05	0.05	0	0.04	0.05	0	0.04	0	0	0.04	5			
28	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0	0	0.033	1	0.033	0.033	1	1	0.033	1	1	1	1	1	1	3		
29	1	1	1	1	1	1	1	1	1	1	1	1	0.06	0.06	1	0.06	1	1	1	0.06	1	1	0.06	1	1	0.06	1	0.06	2	
30	1	1	1	1	1	1	1	1	1	1	1	1	0.06	0.06	1	0.06	1	1	1	0.06	1	1	0.06	1	1	0.06	1	0.06	0	2

4.4.2.2. Intra and inter population genetic diversity by RFLP

Intra and inter population genetic diversity of *M. cuchia* was analyzed based on the 541 bp length of glutamine synthetage gene considering 15 individuals of each population. Two populations were chosen considering Tanguar Haor for P1 and Hakaluki Haor for P2 (Figure 3.28 and Figure 3.29). Same DNA like RAPD was used (Figure 3.24 and Figure 3.25) to study genetic diversity of glutamine synthetage gene considering two restriction enzymes such as +CfrI and +Hpy178III where each enzyme has a suitable single cutting site.

4.4.2.2.1. RFLP band analysis of P1

Fifteen individuals were digested by restriction enzyme +CfrI to detect polymorphism among the individuals of P1. The enzyme +CfrI detected less polymorphism within the individuals of P1 where most of the individual cut with two bands, indicating wild type homozygote, excepts the individuals of 13 and 14 with a single band indicating the polymorphic homozygote (Figure 4.17). Number of bands was revealed from P1 of freshwater mud eel which was digested by +CfrI (Appendix 27).

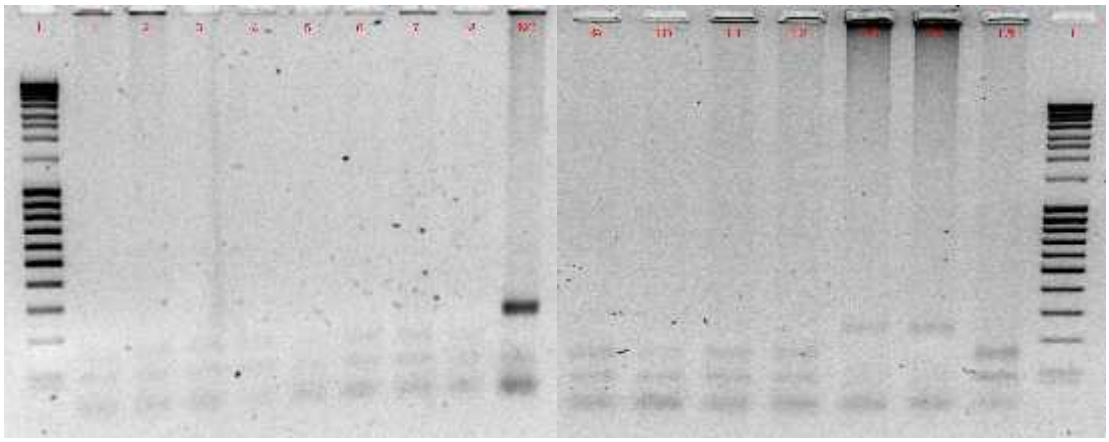

Figure 4.17. Glutamine synthetage digestion by +CfrI in P1 (Upper row= ~541bp, Middle row= ~280bp, Lower row= ~254bp length of DNA, L= Ladder, started with 250bp, NC=Negative control)

The enzyme +Hpy178III detected lack of polymorphism within the individuals of P1 considering same 15 individuals and in this experiment all the individual cut with two bands indicating wild-type homozygote resulting lack of polymorphism was detected (Figure 4.18). Bands were revealed from P1 of freshwater mud eel which was digested by +Hpy178III (Appendix 27).

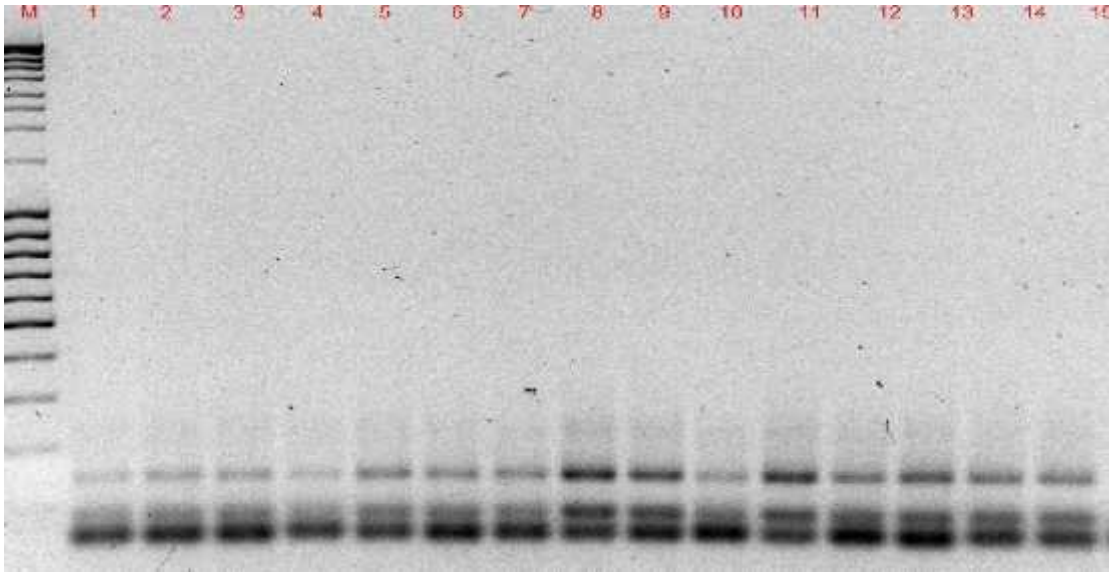

Figure 4.18. Glutamine synthetage digestion by +Hpy178III in P1 (Upper line=~343bp, Lower line=~192bp length of DNA , M= Marker, started with 250bp)

4.4.2.2.2. RFLP band analysis of P2

The enzyme +CfrI detected less polymorphism within the 15 individuals of P2 where two bands were recorded in all the individuals indicating wild type homozygotes, except the individuals of 13, 14 and 15 (Figure 4.19). These three individuals were found with single band indicating the polymorphic homozygote. Number of bands was revealed from P2 which was digested by +CfrI (Appendix 28).

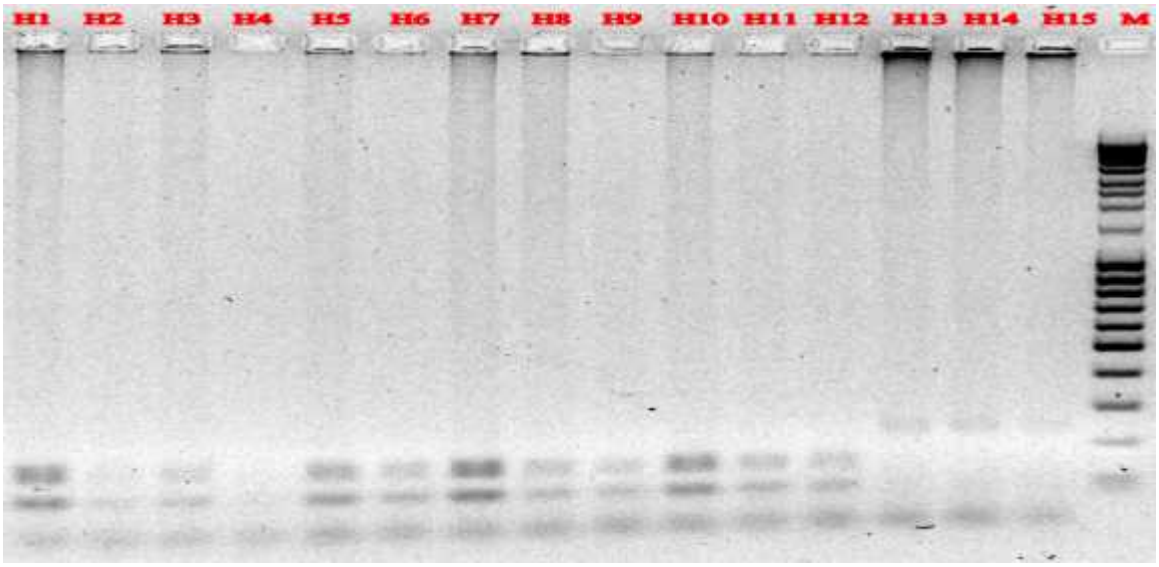

Figure 4.19. Glutamine synthetage digestion by +CfrI in P2 (Upper line= ~541bp, Middle line= ~280bp, Lower line= ~254bp length of DNA, M= Marker, started with 250bp)

Fifteen individuals of P2 of freshwater mud eel were analyzed by the restriction enzyme +Hpy178III where polymorphism was detected in few individuals (Figure 4.20). Uncut or single band was distinguished in the individuals 1, 5, 8, 11, 14 and 15 which were identified as polymorphic homozygote. Wild type homozygote with two bands was observed in the individuals of 2, 3, 6 and 13. Three bands were identified in the individuals of 4, 7, 9, 10 and 12 which were known heterozygote. Bands in number were calculated from P2 which was digested by +Hpy178III (Appendix 28).

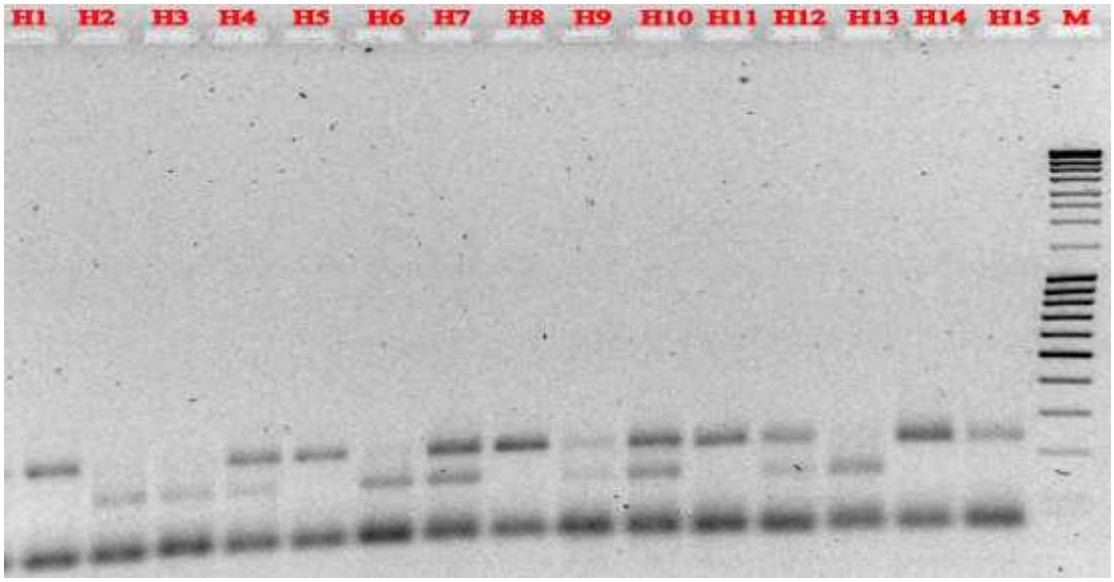

Figure 4.20. Digestion of glutamine synthetage by +Hpy178III in P2 (Upper line= ~ 541bp, Middle line= ~343bp, Lower line= ~192bp length of DNA and M= Marker, started with 250bp)

4.4.2.2.3. Observed genetic diversity among the populations

Based on RFLP bands analysis, proportions of shared fragments (F-value) were calculated among the P1 and P2 of freshwater mud eel. Only three different F-values such as 1, 0.57 and 0.67 were observed in P1 (Appendix 29). The genetic distances were calculated by using three F-values from 15 individuals whereas only three different P-values such as 0, and 0.033 were recorded (Table 4.25). On the basis of genetic distances two groups of individuals with 0 distance values were identified. Most of the individuals were found in one group with zero distance except the individuals 13 and 14 and two groups they were very closely related with 0.02 p value.

Table 4.25. Genetic distance among individuals of P1, calculated from F value. Formula: $P=1-[0.5(-F+ (F^2+8F)^{0.5})]^{1/r}$

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	-														
2	0	-													
3	0	0	-												
4	0	0	0	-											
5	0	0	0	0	-										
6	0	0	0	0	0	-									
7	0	0	0	0	0	0	-								
8	0	0	0	0	0	0	0	-							
9	0	0	0	0	0	0	0	0	-						
10	0	0	0	0	0	0	0	0	0	-					
11	0	0	0	0	0	0	0	0	0	0	-				
12	0	0	0	0	0	0	0	0	0	0	0	-			
13	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	-		
14	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.02	-	
15	0	0	0	0	0	0	0	0	0	0	0	0	0.033	0.033	-

In P2, proportions of shared fragments (F-value) were calculated by analyzing RFLP bands and seven different F-values such as 0, 0.4, 0.45, 0.5, 0.57, 0.67 and 1 were recorded (Appendix 30). The genetic distances were found in P2 by using F-values and different P-values for instance 0.00, 0.02, 0.033, 0.04, 0.05, 0.06 and 1 were originated (Table 4.26). Considering different genetic distances five groups (G) of individuals were revealed with 0 P-values and the observed P-values were 0.00, 0.033, 0.04, 0.06 and 1. In this experiment G1, G2 and G3 were found very close with 0.02 p-values as well as G2 and G4 were found also close. Furthermore, G3 and G4 were also closed with 0.05 P-value. But G4 and 5, G1 and G 3 as well as G1 & G4 were recorded far distance from each other. Finally large genetic distance was recorded between the G1 and other groups.

Table 4.26. Genetic distance among individuals of P2, calculated from F value. Formula: $P=1-[0.5(-F+ (F^2+8F)^{0.5})]^{1/r}$

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1															
2	0.033														
3	0.033	0													
4	0.04	0.05	0.05												
5	0	0.033	0.033	0.04											
6	0.033	0	0	0.05	0.033										
7	0.04	0.05	0.05	0	0.04	0.05									
8	0	0.033	0.033	0.04	0.02	0.04	0.04								
9	0.04	0.05	0.05	0	0.04	0.05	0	0.04							
10	0.04	0.05	0.05	0	0.04	0.05	0	0.04	0						
11	0	0.033	0.033	0.04	0.02	0.033	0.04	0.02	0.04	0.04					
12	0.04	0.05	0.05	0	0.04	0.05	0	0.04	0	0	0.04				
13	1	0.033	0.033	1	1	0.033	1	1	1	1	1	1			
14	0.06	1	1	1	0.06	1	1	0.06	1	1	0.06	1	0.06		
15	0.06	1	1	1	0.06	1	1	0.06	1	1	0.06	1	0.06	0	

4.4.2.2.4. Allelic information for P1 and P2 by PCR-RFLP

Hardy-Weinberg Equilibrium, genotype frequency, allele frequency and intra-locus gene diversity were analyzed by RFLP among the populations considering 2 restriction enzymes (Appendix 31, Appendix 32, Appendix 33 and Appendix 34). Allele frequency was measured by the +Hpy178III in P1 whereas frequency of p and q alleles were found 0 and 1 respectively as well as intra-locus gene diversity was recorded 0 (Table 4.27). Allele frequency of p 0.133 and allele frequency of q 0.867 were observed in P1 as well by the enzyme +CfrI. Intra-locus gene diversity was found 0.231 in P1 by the enzyme +CfrI. On the other hand, the enzymes +Hpy178III and +CfrI revealed the allele frequency of p 0.567 and 0.2 respectively in P2 as well as the allele frequency of q alleles were observed 0.433 and 0.8 respectively. In P2, again intra-locus gene diversity was recorded 0.491 and 0.32 respectively by the enzymes +Hpy178III and +CfrI (Table 4.27). The frequency of q alleles were recorded highest in both the populations than the frequency of p alleles except the enzyme +Hpy178III in P2. However, intra-locus gene diversity was found highest in P2 by both enzymes than P1.

Table 4.27. Allele frequency and intra-locus genetic diversity from RFLP data

Sample	Restriction Enzyme	Allele frequency		Intra-locus gene diversity
		p	q	
P1	+Hpy178III	0	1	0
	+CfrI	0.133	0.867	0.231
P2	+Hpy178III	0.567	0.433	0.491
	+CfrI	0.2	0.8	0.32

4.4.2.2.5. Inter population differentiation

Inter population differentiation was calculated and the result (g_{st} = 0.5299) shows significant differentiation between populations in allele frequencies and it can say that a high percentage of genetic distance was distributed among two populations.

4.4.2.2.6. F-statistics for genetic assortment

F-statistics was analyzed for observing the genetic structure of populations where degree of allele frequency (FIT = 0.7606) and differentiation in allele frequencies between the two populations seems greater (FST = 0.1439), with only a moderate effect of random mating were observed within the populations (FIS = 0.7204) (Appendix 36).

4.4.2.2.7. Genetic status of two populations by PCR-RFLP

Genetic polymorphism was recorded among two populations by the study of RFLP bands, genetic diversity by haplotypes study, allelic informations, inter population differentiation and F-statistics.

4.4.3. Genetic status regarding best population

In this study, RAPD and RFLP based population genetic structure of freshwater mud eel *M. cuchia* in Bangladesh was analyzed. Higher genetic diversity has been recorded in this experimental fish by RAPD assay which is indicating the genetic status of this

fish in Bangladeshi nature by the analysis of genetic diversity (Table 4.10). Considering genetic diversity based population study by RAPD, higher genetic diversity was observed in both the populations (Table 4.22), however, considering the two populations, the genetic diversity was found slightly stronger in population 2 (Hakaluki Haor) than the population 1 (Tanguar Haor) (Table 4.23).

On the other hand, polymorphisms were recorded by RFLP based genetic diversity analysis of partial sequence of glutamine synthetage gene (Table 4.24) while both the enzymes had single cutting point. However, intra-locus gene diversity was found highest in P2 by both enzymes than P1 by analyzing the Hardy-Weinberg Equilibrium, genotype frequency and allele frequency (Table 4.27).

In both cases of RAPD and RFLP higher genetic diversity were observed in general as well as at population level, whereas P2 was recorded stronger genetic status than P1.

Table 4.4. Measurement of gonad and ova diameter

Study option	Male Fish		Female Fish		Testis				Ovary		No. of eggs	Egg Diameter (mm)	
					With sperm duct		Without sperm duct						
	Wt (g)	Lt (cm)	Wt (g)	Lt (cm)	Wt (g)	Lt (cm)	Wt (g)	Lt (cm)	Wt (g)	Lt (cm)		Range	Mean
Feb15-Mar14	90-280	41-70	150-500	50-83	0.5-1.9	11-16	0.35-1.60	4.5-7.2	2.4-6.75	6-10.5	280-621	0.3-2.2	1.075
Mar15-Apr14	90-250	40-75	170-300	65-77	1.4-3.5	12-16	1-2.4	4-7	4.4-11	6-7.6	203-603	0.4-3.2	1.805
Apr15-May14	120-280	43-78	150-400	55-82	3-4.4	13.2-16	1.8-3.2	4.25-7.2	6.1-17.6	6-8	203-742	0.5-4	2.5
May15-June14	180-330	55-80	220-400	60-82	4.6-6.7	12.7-16.5	2.4-4.2	5.75-8.75	9.8-24.5	8-12	385-1495	0.5-4.3	2.49
Treated fish	90-550	44-82	140-520	52-90	2.1-8.1	12-20	1.45-4.7	5-8.2	6.9-20.4	5.9-11.3	155-998	0.5-4	2.195

(Wt = Weight, Lt = Length)

Table 4.9. Inter individual pair wise similarity indices

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	-	6	5	7	4	4	4	3	3	3	4	6	4	4	4	5	5	6	6	6	8	2	3	3	4	5	5	4	4	5
2		-	9	7	5	5	7	7	7	6	8	5	8	3	4	10	8	11	6	5	9	7	7	4	7	6	8	8	6	11
3			-	7	5	6	7	6	4	2	5	5	5	3	5	9	5	7	6	5	6	5	4	4	6	4	6	7	5	4
4				-	4	6	5	6	5	4	5	7	3	5	4	9	3	7	6	4	8	3	5	4	6	5	4	5	4	5
5					-	8	8	5	4	8	6	6	6	5	5	6	3	4	6	5	5	2	4	1	5	2	5	4	1	3
6						-	9	4	6	7	7	8	8	8	6	9	3	2	5	5	5	3	7	1	8	4	6	5	3	4
7							-	12	8	9	14	9	9	5	8	14	6	6	6	10	5	6	5	4	9	3	9	7	4	4
8								-	9	6	11	11	5	5	6	9	8	6	3	5	3	6	3	2	5	3	6	8	4	7
9									-	8	11	8	6	9	7	6	5	4	6	3	5	6	6	2	6	3	6	6	5	7
10										-	11	8	9	9	9	4	3	3	7	8	5	5	5	1	6	3	4	4	2	4
11											-	9	7	7	10	9	6	6	6	12	4	5	6	2	8	6	8	6	5	7
12												-	9	9	8	8	4	6	6	6	4	6	6	2	8	7	7	8	5	6
13													-	4	7	7	7	4	6	6	5	5	6	3	7	6	8	8	6	7
14														-	6	6	4	3	7	4	5	2	7	1	10	5	5	4	3	4
15															-	4	4	3	6	6	5	4	4	2	7	3	6	4	2	5
16																-	6	9	7	9	6	4	7	3	10	4	9	8	3	5
17																	-	9	7	4	4	1	4	4	5	4	8	10	5	11
18																		-	5	5	4	3	4	6	6	3	9	9	5	9
19																			-	7	6	5	6	2	9	4	10	8	4	7
20																				-	7	5	4	5	7	6	8	5	4	4
21																					-	6	5	5	7	5	5	8	3	4
22																						-	5	4	8	5	5	4	3	2
23																							-	4	9	7	6	7	5	6
24																								-	5	5	5	5	5	5
25																									-	8	8	7	7	8
26																										-	8	6	7	10
27																											-	10	8	11
28																												-	5	10
29																													-	10
30																														-

Table 4.10. **Genetic distance among individuals of *M. cuchia***

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	0	0.83	0.80	0.67	0.85	0.84	0.88	0.91	0.90	0.90	0.88	0.82	0.86	0.87	0.86	0.84	0.82	0.81	0.78	0.77	0.70	0.93	0.91	0.87	0.88	0.81	0.84	0.88	0.85	0.84
2		0	0.74	0.79	0.87	0.86	0.84	0.84	0.83	0.85	0.81	0.90	0.79	0.93	0.90	0.75	0.79	0.72	0.85	0.88	0.77	0.81	0.83	0.89	0.84	0.84	0.80	0.80	0.84	0.71
3			0	0.70	0.81	0.76	0.79	0.82	0.88	0.94	0.86	0.86	0.83	0.91	0.83	0.70	0.83	0.78	0.79	0.83	0.81	0.82	0.88	0.83	0.82	0.86	0.81	0.77	0.82	0.88
4				0	0.85	0.74	0.85	0.81	0.83	0.87	0.85	0.79	0.90	0.83	0.86	0.68	0.90	0.77	0.78	0.86	0.70	0.89	0.83	0.82	0.81	0.81	0.88	0.84	0.85	0.84
5					0	0.65	0.75	0.86	0.88	0.71	0.82	0.83	0.79	0.84	0.83	0.82	0.91	0.89	0.79	0.83	0.84	0.94	0.88	**0.96**	0.85	0.94	0.85	0.88	0.97	0.91
6						0	0.70	0.89	0.79	0.75	0.78	0.76	0.69	0.71	0.79	0.69	0.90	0.94	0.83	0.82	0.84	0.90	0.76	**0.96**	0.73	0.86	0.81	0.84	0.90	0.88
7							0	0.67	0.78	0.74	**0.59**	0.78	0.74	0.88	0.77	**0.58**	0.84	0.85	0.84	0.69	0.88	0.83	0.88	0.88	0.76	0.92	0.76	0.82	0.89	0.90
8								0	0.74	0.84	0.70	0.72	0.87	0.88	0.84	0.76	0.77	0.85	0.93	0.86	0.93	0.83	0.93	0.94	0.88	0.92	0.85	0.79	0.89	0.82
9									0	0.75	0.67	0.79	0.82	0.72	0.78	0.84	0.85	0.90	0.82	0.91	0.86	0.81	0.83	0.93	0.84	0.91	0.83	0.83	0.84	0.80
10										0	0.67	0.79	0.70	0.72	0.70	0.90	0.92	0.93	0.78	0.73	0.86	0.84	0.86	**0.97**	0.84	0.91	0.89	0.89	0.94	0.89
11											0	0.78	0.81	0.82	0.70	0.76	0.84	0.85	0.84	**0.60**	0.90	0.86	0.85	0.94	0.79	0.83	0.79	0.85	0.86	0.82
12												0	0.74	0.75	0.77	0.79	0.90	0.85	0.84	0.83	0.90	0.83	0.85	0.94	0.79	0.79	0.82	0.79	0.86	0.85
13													0	0.89	0.77	0.80	0.77	0.89	0.81	0.81	0.86	0.84	0.82	0.89	0.80	0.80	0.76	0.76	0.80	0.79
14														0	0.82	0.84	0.89	0.93	0.79	0.89	0.86	0.94	0.80	**0.97**	0.71	0.85	0.87	0.90	0.91	0.90
15															0	0.89	0.88	0.92	0.81	0.81	0.86	0.88	0.89	0.93	0.80	0.91	0.83	0.89	0.94	0.86
16																0	0.83	0.76	0.80	0.72	0.84	0.89	0.81	0.91	0.72	0.89	0.75	0.78	0.92	0.88
17																	0	0.73	0.77	0.88	0.89	**0.97**	0.89	0.85	0.86	0.88	0.76	0.68	0.84	0.63
18																		0	0.86	0.86	0.90	0.92	0.90	0.79	0.85	0.92	0.75	0.75	0.86	0.75
19																			0	0.77	0.82	0.84	0.82	0.93	0.73	0.88	0.68	0.76	0.88	0.79
20																				0	0.78	0.83	0.89	0.80	0.79	0.79	0.75	0.86	0.87	0.89
21																					0	0.81	0.86	0.82	0.81	0.85	0.87	0.77	0.91	0.90
22																						0	0.85	0.84	0.75	0.83	0.85	0.89	0.90	0.95
23																							0	0.86	0.74	0.77	0.84	0.81	0.85	0.84
24																								0	0.83	0.79	0.83	0.83	0.79	0.83
25																									0	0.75	0.78	0.82	0.79	0.78
26																										0	0.74	0.82	0.74	0.66
27																											0	0.71	0.74	0.67
28																												0	0.85	0.71
29																													0	0.66
30																														0

Table 4.11. Nei's genetic similarity among individuals

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	0	0.29	0.33	0.50	0.27	0.28	0.21	0.16	0.18	0.18	0.21	0.30	0.24	0.23	0.24	0.27	0.30	0.32	0.36	0.38	0.46	0.13	0.17	0.23	0.22	0.32	0.28	0.22	0.26	0.28
2		0	0.42	0.34	0.23	0.24	0.27	0.27	0.30	0.26	0.31	0.19	0.35	0.13	0.17	0.40	0.35	0.44	0.26	0.22	0.38	0.32	0.29	0.21	0.28	0.27	0.33	0.33	0.27	0.45
3			0	0.47	0.31	0.39	0.35	0.30	0.22	**0.11**	0.25	0.24	0.29	0.16	0.29	0.46	0.29	0.36	0.34	0.29	0.32	0.30	0.22	0.29	0.31	0.24	0.32	0.37	0.30	0.21
4				0	0.27	0.41	0.26	0.32	0.29	0.24	0.26	0.35	0.18	0.29	0.24	0.49	0.18	0.38	0.36	0.25	0.46	0.19	0.29	0.31	0.32	0.32	0.22	0.28	0.26	0.28
5					0	0.52	0.40	0.25	0.22	0.44	0.30	0.29	0.34	0.27	0.29	0.31	0.17	0.21	0.34	0.29	0.27	0.12	0.22	**0.07**	0.26	0.12	0.26	0.21	**0.06**	0.16
6						0	0.46	0.21	0.34	0.40	0.36	0.39	0.47	0.44	0.35	0.47	0.18	**0.11**	0.29	0.30	0.28	0.19	0.39	**0.07**	0.42	0.25	0.32	0.27	0.19	0.22
7							0	0.50	0.36	0.41	0.58	0.36	0.42	0.22	0.37	**0.60**	0.28	0.26	0.28	0.48	0.22	0.29	0.22	0.22	0.38	0.15	0.39	0.30	0.20	0.17
8								0	0.41	0.27	0.46	0.44	0.23	0.22	0.28	0.38	0.37	0.26	0.14	0.24	0.13	0.29	0.13	0.11	0.21	0.15	0.26	0.35	0.20	0.30
9									0	0.40	0.50	0.35	0.31	0.44	0.36	0.28	0.26	0.19	0.31	0.16	0.24	0.32	0.29	0.13	0.28	0.16	0.29	0.29	0.27	0.33
10										0	0.50	0.35	0.46	0.44	0.46	0.19	0.15	0.14	0.36	0.42	0.24	0.27	0.24	**0.06**	0.28	0.16	0.19	0.19	0.11	0.19
11											0	0.36	0.33	0.31	0.47	0.38	0.28	0.26	0.28	0.57	0.18	0.24	0.27	0.11	0.34	0.29	0.35	0.26	0.24	0.30
12												0	0.40	0.38	0.36	0.33	0.18	0.24	0.27	0.27	0.17	0.28	0.26	0.11	0.33	0.33	0.29	0.33	0.23	0.25
13													0	0.37	0.30	0.27	0.20	0.14	0.35	0.21	0.24	0.11	0.33	**0.06**	0.45	0.26	0.23	0.19	0.16	0.19
14														0	0.18	0.19	0.21	0.14	0.32	0.32	0.25	0.22	0.20	0.13	0.33	0.17	0.29	0.20	0.11	0.24
15															0	0.21	0.29	0.39	0.33	0.44	0.27	0.20	0.32	0.17	0.43	0.20	0.40	0.36	0.15	0.22
16																0	0.27	0.43	0.37	0.22	0.20	**0.06**	0.20	0.26	0.24	0.22	0.39	0.49	0.28	0.54
17																	0	0.29	0.24	0.24	0.18	0.15	0.18	0.34	0.26	0.15	0.40	0.40	0.25	0.40
18																		0	0.31	0.38	0.30	0.28	0.30	0.13	0.43	0.22	0.49	0.39	0.22	0.34
19																			0	0.38	0.36	0.29	0.21	0.33	0.34	0.34	0.40	0.25	0.23	0.20
20																				0	0.39	0.32	0.24	0.30	0.32	0.26	0.23	0.37	0.16	0.19
21																					0	0.26	0.26	0.28	0.40	0.29	0.26	0.21	0.18	0.10
22																						0	0.21	0.24	0.41	0.37	0.28	0.33	0.26	0.28
23																							0	0.29	0.29	0.34	0.29	0.29	0.34	0.29
24																								0	0.28	0.40	0.36	0.31	0.35	0.36
25																									0	0.26	0.41	0.31	0.41	0.51
26																										0	0.27	0.45	0.41	0.50
27																											0	0.19	0.26	0.45
28																												0	0.15	0.51
29																													0	0.17
30																														0

Table 4.12. Squared Euclidean distances

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	8	29	30
1	0	5.4	4.2	3.7	4.7	4.6	5.5	5.7	5.3	5.3	5.5	5.1	4.8	5.2	4.8	4.58	4.8	5	4.8	4.47	4.36	5.2	5.2	4.24	5.39	4.58	5.1	5.29	5	5.29
2		0	5	5.2	5.6	5.5	6.1	5.9	5.7	5.7	5.7	**6.24**	5.48	6	6	5.48	5.29	4.9	5.66	5.92	5.48	5.48	5.83	5.57	6	5.83	5.74	5.74	5.29	5.2
3			0	4	4.7	4.4	5.1	5.3	5.3	5.7	5.3	5.66	5	5.39	5	4.36	5.2	5	4.8	4.9	5	5	5.39	4.69	5	5	4.9	4.9	4.8	5.48
4				0	4.7	4.1	5.1	5.1	4.9	5.1	5.3	5.1	5	4.8	5	4.58	5.2	4.8	4.58	4.9	4.58	5	5	4.24	5	4.58	5.29	5.1	5	5.29
5					0	**3.6**	4.9	5.5	5.1	4.5	5.1	5.48	4.36	5.2	4.8	4.8	5.39	5.57	4.36	4.69	5.2	5.39	5.39	5.1	5.39	5.2	5.29	5.48	5.39	5.66
6						0	4.6	5.6	4.6	4.6	5	5	4.24	4.47	4.69	4.24	5.29	5.83	4.47	4.8	5.1	5.1	4.69	5	4.69	4.9	5	5.2	5.1	5.74
7							0	4.9	5.1	5.1	4.5	5.66	5.2	5.74	5.2	4.36	5.57	5.92	5.2	4.69	5.92	5.39	5.92	5.48	5.39	5.92	5.29	5.66	5.74	6.16
8								0	5.1	5.7	4.9	5.29	5.57	5.92	5.39	5.39	5.2	5.74	5.57	5.83	**6.24**	5.39	**6.24**	5.66	5.92	6.08	5.83	5.48	5.74	5.66
9									0	4.9	4.7	5.48	5.2	4.8	5	5.57	5.39	5.92	5.2	5.66	5.57	5.2	5.39	5.29	5.57	5.57	5.48	5.29	5.2	5.1
10										0	4.7	5.48	4.58	4.8	4.58	5.92	5.57	6.08	4.8	4.9	5.57	5.39	5.57	5.48	5.57	5.57	5.66	5.83	5.74	5.83
11											0	5.66	5.39	5.57	4.8	5.39	5.57	5.92	5.39	4.24	6.08	5.57	5.74	5.66	5.57	5.39	5.48	5.83	5.57	5.66
12												0	5.2	5.39	5.39	5.74	6.08	6.08	5.57	5.66	5.92	5.57	5.92	5.83	5.74	5.57	6	5.83	5.74	6.16
13													0	5.66	4.9	5.29	4.9	5.66	4.9	5	5.29	5.1	5.1	4.8	5.29	4.9	5	5	4.9	5.2
14														0	5.29	5.48	5.66	6.16	4.9	5.57	5.48	5.83	4.9	5.57	4.9	5.29	5.74	5.57	5.66	5.74
15															0	5.48	5.48	5.83	5.1	5	5.48	5.29	5.66	5	5.29	5.48	5.39	5.74	5.48	5.57
16																0	5.29	5.29	4.9	4.8	5.48	5.48	5.48	5.39	5.1	5.48	5.2	5	5.83	5.92
17																	0	4.9	4.9	5.39	5.48	5.83	5.66	4.8	5.48	5.1	4.8	4.36	4.9	4.36
18																		0	5.66	5.57	5.48	5.66	5.83	4.8	5.66	5.66	5.2	5.2	5.29	5.39
19																			0	4.8	5.1	5.1	5.29	5.2	4.9	5.1	4.58	4.8	5.29	5.39
20																				0	5	4.8	5.57	4.69	5	4.58	4.9	5.29	5.2	5.48
21																					0	5.1	5.29	4.58	5.48	5.29	5.74	5.2	5.66	5.92
22																						0	5.29	4.58	5.1	5.29	5.57	5	5.1	5.74
23																							0	5	5.1	4.69	5.57	5.2	5.29	5.39
24																								0	4.8	4.36	4.9	4.9	4.36	4.69
25																									0	4.69	5.39	5.57	4.9	5.39
26																										0	4.8	5.2	4.47	4.36
27																											0	4.9	4.8	4.69
28																												0	5.39	4.9
29																													0	4.12
30																														0

In this study, some sequential observation has been performed including culture, breeding biology, artificial breeding of freshwater mud eel, *Monopterus cuchia*. Due to any responds was not found with inducing agents it is necessary to study genetic research and the main target of this research was genetic diversity based population studies of freshwater mud eel, *M. cuchia* in Bangladesh for finding a superior population with high genetic diversity where genetic diversity is a raw material for breeding program.

5.1. Rearing and production

The present study was conducted to determine the suitable environment for culture and sustainable production of freshwater mud eel, *M. cuchia*. Considering different environments such as house tanks, earthen ditches, plastic tank and back yard tanks growth performance was recorded highest in earthen ditch-1 in terms of weight gain (in length 17.78 ± 0.24 and in weight 85.07 ± 0.15) and survival (92.5%). Conversely, growth performance in terms of weight gain (in length 11.86 ± 0.26 and in weigh 41.84 ± 0.59) and survival (75%) was lowest in house tank-2. The supplied feed in earthen ditch-1, were earth worms, snails, small dead fish and small frogs. Besides, in house tank-2, chicken viscera, small frogs, snails and small dead fish were supplied. The production performance in the present study was quite satisfactory after six months observation. The second highest growth performance in terms of weight gain was in house tank-1 (in length 15.38 ± 0.13 and in weight 71.15 ± 1.86) whereas supplied feed were given earth worms, small dead fish, small frogs, small live fish (Tilapia fry) and snails. The highest growth 310.63±17.59g was found as weight individually in rice field culture in five month of experiment (Chakraborty *et al.* 2010)

this is differ from the present findings. Although, the environmental conditions were same but different amount of supplied feed has found different impacts on growth performance of fishes from tank-1, 2 and 3. In the present study, it was observed that the earthworms and small live fish (tilapia) were the best among all other supplied foods for better growth performance of this freshwater mud eel, *M. cuchia*. As soon as the feed were supplied they engulfed it quickly. Narejo *et al*. (2003a) reported that this fish which were cultured in cemented cisterns with dead fish and found well growth, however, this study completely disagrees with some previous findings in freshwater mud eel as well as other eel fish (Nasar 1997, Narejo *et al*. 2003a, Golani *et al.* 1988 and Usui 1974) while this study found that earth worms and small live fish (Tilapia fry) were effective for better growth performance of *M. cuchia*. On the other hand, this findings with mostly related with findings of a previous research considering the same types of foods use (Chakraborty *et. al*. 2010).

For better growth and development optimum temperature is a key factor. In this study, increasing weight gain was found during June to November, 2013 with optimum water temperature was 19-31℃, suitable for freshwater eel, *M. cuchia* and similar result was found by Narejo *et.al*. (2003a). Nasar (1997) reported an ideal temperature of 20-35℃ for proper feeding and growth of *M. cuchia*. Usui (1974) reported that below a temperature of 12℃ *A. japonica, A. anguilla and A. rostrata* do not feed and thus do not grow at all. In the present study, the increasing trend of mean growth (final length and weight) was found in earthen ditch-1. Insignificant correlations were observed between the growth parameter in all environments. In this study, paired t-test gives significant values at 95% significant level (P=0.000) in almost all the environments and highly significant result was found in backyard tank-1 (P=0.009). It

may be due to the effect of surroundings. The survival rate of fish fed with small live fish (Tilapia fry), earthworms, frogs, chicken viscera, snails and small dead fish ranged from 75% to 92.5% while highest survival rate was in earthen ditch-1 and lowest in house tank-2. Nahar *et al*. (2012) and Narejo *et al*. (2002) reported quite different survival rate (83%) in *C. gariepinus* and (70%) in snake eel, *P. boro* respectively when fed with dead small fish.

The total production of fish ranged from 2.1 kg to 4.07kg by 6 months of this experiment where Narejo *et al*. (2003a) found the total production of fish ranged from 0.241 to 0.624 kg/m2/year. Besides, highest production of fish was in earthen ditch-1 (4.07 kg) might be due to greater survival rate and lowest production was in house tank-2 (2.1 kg). The second highest production was in house tank-1 (3.3 kg) followed by earthen ditch 2 (3.2 kg). In case of earthen ditch-1, survival rate, growth performance and production were higher than the other environments. In earthen ditches there was enough space for mud eel to move around and hide. This natural environment allows mud eel to mature better than the others. In breeding house, three tanks were in same environmental conditions, but, different foods in different amounts cause variation in growth performance and production of the three tanks. The production from plastic tank is quite well (2.87 kg) while the food such as live small fish (Tilapia fry), earth worms and small frog were given in plastic tank. In backyard tank 1 and 2 the amount of given food were same. So, growth performance was nearly 2.16 kg and 2.12 kg respectively. It was clear from the present study that mud eel shows its best growth performance and production in natural environment.

5.2. Breeding biology and artificial breeding

Sexual differentiation of different commercial fishes even though some other eels such as *Anguilla Reinhardtii* (Berumer 1979), *A. anguilla* (Sinha, and Jones 1967) and *Monopterous albus* (Huang *et al*. 2008) were observed traditionally but no record was available for this *M. cuchia*. In this experiment male and female *M. cuchia* was separated by external, internal and histological characteristics. As *M. albus* is recorded hermaphrodite where bisexual characteristic was found in *M. cuchia* through the present study. Sex-specific growth trajectories in freshwater eels are well documented by different above researches. The prevailing view is that females grow faster than males (Vøllestad and Jonsson 1986, Poole and Reynolds 1996, Holmgren et al. 1997, Oliver 1997, Oliveira and McCleave 2002) while similar results were found also in this experiment in *M. cuchia*.

In the present study the experiment was described for the observation of breeding biology and induced breeding of freshwater mud eel, *M. cuchia* in cemented tanks, hapa and in the laboratory condition. The freshwater mud eel, *M. cuchia* (Hamilton 1822) is a rare species of air breathing teleostean fish in Bangladesh and India with spawning period confined down to peak summer (Khan 2008). Biological and reproductive aspects in synbranchids are also poorly studied, except by a few papers dealing with aspects of reproductive biology, larval development and gas exchange in a few species. Observation of breeding habits and larval development were also provided for the Asian synbranchid *M. albus* (Wu and Liu 1942) and *M. cuchia* (Banerji *et al.* 1981). It was observed on that the reproductive biology of air-breathing freshwater mud eel, *M. cuchia* from Bangladesh by Singh *et al.* (1989) and Narejo *et*

al (2003c) where the ova diameter was found between 0.3 mm to 4 mm but in the present study almost same result on the ova diameter was found with the range of 0.3 mm to 4.3 mm. In the present study the average ova diameter was recorded 1.07, 1.81, 2.5 and 2.49 but in case of hormonal induced fish the egg diameter was comparatively lower (2.19) than the same duration of non- injected fish (2.5). It has shown that the egg diameter was gradually increased during the breeding season of freshwater mud eel, *M cuchia.* Eggs were spherical and uniform in diameter. Similar findings were also reported by Nabi and Hossain (1996) in *Macrognathus aculeatus* and Suresh *et al*. (2006) in *Macrognathus pancalus.* The peak of Gonadosomatic Index (GSI) recorded during April to June for female (7.52 ± 1.15) and male (5.50 ± 1.25) by Narejo *et al*. (2003c) but in present study the peaks of Gonadosomatic Index (GSI) was recorded differ during February to June for female (5.1 ±1.09) and male (2.14±0.2). In case of spiny eel, *Macrognathus aral*, the peak value of GSI attained during May for male (1.3) and August for female (12.4) (Abujam and Biswas 2011), which was far from the present findings. In the present study it was also found that the mean of GSI for the Induced fish was higher in case of male both in 5 days (2.32 ± 0.71) and 10 days (2.86 ± 0.57) experiment than the control fish (2.26 ± 0.52) while in case of female the GSI of the control fish (4.26 ± 0.65) was higher than a 5 day treated fish (4.1 ± 0.31) but lower than a 10 day treated fish (4.74 ± 0.65). Therefore, it may be said that the induced hormone influence the gonad development.

Estimation of body length, body weight and fecundity are the prerequisite in successful breeding program. Bagenal and Braum (1978) had reported that fecundity in fish species characteristically varied among individually of the same size and age and the present findings are agreed with this statement. Fagede *et al*. had also

suggested that fecundity variation may be due to differential food (Fagade *et al*. 1984). In the present study the individual fecundity recorded from 1495 (65 cm / 310g) to 155 (240 cm / 69g) while relative fecundity ranged from 2.64 to 12.45 but for this air-breathing freshwater mud eel, individual fecundity recorded from 5480 (88.5cm /650g) to 260 (41.8cm / 88g) by Narejo *et al*. (2003c). Nasar reported the fecundity of *M. cuchia* ranging from 118-687 eggs (1989) but in present study it was found much higher than that. The fecundity also varied with the seasons, climate conditions and environmental habitat, nutritional status and genetic potential (Bromage *et al*. 1992). It was observed during the present study, the same length and weight fish does not possess same amounts of eggs. The result of induced breeding was successfully observed in different eel fish such as *M. albus, Angilla anguilla, Anguilla japonica*, etc. Though rice field eel, *M. albus* were spawned by the injection of HCG (1000, 1500 and 2000 UI/ kg fish) and LH-RHa (50, 100 and 150 µg / kg fish) however, the spawning rate was found highest (75%) in case of 150 µg LHRHa / kg fish and 2000 UI HCG / kg fish by Huong *et al*. (2008). Also, it was found that the *M. albus* can breed in captivity without using chemical stimuli, natural reproduction is considered the most optimum solution (IIRR 2001), but the success rate was very low and it is not commercially usable. In case of *M. cuchia*, induced breeding was observed and found unwilling to spawn by hormonal treatments through different doses of inducing agents and same result was found without using chemical stimuli in captive condition. Same results were also found by Shuvra (2011) where spawning activities were not seen by inducing agents. Artificial propagation of eels has long been attempted not only to address fundamental questions on the reproductive biology of these fish, but also from a fisheries management and eel aquaculture perspective. Increasing fishing pressures on eels have led to reduced recruitment of juveniles of

several anguillid species, limiting the industry and potentially leading to ecological impacts. Despite these research efforts, however, captive breeding of eels has not yet been achieved. Sexually development in freshwater eels is halted when these fish are held in captivity (Dufour *et al*. 1988), but this arrest can be overridden by hormone treatment. Same results were found in this experiment when PG, HCG, GnRH and ovulin were used in captive condition. Boethius and Boethius (1976) were able to mature males of European eel and the species was injected weekly with carp pituitary and maintaining them in sea water at a temperature of about 14°C. Several other workers have subsequently succeeded in maturing the male, as well as obtaining the release of mature eggs from females, but artificial fertilization of the European eel has not been succeeded. Same results were found in this experiment where induced breeding was not succeeded. But, The Japanese eels *Anguilla japonica* have been stimulated to spawn by hormonal treatments and by keeping the brood fish in sea water at a temperature of about 23°C (Yamamoto *et al*. 1975), but the larvae could be reared only until the sixth day. Although induced breeding was not performed successfully in freshwater mud eel, *M. cuchia*, however, successful results were recorded for induction of vitallogenesis in female European eels. But in the present study eggs and oviduct tissues were found damage in almost all the induced female fish.

5.3. Molecular species identification

Efficient identification of this eel species is critical for aquaculture management as well as for eel conservation particularly in Bangladesh (Dudu 2010). Thus, identification of *M. cuchia* needs to be supported by molecular characterization

instead of conventional methods (Huang *et al*. 2001). Inexpensive, simple, rapid PCR based techniques mitochondrial 16s ribosomal RNA could be used to identify *M. cuchia* which is already designed a universal primer for species identification (Kitano *et al*. 2007). 16s mitochondrial DNA is a universal primer which can successfully amplify the expected PCR products from various kinds of vertebrates including mammals, birds, reptiles, amphibians, fish, etc, and the sequenced segments contained sufficient nucleotide difference to identify each animal species (Kitano *et al*. 2007). Different molecular techniques already established to identify freshwater eels such as RFLP analyses of PCR amplified DNA, fragments and allele-specific PCR from mitochondrial DNA (Lin *et al*. 2002). 12S rRNA, 16S rRNA, D-loop and cytochrome b genes are generally used for fish identification. Yang *et al*. (2014) identified 11 species of animals including *M. albus* through mitochondrial 12S and 16S ribosomal RNA genetic analysis. The phylogenetic analysis of cytochrome C oxidase subunit I (COI) were observed from seven freshwater eels to identify the molecular differences of *M. cuchia* and *M. albus* (Devi *et al*. 2014). In this research successfully amplify 16S rRNA gene and find all this individuals at the same length of DNA with 250 bp long. 541 bp long partial sequence of glutamine synthestase gene was expressed in *M. cuchia* (Saha *et al*. 2010) which was used in this study for the identification of this fish. Among two primers glutamine synthetase contains GC content higher than the 16s mitochondrial DNA. However, in this experiment the same length 541 bp long DNA band was found in all individuals. In this research 16S rRNA and glutamine synthetage gene was used for the first time and a successful technique was developed for species identification of this fish.

5.4. Genetic diversity based population studies

To my knowledge, the present study is the first attempt to determine the genetic diversity based population genetic structure of freshwater mud eel, *M. cuchia* in Bangladesh by RAPD assay and the partial sequence of glutamine synthetage gene.

5.4.1. RAPD assay

In this study, RAPD based markers were used to genetic study of freshwater mud eel, *M. cuchia* in Bangladesh considering thirty individuals with eight primers (B 03, OPF 14, C 04, OPB 05, OPB 08, OPB 19, OPB-12 and UBC122). Different lengths of bands were observed in different primers with different ranges where a total of 735 bands with 228 polymorphic loci were detected and polymorphisms were revealed by all the primers. Miah *et al.* (2013b) studied genetic diversity of freshwater mud eel, *M. cuchia* using three RAPD primers where fish ware collected from Tanguar Haor ecology, Sunamganj, while genetic diversity was observed higher like the findings of this research. Experimented data indicated that intra-specific polymorphism were observed 100% in this study, though polymorphism was found by the study of Wei *et al.* (2006), Alam *et al.* (2010) and Miah *et al.* (2013b), however, their research showed monomorphic loci as well. Intra and inter population genetic diversity was analyzed based on RAPD assay considering the ecological habitats of Tanguar Haor (P1) and Hakaluky Haor (P2) respectively. Again same eight arbitrary primers (B 03, OPF 14, C 04, OPB 05, OPB 08, OPB 19, OPB-12 and UBC122) were used. A total of 353 bands with 147 polymorphic loci were detected in P1 whereas a total 371 bands with 153 polymorphic loci were found in P2. Polymorphisms were revealed by

all the primers in P1 and P2 while a single monomorphic locus were seen in P1 resulting highest polymorphism (100%) showed by all the primers in P2 and 95% polymorphism was observed P1. Genetic Diversity of seven populations of rice field eel (*Monopterus albus*) in China based on RAPD analysis by thirteen arbitrate primers where 122 polymorphic loci were detected by Wei *et al*, (2006). Yin *et al*. (2005) assessed the genetic differentiation and variation of the wild and raised swamp eels *M. albus* using RAPD technique and the results showed the percent polymorphic loci was 44.79% and 36.5% while Li *et al*. (2013) found the percent polymorphic loci of wild samples was 60.6–71% and cultured samples was 54–56.3% by ISSR analysis. This research was disagreed with the findings of Yin *et al*. (2005), Wei *et al.* (2006), Alam *et al.* (2010), Li *et al.* (2013) and Miah *et al.* (2013b), while lowest polymorphism was observed there. Though little bit higher polymorphism was found by Alam *et al.* (2010) in a population in Bangladesh and Ruzainah *et al.* (2003) was also found higher genetic diversity with RAPD fingerprinting of two eel loaches, *Pangio piperata* and *Pangio filinaris.* But this research found higher genetic diversity among two populations. In the rice field eel (*Monopterus albus*), 30 microsatellites were analyzed by AFLP of sequences where 13 loci exhibited polymorphism and these loci should provide sufficient level of genetic variation (Li *et al*. 2007), while lots of polymorphic loci were recorded in the present study of *M. cuchia* by RAPD assay. The genetic diversity of Asian swamp eel *Monopterus albus* were analyzed with 16 polymorphic novel microsatellites with 11 loci in two natural populations whereas the observed heterozygosity was 0.65 (Lei *et al*. 2012) which was also lowest from the present study in *M. cuchia* by RAPD analysis.

Inter individual pair wise similarity of this freshwater mud eel was recorded by 13 different groups where highest and lowest similarity was found 14 and 1 respectively. Inter individual pair wise similarity among the individuals of P1 was found 9 groups with highest and lowest values were observed 14 and 2 respectively whereas in P2, 13 groups of inter individual pair wise similarity were recorded between the values from 1 to 11. The averages inter individual pair wise similarity was observed 6.46 and 6.08 in P1 and P2 respectively, while only 3 groups of individuals were observed with the ranged of 0 to 2 by Miah *et al*. (2013b). Highest genetic diversity was recorded by the study of basic genetic diversity analysis while the average genetic diversity was found 0.8173716. Different genetic distance values were recorded in P1 and P2 while the average genetic distances were calculated 0.80 in P1 and 0.816 in P2 respectively by this study whereas almost similar results were found by Wei *et al*. (2006) and Miah *et al*. (2013b). Different values of Nei's genetic similarities were found similar results were observed between the present study as well as Miah *et al*. (2013b). Several linkage distances of values were found between the ranges of 3.6 to 6.24 by basic study of genetic diversity whereas the average linkage distances were found 5.115 and 5.141 respectively in P1 and P2. However, higher linkage distances were recorded in Tanguar Haor (Miah *et al*. 2013b). A genetic relationship in the basic study was observed by cluster analysis where 6 by 11 clades were generated considering the specific linkage distance. Relationship was made among populations where 5 clusters were created by 5 clades in Tanguar Haor population (P1) and 5 clusters and 5 clades were generated in Hakaluki Haor (P2), where, only 3 clades and 4 clusters were found in the few individuals of Tanguar Haor (Miah *et al*. 2013b). In this study, genotypes and alleles frequency were observed where the frequency of q alleles were recorded highest in both the populations than the frequency of p alleles. Considering intra-locus

gene diversity the average gene diversity was recorded highest 0.164635264 in P2 and lowest 0.150829501 in P1. Overall, higher genetic diversity has been recorded in this experimental fish which is indicating the good genetic status of this fish in Bangladeshi nature particularly at the ecological habitats of Sylhet region. Considering two populations, it was observed that the genetic diversity was little bit stronger in P2 (Hakaluki Haor) than the population 1 (Tanguar Haor). Wei *et al*. (2006), Lei *et al.* (2012) and Li *et al.* (2013) indicated that most of the total genetic variances of different population existed within populations and some variations existed among populations and the present results agreed with within populations. Miah *et al*. (2013b) found less genetic variability than the present study from the population (Tanguar Haor, Sunamgonj).

5.4.2. PCR-RFLP analysis

In this study, PCR products of 30 individuals of freshwater mud eel, *M. cuchia* were digested with the restriction enzymes of +CfrI and +Hpy178III for RFLP analysis. These two enzymes were digested the glutamine synthetage gene had an approximate size of 541 bp and polymorphism was detected in few individuals. The enzyme +CfrI detected a few polymorphisms within the individuals whereas two bands were found with wild type homogygotes and only five individuals were observed with a single band indicating the polymorphic homogygote. On the other hand, the enzyme +Hpy178III cut most of the individuals with two bands considering wild type homogygotes, polymorphism was detected in four individuals with single band and these individuals might be polymorphic homozygote. In addition heterozygosity with three bands were also seen in few individuals the enzyme +Hpy178III as well. The

genetic diversity was observed by using the RFLP band analysis considering the measurement of genetic distances (P-value) from the proportions of shared fragments (F-value). Genetic distances (P-value) were calculated from 30 individuals and seven different P-values values such as 0.00, 0.02, 0.033, 0.04, 0.05, 0.06 and 1 were found by using the seven different F-values such as 0.00, 0.4, 0.45, 0.5, 0.57, 0.67 and 1. On the basis of genetic distances, four different groups of individuals were identified with the p-values of 0, 0.033, 0.05 and 1. The genetic distance between individuals in group one and group two was found at P-value 0.02 with closely related. Group two was also at short genetic distance (P-value 0.04) with individuals of group three. The genetic distance between the individuals of group 3 and 4 was found 0.06 that means individuals of group 4 was far distance from the individuals of group 3 which was larger distance from each other. Individuals of group one with group 3 and 4 were relatively far from each other at a distance of P-value 0.04 and 0.06 respectively. From the above study, polymorphism was recorded among the individuals of this experimental freshwater mud eel in Bangladesh by the study of glutamine synthetage gene.

Intra and inter population genetic diversity of *M cuchia* was analyzed based on the 541 bp length of glutamine synthetage gene considering 15 individuals of each population whereas two populations were chosen considering the ecological of habitats of Tanguar Haor for population 1 (P1) and Hakaluki Haor for population 2 (P2). Two same restriction enzymes such as +CfrI and +Hpy178III were used to detect polymorphism of glutamine synthetage gene at population level. The enzyme +CfrI detected less polymorphism within the individuals of P1 where most of the

individual cut with two bands, indicating wild type homozygote, excepts the individuals of 13 and 14 with a single band indicating the polymorphic homozygote. The enzyme +Hpy178III detected lack of polymorphism within the individuals of P1 with two bands indicating wild-type homozygote. The enzyme +CfrI detected a lack of polymorphism among the individuals of P2 where two bands were recorded in all the individuals indicating wild type homozygotes, except the individuals of 13, 14 and 15 with single band indicating the polymorphic homozygote. Again by the enzyme +Hpy178III polymorphism was detected among the individuals with single, double and triple bands indicating polymorphic homozygote, wild type homozygote and heterozygote. Genetic diversity among the two populations was analysis based on RFLP bands considering the proportion of shared fragments (F-value) and P-values. Two different P-values such as 0, and 0.033 were recorded from three different F-values such as 1, 0.57 and 0.67 in P1 where two groups of individuals with 0 distance values were identified and theses two groups were very closely related with 0.02 p value in P1. In P2, the genetic distance with different P-values for instance 0.00, 0.02, 0.033, 0.04, 0.05, 0.06 and 1 were originated from seven different F-values (0, 0.4, 0.45, 0.5, 0.57, 0.67 and 1. Considering different genetic distances five groups (G) of individuals were revealed with 0 P-values and the observed P-values were 0.00, 0.033, 0.04, 0.06 and 1. In this experiment G1, G2 and G3 were found very close with 0.02 p-values as well as G2 and G4 were found also close. Furthermore, G3 and G4 were also closed with 0.05 P-value. But G4 and G5, G1 and G 3 as well as G1 & G4 were recorded far distance from each other. Finally large genetic distance was recorded between the G1 and other groups. Allelic Information among two populations was observed by PCR-RFLP considering the analysis of Hardy-Weinberg Equilibrium, genotype frequency, allele frequency and intra-locus gene diversity.

Allele frequency was measured by the enzyme +Hpy178III in P1 whereas frequencies of p and q alleles were found 0 and 1 respectively as well as intra-locus gene diversity was recorded 0. Allele frequency of p 0.133 and allele frequency of q 0.867 were observed in P1 by the enzyme +CfrI. Intra-locus gene diversity was found 0.231 in P1 by the enzyme +CfrI. On the other hand, the enzymes +Hpy178III and +CfrI revealed the allele frequency of p 0.567 and 0.2 respectively in P2 as well as the allele frequency of q alleles were observed 0.433 and 0.8 respectively. In P2, again intra-locus gene diversity was recorded 0.491 and 0.32 respectively by the enzymes +Hpy178III and +CfrI. The frequency of q alleles were recorded highest in both the populations than the frequency of p alleles except the enzyme +Hpy178III in P2. However, intra-locus gene diversity was found highest in P2 by both enzymes than P1. Inter population differentiation for one locus was calculated and the result (gst = 0.5299) shows significant differentiation between populations in allele frequencies and it can say that a high percentage of genetic distance is distributed among two populations. F-statistics was analyzed for observing the genetic structure of populations where degree of allele frequency (F_{IT} = 0.7606) and differentiation of allele frequencies between two populations seems greater (F_{ST} = 0.1439), with only a moderate effect of random mating were observed within the populations (F_{IS} = 0.7204). Therefore, in this study by RFLP of glutamine synthetage gene polymorphism was found. Genetic polymorphism was recorded among two populations by the study of RFLP bands, genetic diversity by haplotypes, allelic informations, inter population differentiation and F-statistics, however, overall higher polymorphism was observed in P2 as well.

According to the Wei *et al*. (2006), genetic differentiation existed among all the populations (g^{st} = 0.1798) of *M. albus* whereas the significant differentiation of *M. cuchia* was found between the experimental populations (g_{st} = 0.5299) indicating that a high percentage of genetic distance was distributed. Genetic diversity of *M. albus* in a wild population was investigated by employing mtDNA polymorphisms (Cai *et al*. 2008) where the genetic differentiation between the population (F_{ST} = 0.29167 to 0.55126), was higher than that among the other three (F_{ST}=0.04196 to 0.05984) indicating little genetic differentiation was found among the populations. The genetic differentiation between the two populations of *M. albus* was also found significant (F_{ST} = 0.24) (Lei *et al*. 2012), but in the present study it was analyzed for observing the genetic structure of populations where degree of allele frequency (F_{IT} = 0.7606) and differentiation of allele frequencies between two populations seems greater (F_{ST} = 0.1439), with only a moderate effect of random mating were observed within the populations (F_{IS} = 0.7204). Therefore, in this study by RFLP of glutamine synthetage gene genetic polymorphism was recorded higher among two populations. This finding was better than the findings Yang *et al*. (2005) and Lu *et al*. (2005). Li *et al.* (2011) was studied hepcidin gene and they also found polymorphism in rice field *M. albus*. Tok *et al*. (2009) analyzed glutamine accumulation and up-regulation of glutamine synthetase activity in the swamp eel, *M. albus*, however, no genetic diversity of glutamine synthetage gene of *M. albus* was studied. Major histocompatibility complex (MHC) class IIA gene was analyzed in swamp eel *M. albus* and only seven alleles were identified (Li *et al*. 2014) but in this research though highest 3 alleles were recorded in few individuals of *M. cuchia* considering glutamine synthetage gene while expected two alleles in each of the individuals, however, polymorphism was found among the populations which is good for the experimental populations for breeding

and adaptation. Though induced expression of downstream luc gene activities of *Monopterus albus* and *Channa straitus* extracts were analyzed but no genetic diversity was found by the study of this gene (Atif *et al*. 2015).

6.1. Summary

The freshwater mud eel, *Monopterus cuchia* (Hamilton, 1822) belongs to the family synbranchidae of the order synbranchiformes (Rosen and Greenwood, 1976; Munshi *et al*., 1987) which is found in different open water resources such as rivers, haors, baors, beels, canals, floodplains and rice fields in Bangladesh (Rahman, 1989). It has great demands in foreign markets and the main advantage is that this fish are transported in live through a small basket or plastic jar while this fish maintain duel system of oxygen uptake for respiration resultant this fish can survive 90 to 132 days without food (Liem 1967, Pullin and Shehadan, 1980). This tasty fish is a high-value export fishery item of Bangladesh and its trade involving 15 countries in the world, with high demand in China, Malaysia, Singapore, Japan and Taiwan (Hasan *et al*., 2012). This fish can also play a unique role for socio-economic welfare of the area that can help to poor people for their livelihood development.

This fish is now a red listed species in Bangladesh (IUCN, 2000) because of commercial fishing while different capture techniques are established to collect this fish but production technique of this fish is not established any more, though, the preliminary observations in cemented cistern on growth, survival and diet condition of this mud eel has been observed (Narejo *et al*., 2003b; Narejo *et al*., 2003c). For sustainable production and conservation of this fish in Bangladesh it is necessary to supply available fry and artificial fry production of this fish is very important while unsuccessful induced breeding was recorded (Unpublished data). Due to this fish is not responding with inducing agents, therefore, genetic research at population level is most important while only DNA fingerprinting were observed in narrow sense using

RAPD marker in China (He *et.al*. 2004) and in Bangladesh (Alam *et al*. 2010). Therefore, in this research population genetic structure of freshwater mud eel was observe for finding a better population with high genetic diversity that will be used in future for artificial breeding. In the present research, some associated observations including culture, breeding biology and artificial breeding of this freshwater mud eel was also conducted.

The specific objectives of the research were:

1. to observe the suitable environments for survival, growth and production of the fish in Bangladesh
2. to observe the breeding biology of mud eel, *Monopterus cuchia* in natural conditions as well as under hormonal treatments
3. to observe breeding performance through inducing agents
4. to perform molecular based species identification for resolving morphological ambiguity
5. to investigate genetic diversity among individuals of *Monopterus cuchia*
6. to observe the genetic population of *Monopterus cuchia* collected from different ecological habitats
7. to make relationships among populations as well as individuals
8. to identify better population for breeding program from different ecosystems in Bangladesh

Based on above objectives the summary of the research were discussed sequentially.

6.1.1. Rearing and production

This experiment was conducted to study the effect of different environments on rearing and production performance of freshwater mud eel, *M. cuchia*. The study was conducted over a period of six months from June to November 2013. Different environments such as House tanks, Earthen ditches, Plastic tank and Back yard tanks were used. Except the earthen ditch-2 (control), all environments were fed by different biological food such as small live fish (Tilapia fry), earth worms, small dead fish, snails, small frogs and chicken viscera in different amounts. 40 fishes (approximately 1kg in weight) were stocked into each of the environment. At the end of the experiment, the highest mean of final length (32.89±2.65423) and final weight (109.69±2.92777) were obtained in earthen ditch-1. The weight gain in length 17.78 ± 0.24 and in weight 85.07 ± 0.15 with 92.5% survival and production (4.07kg) were also highest in earthen ditch-1. However, earthenditch-2 which was used as control has also shown satisfactory results in terms of weight gain in length and weight 14.1 ± 0.48 and 66.66 ± 0.82 respectively with 87.5% survival. On the other hand, the lowest mean growth considering final length (28.45±2.80092) and weight (67.24±3.79328) were recorded in house tank-2 while the weight gain in length (11.86 ± 0.26) and in weigh (41.84 ± 0.59) were also observed in house tank-2 with the production of 2.02kg by 75% survival. Based on Pearson Correlation coefficient there were no significant correlations between lengths and weights of fish in different culture regimes. Significant value (P=0.000) of each of the environments implies that *M. cuchia* can be reared significantly except back yard tank-1 (P=0.009). But, in case of earthen ditch-1 the highest value was found in both length and weight with 5% significance level due to supplied live feeds. On the basis of the better growth,

survival and production, it is suggested that the earthen ditch is one of the suitable culture environments of *M. cuchia*. From this study, it was also found that the live feed are suitable for obtaining highest growth performance based on the findings of plastic tank, house tanks 1 and house tank 3.

6.1.2. Breeding biology and artificial breeding

This study was carried out for sexual differentiation of freshwater mud eel, *M. cuchia* which is most important for induced breeding. A successful protocol was developed to identify male and female *M. cuchia* through morphological, anatomical and histological analysis. In this study, breeding biology and induced breeding of freshwater mud eel, *M. cuchia* was observed during the experimental period from February to June, 2013. Breeding biology of freshwater mud eel, *M. cuchia* was considered in terms of gonadosomatic index, length-weight relationship of gonad, ova diameter and fecundity. The ova diameter was recorded from 0.3 mm to 4.30 mm and the individual fecundity was recorded from 155 to 1495 while relative fecundity was found from 2.64 to 12.45. The fecundity related to body weight and length of fish was also discussed. A peak of GSI was observed 2.14±0.2 in male and 5.1 ±1.09 in female. Induced breeding of freshwater mud eel, *M. cuchia* was also practiced with different doses of different inducing agents like pituitary gland (PG), human chorionic gonadotropin (HCG), Gonadotropin releasing hormone (GnRH) and Ovuline-a synthetic hormone in different environmental conditions. However, it was observed that the artificial breeding of freshwater mud eel, *M. cuchia* was not yet succeeded through inducing agents in captive conditions, rather the inducing agent showed

negative impacts on fecundity and ovarian tissues. It was seen that mature eggs in the oviduct were reduced, absorbed and some eggs were found in spoiled condition.

6.1.3. Molecular species identification

This study was carried out for molecular identification of freshwater mud eel, *M. cuchia* which is most important for induced breeding. Traditional classification of freshwater eels has always been obscured and unreliable due to their morphological ambiguity. A rapid and cost effective molecular markers, mitochondrial 16S rRNA and glutamine synthetase gene was used to establish molecular standards for identification of this fish. Similar bands were seen in all the individuals at the level of 250bp length by using 16s mitochondrial DNA of 24 individuals and 544bp length for partial sequence of glutamine synthetage gene in 11 individuals.

6.1.4. Genetic diversity based population studies

6.1.4.1. Genetic diversity by RAPD

In this study, RAPD based markers were used to genetic study of freshwater mud eel, *M. cuchia* in Bangladesh considering thirty individuals with eight primers (B 03, OPF 14, C 04, OPB 05, OPB 08, OPB 19, OPB-12 and UBC122). DNA profiling was scored as 1 if the presence of bands or 0 if the absence of bands and different lengths of bands were observed in different primers with different ranges where a total of 735 bands with 228 polymorphic loci were detected and only polymorphisms were revealed by all the primers. The highest number of bands (120) was amplified by the

primer C04 and the lowest number of bands (49) was amplified by the primer UBC122. Inter individual pair wise similarity of this freshwater mud eel was recorded by 13 different groups where highest and lowest similarity was found 14 and 1 respectively. 36 groups of genetic diversity were measured in the experimental individuals whereas the lowest genetic distance was found 0.58 and the highest genetic distance was recorded 0.97 while the average genetic diversity was found 0.8173716. Different values of Nei's genetic similarities were found whereas highest and lowest values were recorded 0.60 and 0.06 respectively. In addition, linkage distance was computed and again different groups of values were found between the ranges of 3.6 to 6.24. A genetic relationship among individuals was observed by cluster analysis where six clusters by 11 clades were generated considering the specific linkage distance. From all the findings, it was observed that, higher genetic diversity has been recorded in this experimental fish which is indicating the good genetic status of this fish in Bangladeshi nature particularly at the ecological habitats of Sylhet region.

6.1.4.2. Genetic diversity at population level by RAPD assay

In this study, intra and inter population genetic diversity were analyzed based on RAPD assay considering 2 populations whereas 15 individuals were considered for each population. The ecological habitats of Tanguar Haor and Hakaluky Haor were consider as population 1 (P1) and population 2 (P2) respectively. Again eight arbitrary primers (B 03, OPF 14, C 04, OPB 05, OPB 08, OPB 19, OPB-12 and UBC122) were used to amplify the genomic DNA of two experimental populations. DNA profiling was scored as 1 if the presence of bands or 0 if the absence of bands

on the gel. Bands were located in different lengths in different primers in two populations. A total of 353 bands with 147 polymorphic loci were detected in P1 whereas a total 371 bands with 153 polymorphic loci were found in P2. The highest number of bands (62) was amplified by the primer OPB 14 and lowest number of bands (27) was observed by the primers OPB 12 and UBC 122 in P1. On the other hand, in P2, highest number of bands (60) was recorded by using the primer C04 and the lowest number of bands (22) was amplified from the primer UBC 122. Polymorphisms were revealed by all the primers in P1 and P2 while a single monomorphic locus were seen in P1 resulting highest polymorphism (100%) showed by all the primers among the tested populations, though, 95% polymorphism was observed by using the primer OPB 08 in P1. Inter individual pair wise similarity among the individuals of P1 was found 9 groups with highest and lowest values were observed 14 and 2 respectively whereas in P2, 13 groups of inter individual pair wise similarity were recorded between the values from 1 to 11. The averages inter individual pair wise similarity was observed 6.46 and 6.08 in P1 and P2 respectively. The 27 different genetic distance values (from 0.59 to 0.94) were recorded in P1 and the 29 different distance values (from 0.63 to 0.97) was found in P2 while the average genetic distances were calculated 0.80 in P1 and 0.816 in P2 respectively. Nei's genetic similarity in P1 was ranged 0.11 – 0.58 with 34 diverse values and in P2 was ranged between 0.06 – 0.54 with 36 different values and in an average were originated 0.323 in P1 and 0.301 in P2 respectively. A total 35 and 18 different linkage distances were estimated in P1 and P2 respectively whereas the average linkage distances were found 5.115 and 5.141 respectively in P1 and P2. Again the lowest and highest linkage distances were found 3.6 and 24 in P1 as well as 4.12 and 5.92 in P2. A genetic relationship was made by using linkage distance where five clusters were

created from 5 clades in P1 and five clusters and five clades were generated in P2. Based on allelic information, the frequency of q alleles were recorded highest in both the populations than the frequency of p alleles. Based on the intra-locus gene diversity the average highest gene diversity was observed in P2 in most of the primers, except the primers OPF 14, OPB 19 and OPB 12. However, the average gene diversity was found highest 0.164635264 in P2 and lowest 0.150829501 in P1. According to the genetic diversity based population study, overall higher genetic diversity was recorded in both the populations. However, considering two populations, it was observed that the genetic diversity was little bit stronger in P2 (Hakaluki Haor) than the population 1 (Tanguar Haor).

6.1.4.3. Genetic diversity by RFLP

In this study, PCR products of 30 individuals of freshwater mud eel, *M. cuchia* were digested with the restriction enzymes of +CfrI and +Hpy178III for RFLP analysis. These two enzymes were digested the glutamine synthetage gene had an approximate size of 541 bp and polymorphism was detected in few individuals. The enzyme +CfrI detected a few polymorphisms within the individuals whereas two bands were found with wild type homogygotes and only five individuals were observed with a single band indicating the polymorphic homogygote. On the other hand, the enzyme +Hpy178III cut most of the individuals with two bands considering wild type homogygotes, polymorphism was detected in four individuals with single band and these individuals might be polymorphic homozygote. In addition heterozygosity with three bands were also seen in few individuals the enzyme +Hpy178III as well. The genetic diversity was observed by using the RFLP band analysis considering the measurement of genetic distances (P-value) from the proportions of shared fragments

(F-value). Genetic distances (P-value) were calculated from 30 individuals and seven different P-values values such as 0.00, 0.02, 0.033, 0.04, 0.05, 0.06 and 1 were found by using the seven different F-values such as 0.00, 0.4, 0.45, 0.5, 0.57, 0.67 and 1. On the basis of genetic distances, four different groups of individuals were identified with the p-values of 0, 0.033, 0.05 and 1. The genetic distance between individuals in group one and group two was found at P-value 0.02 with closely related. Group two was also at short genetic distance (P-value 0.04) with individuals of group three. The genetic distance between the individuals of group 3 and 4 was found 0.06 that means individuals of group 4 was far distance from the individuals of group 3 which was larger distance from each other. Individuals of group one with group 3 and 4 were relatively far from each other at a distance of P-value 0.04 and 0.06 respectively. From the above study, polymorphism was recorded among the individuals of this experimental freshwater mud eel in Bangladesh by the study of glutamine synthetage gene.

6.1.4.4. Genetic diversity at population level by PCR-RFLP

Here the, intra and inter population genetic diversity of *M. cuchia* was analyzed based on the 541 bp length of glutamine synthetage gene considering 15 individuals of each population whereas two populations were chosen considering the ecological of habitats of Tanguar Haor for population 1 (P1) and Hakaluki Haor for population 2 (P2). Two same restriction enzymes such as +CfrI and +Hpy178III were used to detect polymorphism of glutamine synthetage gene at population level. The enzyme +CfrI detected less polymorphism within the individuals of P1 where most of the individual cut with two bands, indicating wild type homozygote, excepts the

individuals of 13 and 14 with a single band indicating the polymorphic homozygote. The enzyme +Hpy178III detected lack of polymorphism within the individuals of P1 with two bands indicating wild-type homozygote. The enzyme +CfrI detected a lack of polymorphism among the individuals of P2 where two bands were recorded in all the individuals indicating wild type homozygotes, except the individuals of 13, 14 and 15 with single band indicating the polymorphic homozygote. Again by the enzyme +Hpy178III polymorphism was detected among the individuals with single, double and triple bands indicating polymorphic homozygote, wild type homozygote and heterozygote. Genetic diversity among the two populations was analysis based on RFLP bands considering the proportion of shared fragments (F-value) and P-values. Two different P-values such as 0, and 0.033 were recorded from three different F-values such as 1, 0.57 and 0.67 in P1 where two groups of individuals with 0 distance values were identified and theses two groups were very closely related with 0.02 p value in P1. In P2, the genetic distance with different P-values for instance 0.00, 0.02, 0.033, 0.04, 0.05, 0.06 and 1 were originated from seven different F-values (0, 0.4, 0.45, 0.5, 0.57, 0.67 and 1. Considering different genetic distances five groups (G) of individuals were revealed with 0 P-values and the observed P-values were 0.00, 0.033, 0.04, 0.06 and 1. In this experiment G1, G2 and G3 were found very close with 0.02 p-values as well as G2 and G4 were found also close. Furthermore, G3 and G4 were also closed with 0.05 P-value. But G4 and G5, G1 and G 3 as well as G1 & G4 were recorded far distance from each other. Finally large genetic distance was recorded between the G1 and other groups. Allelic Information among two populations were observed by PCR-RFLP considering the analysis of Hardy-Weinberg Equilibrium, genotype frequency, allele frequency and intra-locus gene diversity. Allele frequency was measured by the enzyme +Hpy178III in P1 whereas

frequencies of p and q alleles were found o and 1 respectively as well as intra-locus gene diversity was recorded 0. Allele frequency of p 0.133 and allele frequency of q 0.867 were observed in P1 by the enzyme +CfrI. Intra-locus gene diversity was found 0.231 in P1 by the enzyme +CfrI. On the other hand, the enzymes +Hpy178III and +CfrI revealed the allele frequency of p 0.567 and 0.2 respectively in P2 as well as the allele frequency of q alleles were observed 0.433 and 0.8 respectively. In P2, again intra-locus gene diversity was recorded 0.491 and 0.32 respectively by the enzymes +Hpy178III and +CfrI. The frequency of q alleles were recorded highest in both the populations than the frequency of p alleles except the enzyme +Hpy178III in P2. However, intra-locus gene diversity was found highest in P2 by both enzymes than P1. Inter population differentiation for one locus was calculated and the result (gst = 0.5299) shows significant differentiation between populations in allele frequencies and it can say that a high percentage of genetic distance is distributed among two populations. F-statistics was analyzed for observing the genetic structure of populations where degree of allele frequency (F_{IT} = 0.7606) and differentiation of allele frequencies between two populations seems greater (F_{ST} = 0.1439), with only a moderate effect of random mating were observed within the populations (F_{IS} = 0.7204). Therefore, in this study by RFLP of glutamine synthetage gene polymorphism was found. Genetic polymorphism was recorded among two populations by the study of RFLP bands, genetic diversity by haplotypes, allelic informations, inter population differentiation and F-statistics, however, overall higher polymorphism was observed in P2 as well.

6.1.5. Genetic status regarding best population

In this study, RAPD and RFLP based population genetic structure of freshwater mud eel *M. cuchia* in Bangladesh was analyzed. Higher genetic diversity has been recorded in this experimental fish by basic RAPD assay which is indicating the genetic status of this fish in Bangladeshi specially in experimental ecology habitats. Considering genetic diversity based population study by RAPD, higher genetic diversity was observed in both the populations, however, considering the two populations, the genetic diversity was found slightly stronger in population 2 (Hakaluki Haor) than the population 1 (Tanguar Haor). On the other hand, polymorphisms were recorded by RFLP based basic diversity analysis of partial sequence of glutamine synthetage gene while both the enzymes had single cutting point. However, intra-locus gene diversity was found highest in P2 by both enzymes than P1 by analyzing the Hardy-Weinberg Equilibrium, genotype frequency and allele frequency. In both cases of RAPD and RFLP study higher genetic diversity were observed in general as well as at populations level, where P2 was recorded stronger genetic status than P1.

6.2. Conclusions

Finally following findings are concluded:

1. Different environments such as House tanks, Earthen ditches, Plastic tank and Back yard tanks can be used for rearing of this fish. However, basis of the better growth, survival rate and production of this finding, it is suggested that earthen ditches are suitable for the best culture and production of *M. cuchia*. It was also found that live feed are suitable for obtaining highest growth performance.

2. Breeding biology of freshwater mud eel, *M. cuchia* was studied in terms of gonadosomatic index, length-weight relationship of gonad, ova diameter and fecundity and a successful protocol was developed to identify male and female *M. cuchia*.

3. Induced breeding of *M. cuchia* was also practiced with different doses of different inducing agents like PG, HCG, GnRH and Ovuline in different environmental conditions. Though artificial breeding of this fish was not yet succeeded, however, it will be helpful to future research regarding seed production through inducing agents.

4. Traditional classification of freshwater eels has always been obscured and unreliable due to their morphological ambiguity, therefore, a rapid and cost effective molecular standards has been established for identification of this fish based on 16S rRNA and Glutamine Synthetage gene.

5. A basic RAPD observation, higher genetic diversity has been recorded in this experimental fish which is indicating the genetic status of this fish in Bangladesh nature.

6. Basic analysis of RFLP, polymorphism in partial sequence of glutamine synthetage gene has been distinguished of this experimental fish, however, more research is essential to know the genetic status of this gene in details.

7. According to the genetic diversity based population study of this fish through RAPD and RFLP assay, higher genetic diversity was recorded in population 2 (Hakaluki Haor) than the population 1 (Tanguar Haor) as well.

6.3. Recommendations

As the freshwater mud eel is a well established export product that will be made a great contribution to the national economy of Bangladesh and its culture could be considered as an alternative option for poor people and immerging trade for fishery product traders while eel aquaculture industry is completely absent only capture based fishery practice are performing in Bangladesh. As artificial breeding programme was not succeeded yet therefore genetic study should be considered for developed of breeding programme which will be ultimate help for eel aquaculture, production and protection of this fish in Bangladesh. Artificial breeding may have several causes to unsuccessful spawning responds such as provably imbalance of environmental conditions, physical stress and diseases etc. Another reason can be considered that its need more time with different inducing agents with different doses. Special consideration should be taken for tanks, hapa, high quality water, recycling system, good aeration, sufficient food supply, avoid crowding etc. Though higher genetic diversity was observed, however, more genetic research will be studied with lots of RAPD primers considering the samples collected from different more ecological habitats. Even if, polymorphism was observed in the experimental fish but more genetic research will be conducted with more restriction enzymes for GS as well as other different genes considering the samples collected from different more ecological habitats of Bangladesh.

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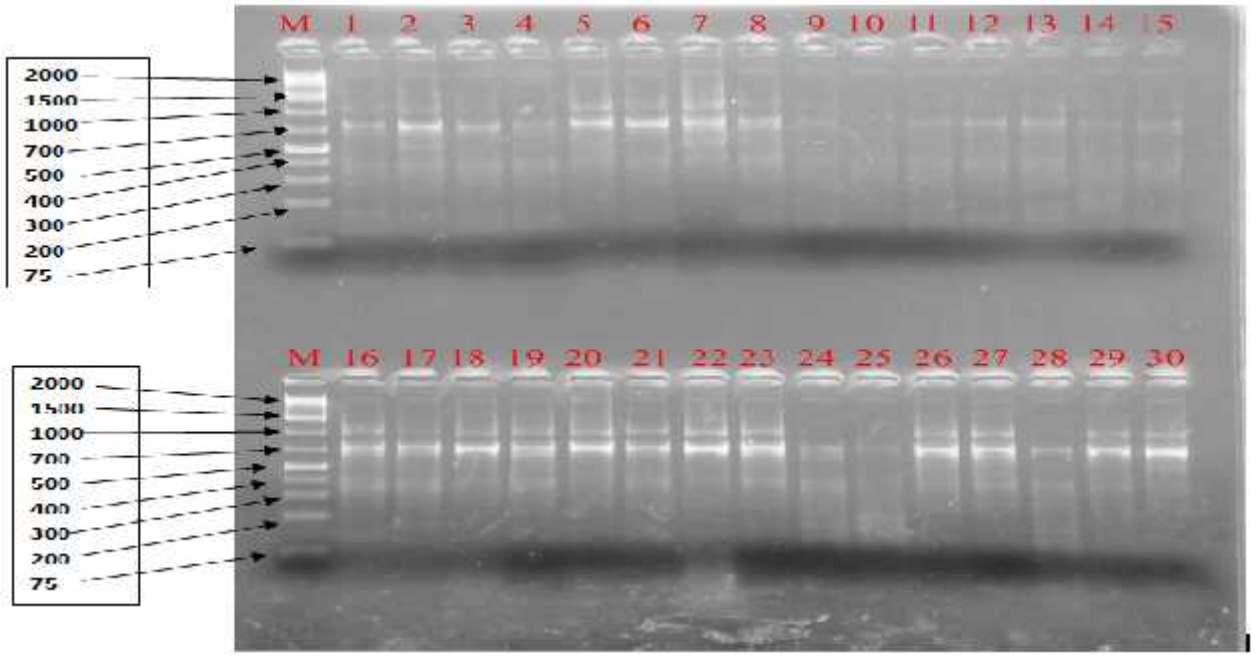
Appendix 1a. Gel image of RAPD primer B03

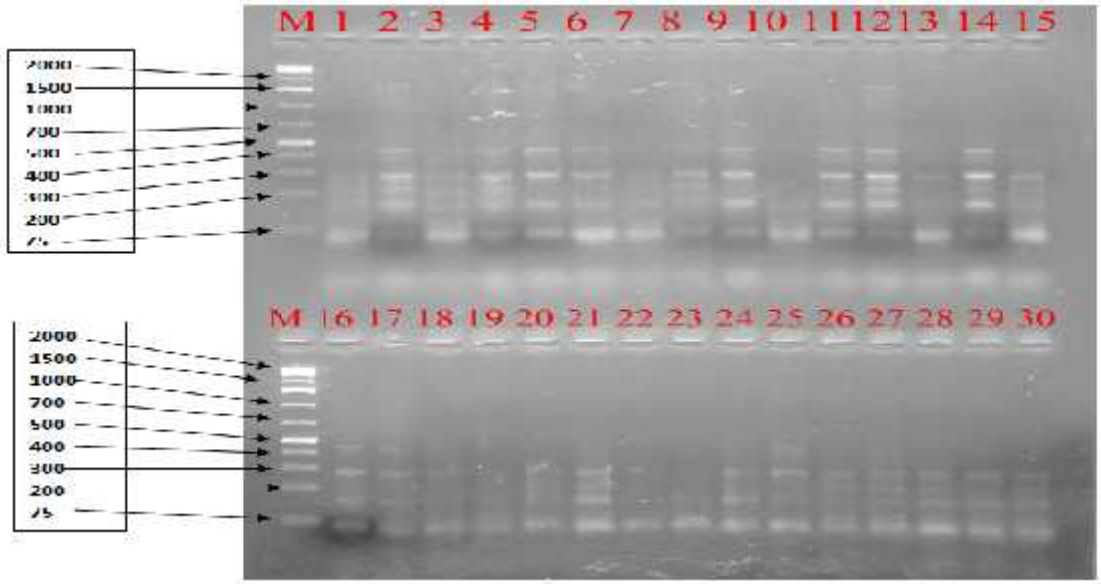


Appendix 1b. Data scoring from gel image of RAPD primer B03

LDNA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
151	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
329	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	1	0	0	0
348	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
372	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
391	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
412	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
442	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
650	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
685	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
705	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0
719	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	0	0	1	0	0	0	0	0
738	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0
755	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
802	1	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
826	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
838	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
867	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
901	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1029	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	1	1
1047	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0
1074	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
1109	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
1153	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1248	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2027	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
2084	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
2114	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0
2144	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
2174	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0
2220	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
2265	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
2444	0	0	0	0	1	0	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2476	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2508	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2574	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2608	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

LDNA= Length of DNA

Appendix 2a. Gel image of RAPD primer OPF 14

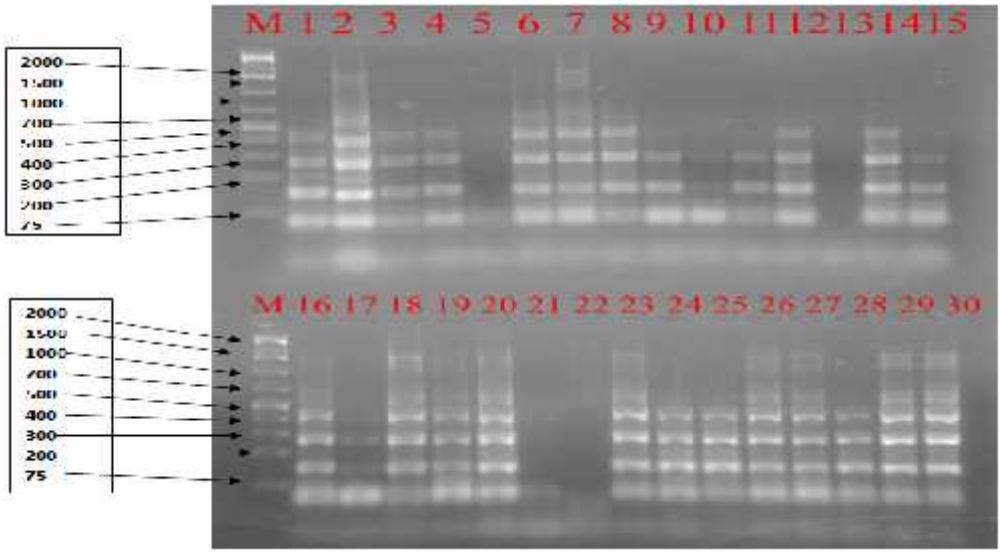


Appendix 2b. Data scoring from the gel image of RAPD primer OPF 14

LDNA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
71	1	1	1	1	0	1	1	0	0	0	1	1	1	0	1	0	0	1	0	0	0	1	0	1	0	1	1	1	1	1
80	0	0	0	0	1	0	0	1	1	1	0	0	0	1	0	1	1	0	1	1	1	0	1	0	1	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
115	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1
124	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
131	0	1	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0
139	0	0	0	1	1	1	1	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
151	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
170	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
181	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
199	0	0	0	1	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
212	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0
224	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0
241	0	1	1	0	0	0	1	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
251	1	0	0	1	1	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0
261	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
280	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
360	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
372	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
404	0	0	1	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
412	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
423	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
455	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1566	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

LDNA= Length of DNA

Appendix 3a. Gel image of RAPD primer C04

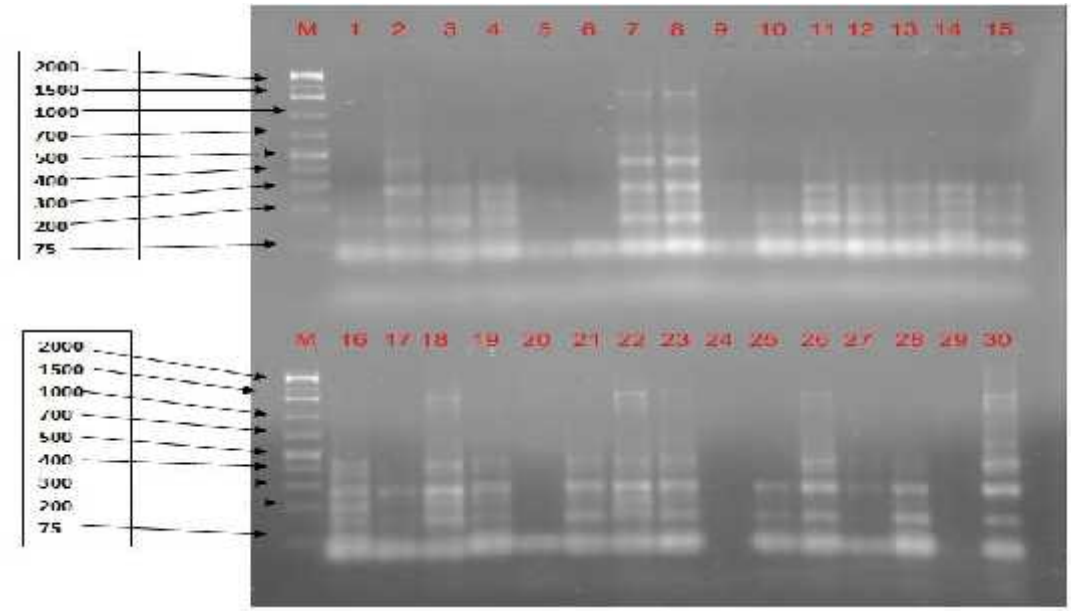


Appendix 3b. Data scoring from the gel image of RAPD primer C04

LDNA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
40	0	1	0	0	1	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	1	0
71	0	1	1	1	0	0	1	0	1	0	0	0	0	0	1	0	1	0	0	1	1	1	1	1	1	1	1	1	0	1
80	0	0	0	0	1	1	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
124	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	1	1	0	0
131	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	1	1
139	1	0	0	0	0	1	1	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
151	0	0	0	0	0	0	0	1	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
170	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
199	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
224	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	1	1	0	1
241	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	1	0	1	0	0	1	0
251	1	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
270	0	0	0	0	0	0	0	1	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
360	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
383	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	1	0	0
391	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	1	0	0	1
423	0	0	1	1	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
442	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
520	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
544	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0
571	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
635	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1315	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1409	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
1473	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

LDNA= Length of DNA

Appendix 4a. Gel image of RAPD primer OPB 05

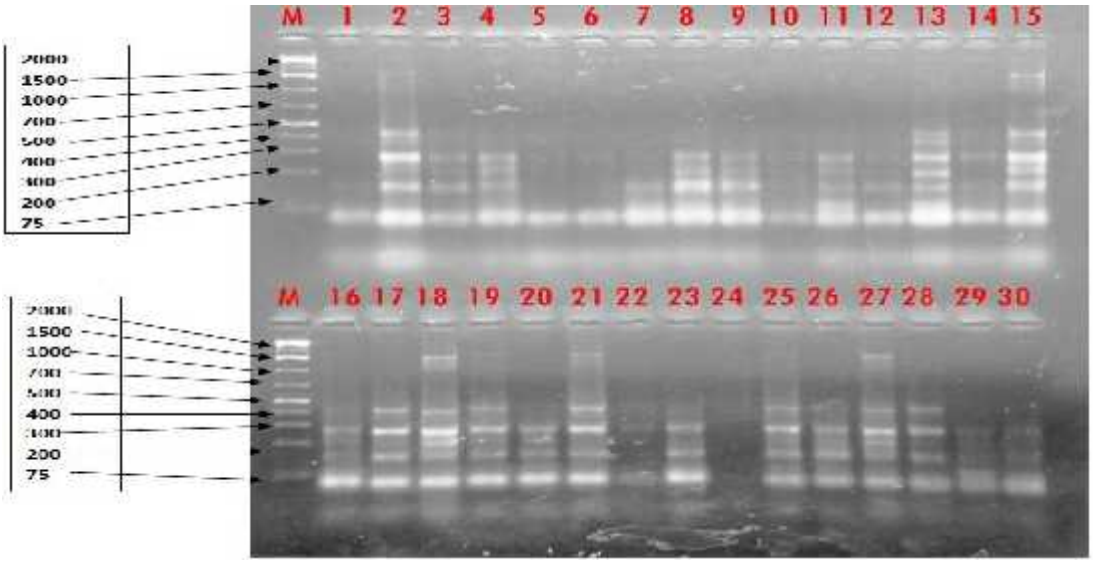


Appendix 4b. Data scoring from the gel image of RAPD primer OPB 05

LDNA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
71	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1
80	0	0	0	0	1	1	1	1	0	1	1	0	1	0	1	0	0	0	1	1	1	0	1	0	0	0	1	1	0	0
90	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
124	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
131	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	1
139	1	0	0	1	0	0	0	0	0	1	1	1	0	1	0	0	0	0	1	0	1	0	1	0	0	1	0	0	0	0
151	0	0	0	0	0	0	1	1	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
170	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
191	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
199	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
212	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
224	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	1	0	0
241	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	1
251	0	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	1	0	0	1	0	0	0	0
261	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
270	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
280	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
348	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
391	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	1	0	0
412	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1
423	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
442	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
455	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
544	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
705	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
719	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1409	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
1501	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
1517	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1533	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
1566	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
1581	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1605	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

LDNA= Length of DNA

Appendix 5a. Gel image of RAPD primer OPB 08

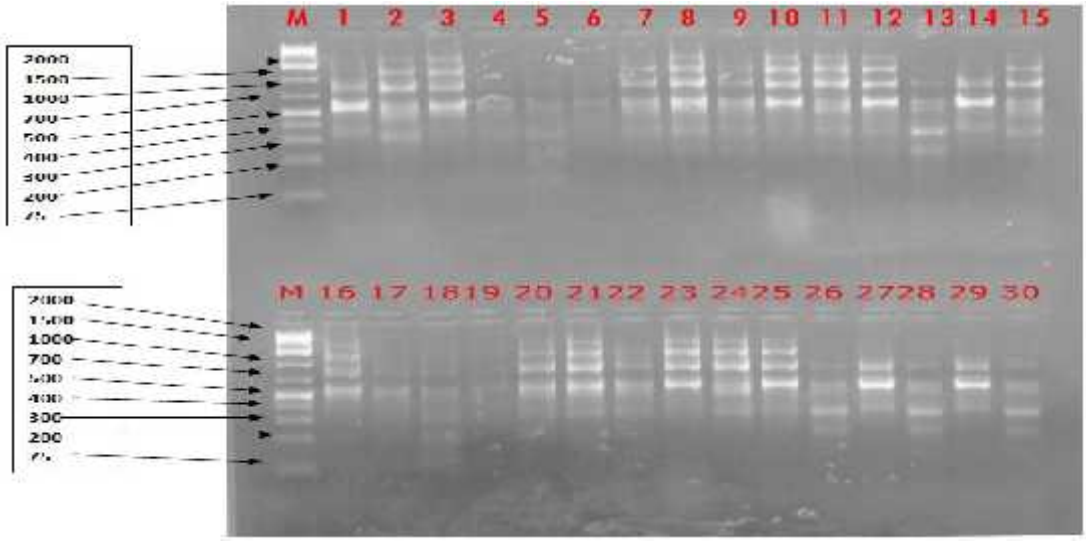


Appendix 5b. Data scoring from the gel image of RAPD primer OPB 08

LDNA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
60	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	0	0	0	0	1	0	1	0	1	1
71	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1	0	0	1	0	1	0	0
115	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
124	1	1	1	1	0	0	1	1	0	0	1	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0
139	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	0	0	0	1	1	0	0	0	0
160	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
170	0	0	0	1	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
191	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
199	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
212	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1
232	0	1	0	0	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0
241	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1	1	0	1	0	1	0	0	0	0	0	0	1	1	0	0
261	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
270	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
318	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
348	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
372	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
391	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0
412	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
423	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0
1339	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
1517	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
1605	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

LDNA= Length of DNA

Appendix 6a. Gel image of RAPD primer OPB 19

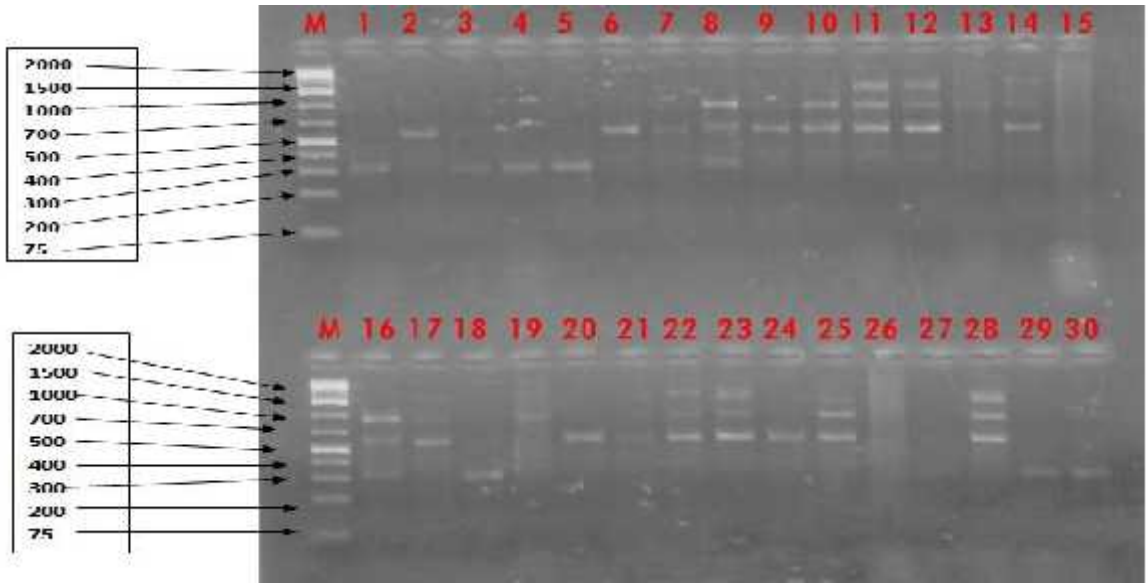


Appendix 6b. Data scoring from the gel image of RAPD primer OPB 19

LDNA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
170	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
212	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
232	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
301	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
329	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	1	0	1
348	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
360	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
383	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
404	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
520	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
544	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
571	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1
589	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
605	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
618	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
635	0	0	0	0	1	1	1	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
650	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	1	1	0	0	0
685	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
826	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
890	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
926	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
959	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0
976	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0
993	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1015	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
1029	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
1047	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
1074	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
1248	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1295	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
1315	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1339	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1362	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
1396	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
1422	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
1449	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
1728	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1742	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1766	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1836	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
1884	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
1920	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

LDNA= Length of DNA

Appendix 7a. Gel image of RAPD primer OPB 12

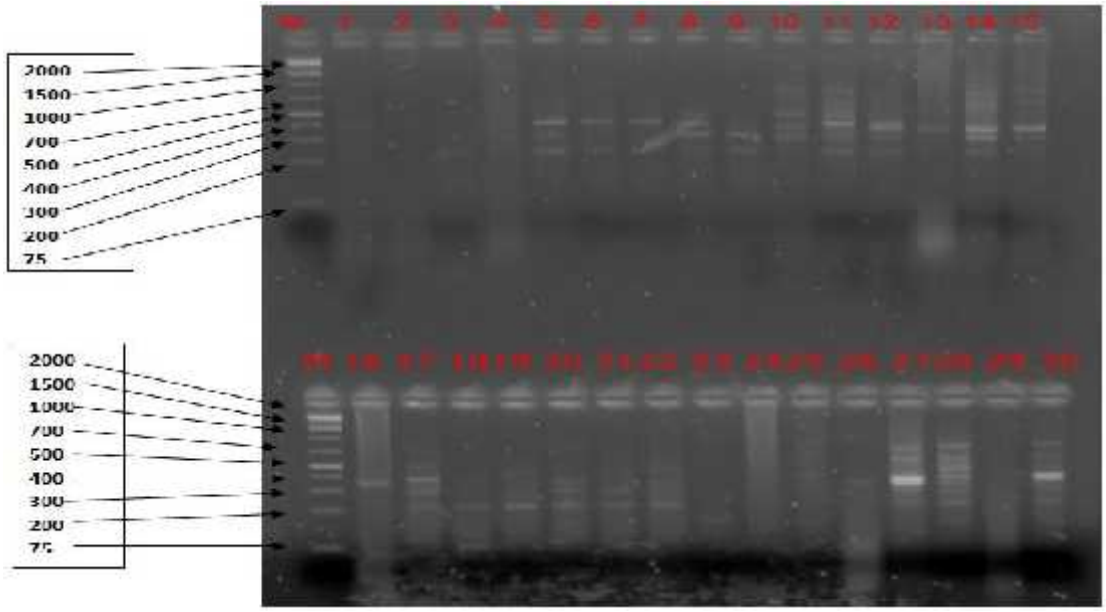


Appendix 7b. Data scoring from the gel image of RAPD primer OPB 12

LDNA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
280	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
310	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
318	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1
348	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
571	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
605	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
618	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
635	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
650	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
685	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
705	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
867	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
926	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
993	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
1015	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
1047	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
1074	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
1109	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1129	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
1153	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
1181	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1248	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1422	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
1581	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
1605	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1656	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
1675	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1710	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1728	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
1766	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
1920	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2703	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0

LDNA= Length of DNA

Appendix 8a. Gel image of RAPD primer UBC 122

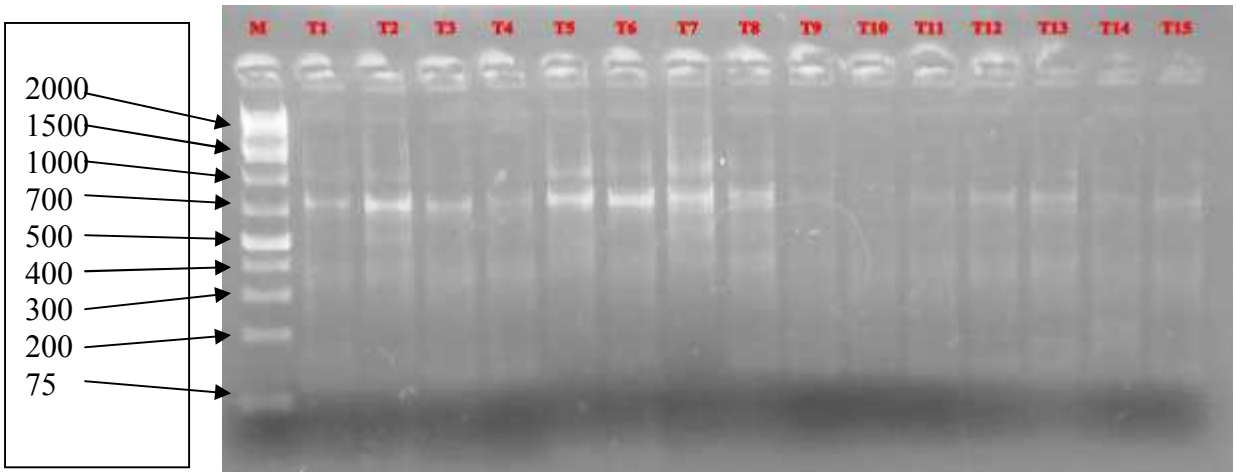


Appendix 8b. Data scoring from the gel image of RAPD primer UBC 122

LDNA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
310	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	1	1	0	0
318	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
329	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	1	0	1
348	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	1	0	0
360	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1
520	1	0	0	0	1	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
544	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
571	0	0	0	0	0	0	0	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
589	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
605	0	0	0	0	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
518	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
635	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

LDNA= Length of DNA

Appendix 9a. DNA profiling on the gel of RAPD primer B03 for P1



Appendix 9b. Data scoring from gel DNA of RAPD primer B03 for P1

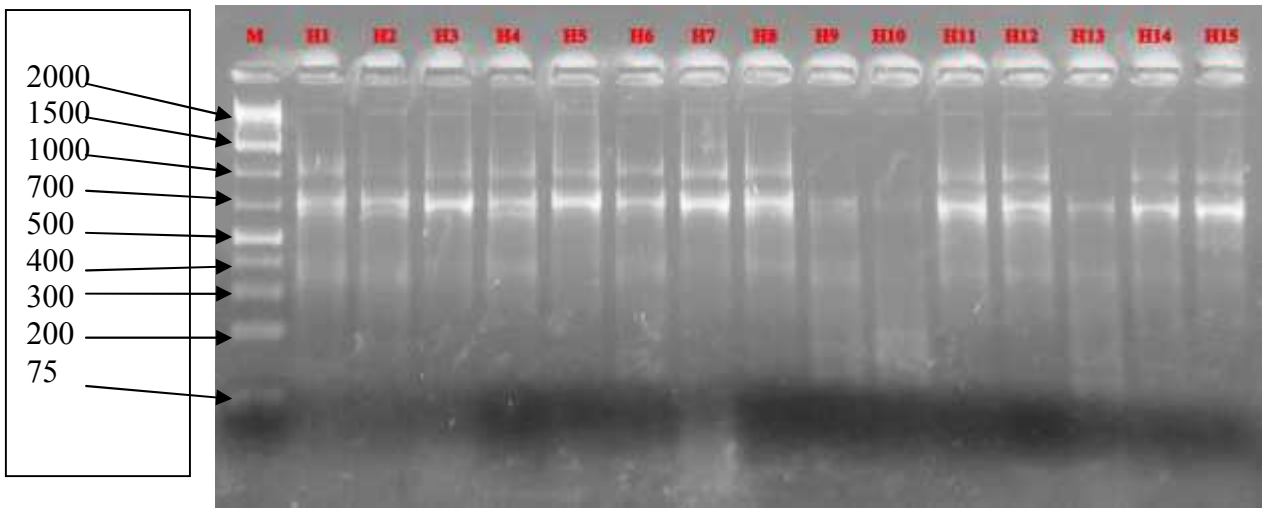
T	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
372	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
391	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
412	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
442	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
705	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
802	1	0	1	0	1	0	0	0	0	0	0	0	0	0	1
826	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
838	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
867	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0
901	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0
1153	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0
1248	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
2027	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
2265	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
2444	0	0	0	0	1	0	1	1	0	1	0	0	1	0	0
2476	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
2508	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
2574	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
2608	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0

T= Tanguar Haor= P1

Appendix 9c. Allelic information of P1 from the primer B03

Situation of Locus	Individuals			Genotype Frequency		Allele Frequency		Hj	Hi
	n	n1	n2	p1	p2	q	p	$(1-p^2-q^2)$	
372	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	0.12023389
391	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
412	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
442	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
705	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
802	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
826	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
838	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
867	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
901	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
1153	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
1248	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
2027	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
2265	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
2444	15	5	10	0.33333333	0.66666667	0.81649658	0.18350342	0.29965983	
2476	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
2508	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
2574	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
2608	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	

Appendix 10a. DNA profiling on the gel of RAPD primer B03 for P2



Appendix 10b. Data scoring from gel DNA of RAPD primer B03 for P2

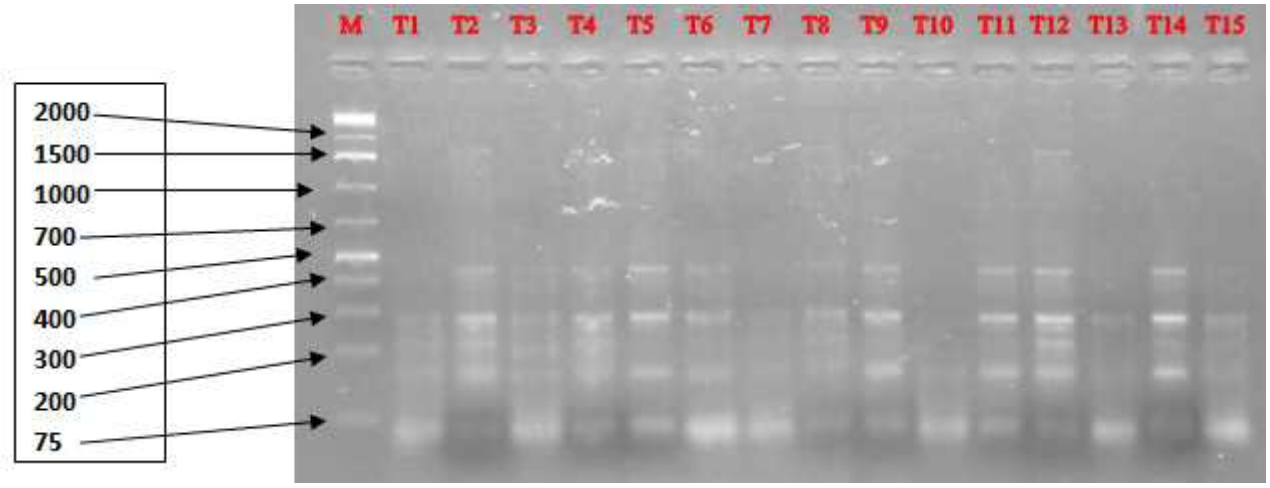
H	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
151	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
329	1	0	0	0	0	0	0	0	1	0	1	1	0	0	0
348	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
391	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
650	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
685	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
705	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0
719	1	0	0	1	1	1	1	0	0	1	0	0	0	0	0
738	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0
755	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
1029	0	0	0	0	0	0	0	1	0	0	1	1	0	1	1
1047	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0
1074	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
1109	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
1153	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2027	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
2084	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
2114	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0
2144	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
2174	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0
2220	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
2265	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0

H= Hakaluki Haor= P2

Appendix 10c. Allelic information of P2 from the primer B03

Situation of Locus	Individuals			Genotype Frequency		Allele Frequency		Hj	Hi
	n	n1	n2	p1	p2	q	p	(1-p2-q2)	
151	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
329	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
348	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
391	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
650	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
685	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
705	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
719	15	6	9	0.4	0.6	0.77459667	0.22540333	0.34919334	
738	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
755	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
1029	15	5	10	0.33333333	0.66666667	0.81649658	0.18350342	0.29965983	0.14273646
1047	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
1074	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
1109	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1153	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
2027	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
2084	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
2114	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
2144	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
2174	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
2220	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
2265	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	

Appendix 11a. DNA profiling on the gel of RAPD primer OPF 14 for P1



Appendix 11b. Data scoring from gel DNA of RAPD primer OPF 14 for P1

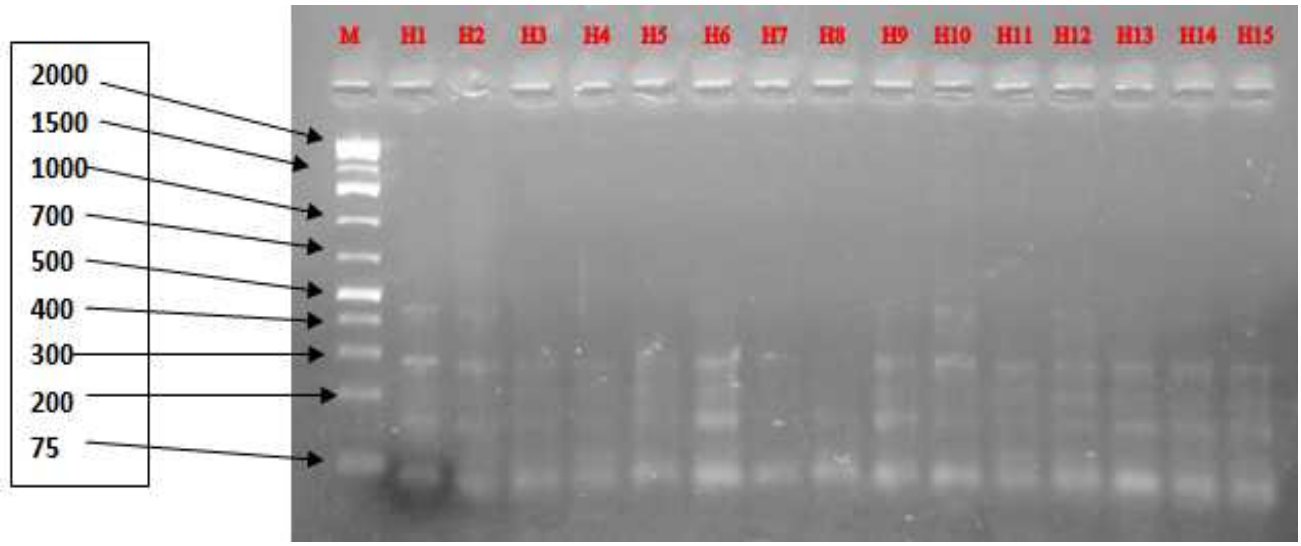
T	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
71	1	1	1	1	0	1	1	0	0	0	1	1	1	0	1
80	0	0	0	0	1	0	0	1	1	1	0	0	0	1	0
124	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1
131	0	1	1	0	0	0	0	0	0	1	0	0	1	0	0
139	0	0	0	1	1	1	1	0	1	0	0	0	0	1	0
151	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0
170	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
181	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
199	0	0	0	1	0	0	0	1	0	0	0	1	0	1	0
241	0	1	1	0	0	0	1	0	0	0	1	0	1	0	0
251	1	0	0	1	1	1	0	0	0	0	0	1	0	0	0
261	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1
280	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
404	0	0	1	0	0	1	0	0	0	0	0	1	0	1	0
412	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
423	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
455	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0
1566	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0

T= Tanguar Haor= P1

Appendix 11c. Allelic information of P1 from the primer OPF 14

Situation of Locus	Individuals			Genotype Frequency		Allele Frequency		Hj	Hi
	n	n1	n2	p1	p2	q	p	(1-p2-q2)	
71	15	10	5	0.66666667	0.33333333	0.57735027	0.42264973	0.48803387	
80	15	5	10	0.33333333	0.66666667	0.81649658	0.18350342	0.29965983	
124	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
131	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
139	15	6	9	0.4	0.6	0.77459667	0.22540333	0.34919334	
151	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
170	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
181	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
199	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	0.20492367
241	15	5	10	0.33333333	0.66666667	0.81649658	0.18350342	0.29965983	
251	15	5	10	0.33333333	0.66666667	0.81649658	0.18350342	0.29965983	
261	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
280	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
404	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
412	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
423	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
455	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
1566	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	

Appendix 12a. DNA profiling on the gel of RAPD primer OPF 14 for P2



Appendix 12b. Data scoring from gel DNA of RAPD primer OPF 14 for P2

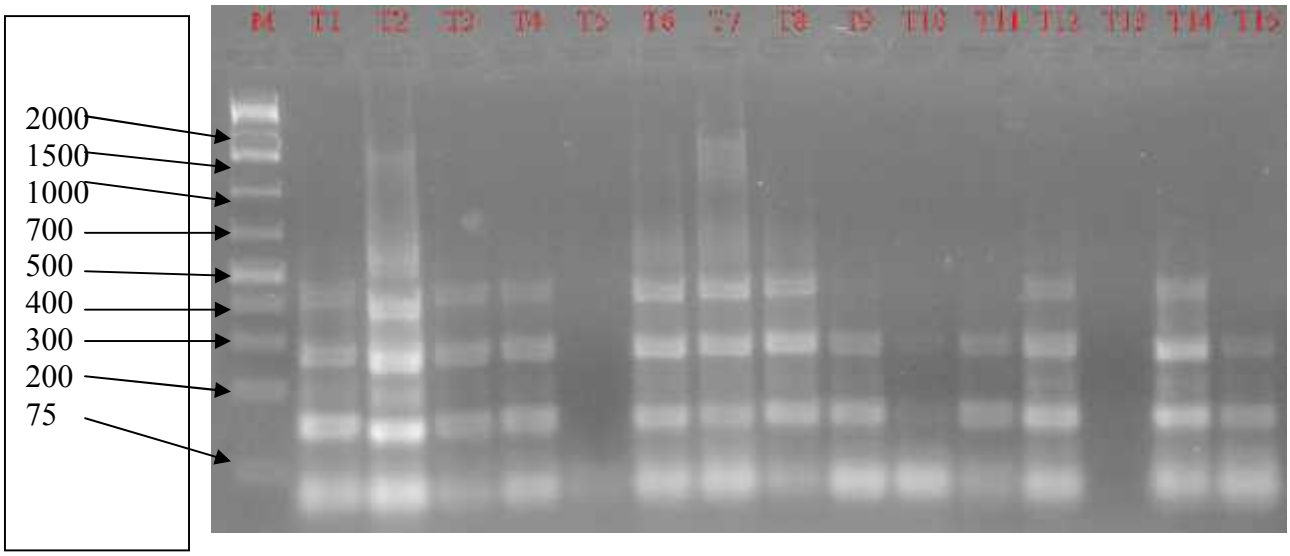
H	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
71	0	0	1	0	0	0	1	0	1	0	1	1	1	1	1
80	1	1	0	1	1	1	0	1	0	1	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
115	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1
131	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0
139	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
151	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
170	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
181	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
199	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
212	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0
224	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0
241	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
251	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0
360	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
372	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
404	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
423	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

H= Hakaluki Haor= P2

Appendix 12c. Allelic information of P2 from the primer OPF 14

Situation of Locus	Individuals			Genotype Frequency		Allele Frequency		Hj	Hi
	n	n1	n2	p1	p2	q	p	(1-p2-q2)	
71	15	8	7	0.53333333	0.46666667	0.68313005	0.31686995	0.43292677	
80	15	7	8	0.46666667	0.53333333	0.73029674	0.26970326	0.39392682	
90	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
115	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
131	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
139	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
151	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
170	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
181	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	0.15227182
199	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
212	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
224	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
241	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
251	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
360	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
372	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
404	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
423	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	

Appendix 13a. DNA profiling on the gel of RAPD primer C04 for P1



Appendix 13b. Data scoring from gel DNA of RAPD primer C04 for P1

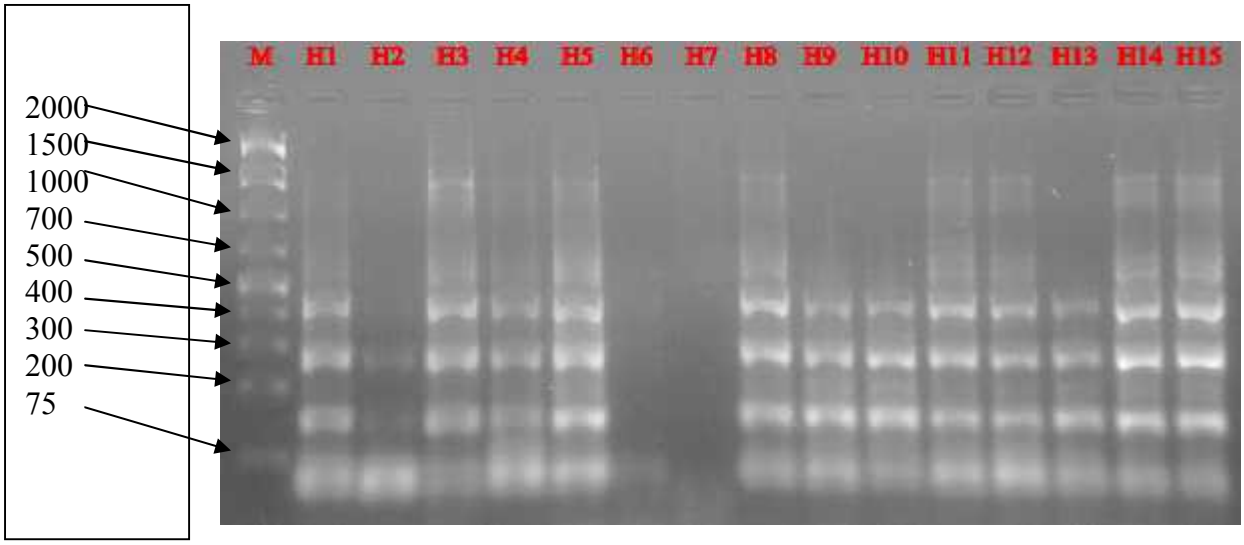
T	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
40	0	1	0	0	1	1	0	0	1	1	0	0	1	0	0
60	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
71	0	1	1	1	0	0	1	0	1	0	0	0	0	0	1
80	0	0	0	0	1	1	0	1	0	0	1	1	0	0	0
124	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
131	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
139	1	0	0	0	0	1	1	0	1	0	0	1	0	1	0
151	0	0	0	0	0	0	0	1	0	1	1	0	0	0	1
170	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0
199	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
224	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
241	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
251	1	0	0	1	0	1	0	0	0	0	0	0	0	1	0
270	0	0	0	0	0	0	0	1	1	1	1	1	0	0	1
360	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
391	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
423	0	0	1	1	0	1	1	0	0	0	0	0	0	1	0
442	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0
520	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
635	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
1315	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
1473	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0

T= Tanguar Haor= P1

Appendix 13c. Allelic information of P1 from the primer C04

Situation of Locus	Individuals			Genotype Frequency		Allele Frequency		Hj	Hi
	n	n1	n2	p1	p2	q	p	(1-p2-q2)	
40	15	6	9	0.4	0.6	0.77459667	0.22540333	0.34919334	
60	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
71	15	6	9	0.4	0.6	0.77459667	0.22540333	0.34919334	
80	15	5	10	0.33333333	0.66666667	0.81649658	0.18350342	0.29965983	
124	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
131	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
139	15	6	9	0.4	0.6	0.77459667	0.22540333	0.34919334	
151	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
170	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
199	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
224	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	0.16625388
241	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
251	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
270	15	6	9	0.4	0.6	0.77459667	0.22540333	0.34919334	
360	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
391	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
423	15	5	10	0.33333333	0.66666667	0.81649658	0.18350342	0.29965983	
442	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
520	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
635	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1315	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1473	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	

Appendix 14a. DNA profiling on the gel of RAPD primer C04 for P2



Appendix 14b. Data scoring from gel DNA of RAPD primer C04 for P2

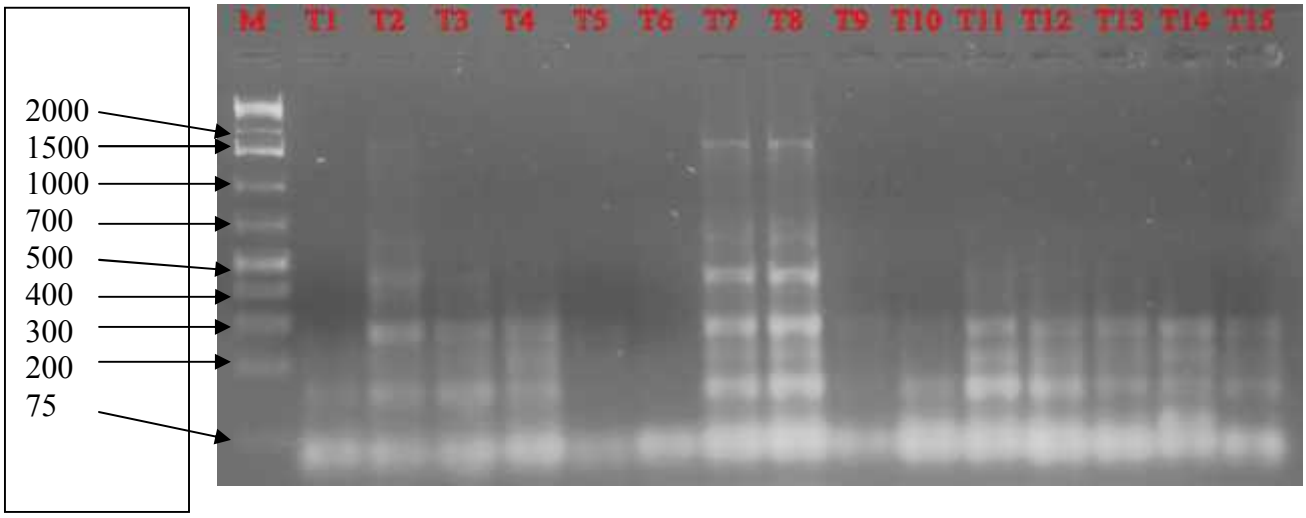
H	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
60	1	0	1	1	0	0	0	0	0	0	0	0	0	1	0
71	0	1	0	0	1	1	1	1	1	1	1	1	1	0	1
124	1	1	1	1	1	0	0	0	0	0	0	1	1	0	0
131	0	0	0	0	0	0	0	1	1	1	1	0	0	1	1
224	1	0	1	0	0	0	0	0	0	1	0	1	1	0	1
241	0	1	0	1	1	0	0	0	1	0	1	0	0	1	0
251	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
360	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
383	1	0	1	0	0	0	0	0	0	1	0	0	1	0	0
391	0	0	0	1	0	0	0	0	1	0	1	1	0	0	1
423	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
520	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
544	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0
571	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
1409	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0

H= Hakaluki Haor= P2

Appendix 14c. Allelic information of P2 from the primer C04

Situation of Locus	Individuals			Genotype Frequency		Allele Frequency		Hj	Hi
	n	n1	n2	p1	p2	q	p	(1-p2-q2)	
60	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
71	15	11	4	0.73333333	0.26666667	0.51639778	0.48360222	0.49946223	
124	15	7	8	0.46666667	0.53333333	0.73029674	0.26970326	0.39392682	
131	15	6	9	0.4	0.6	0.77459667	0.22540333	0.34919334	
224	15	6	9	0.4	0.6	0.77459667	0.22540333	0.34919334	
241	15	6	9	0.4	0.6	0.77459667	0.22540333	0.34919334	
251	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	0.22938291
360	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
383	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
391	15	5	10	0.33333333	0.66666667	0.81649658	0.18350342	0.29965983	
423	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
520	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
544	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
571	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1409	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	

Appendix 15a. DNA profiling on the gel of RAPD primer OPB 05 for P1



Appendix 15b. Data scoring from gel DNA of RAPD primer OPB 05 for P1

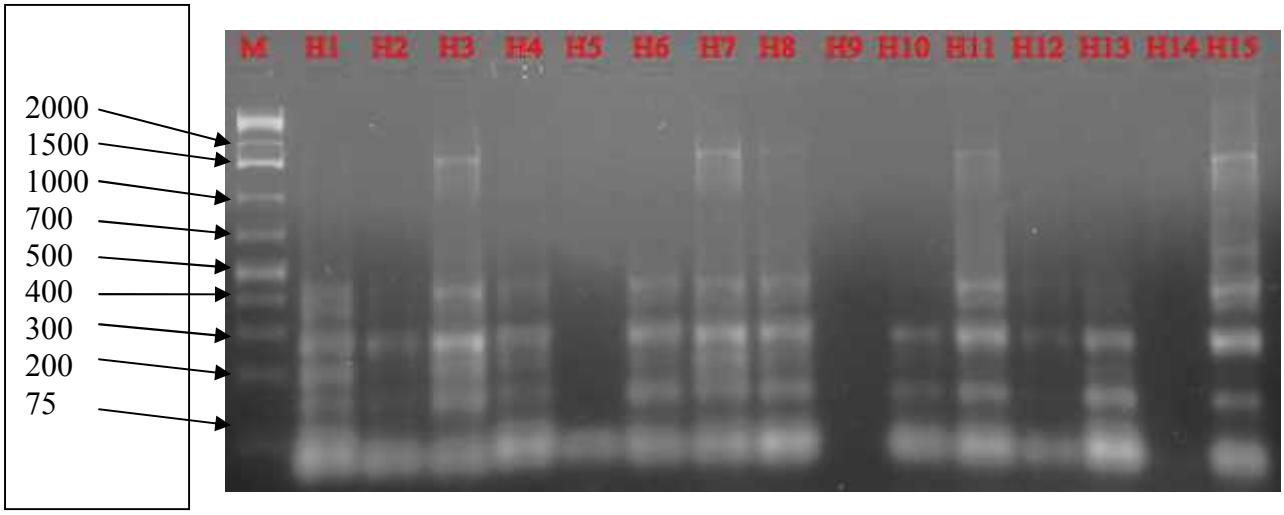
T	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
71	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	1	1	1	1	0	1	1	0	1	0	1
90	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0
131	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
139	1	0	0	1	0	0	0	0	0	1	1	1	0	1	0
151	0	0	0	0	0	0	1	1	0	0	0	0	1	0	1
191	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
199	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0
212	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
251	0	1	1	1	0	0	0	0	0	0	0	0	1	0	0
261	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1
270	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0
280	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
423	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
442	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
455	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
705	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
719	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
1517	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
1581	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
1605	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

T= Tanguar Haor= P1

Appendix 15c. Allelic information of P1 from the primer OPB 05

Situation of Locus	Individuals			Genotype Frequency		Allele Frequency		Hj	Hi
	n	n1	n2	p1	p2	q	p	(1-p2-q2)	
71	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
80	15	8	7	0.53333333	0.46666667	0.68313005	0.31686995	0.43292677	
90	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
131	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
139	15	6	9	0.4	0.6	0.77459667	0.22540333	0.34919334	
151	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
191	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
199	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
212	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
251	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	0.1515266
261	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
270	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
280	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
423	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
442	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
455	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
705	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
719	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1517	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1581	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1605	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	

Appendix 16a. DNA profiling on the gel of RAPD primer OPB 05 for P2



Appendix 16b. Data scoring from gel DNA of RAPD primer OPB 05 for P2

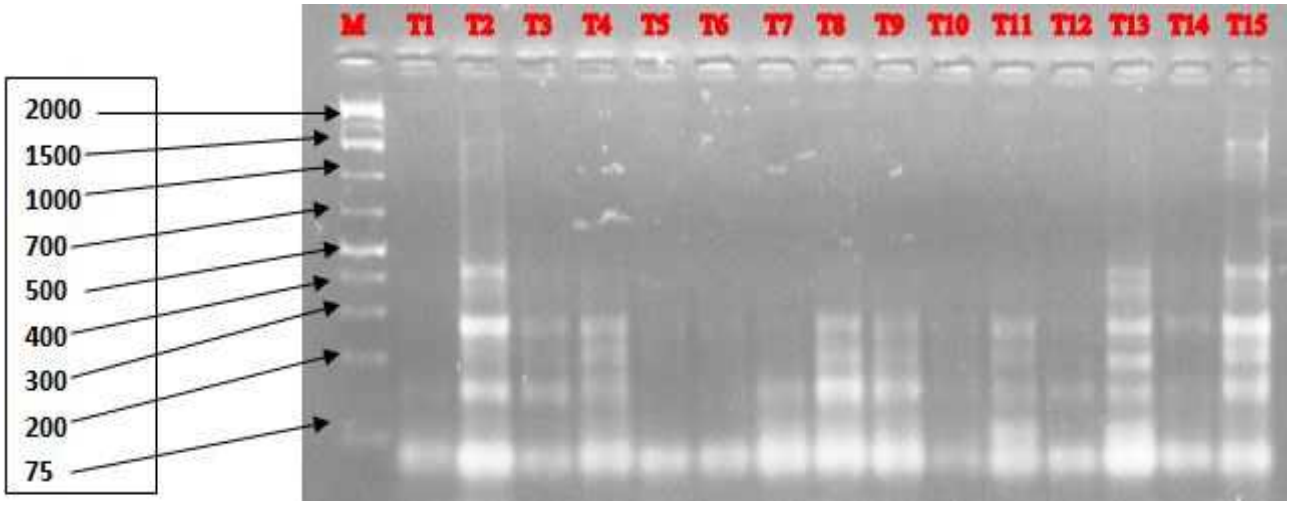
H	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
71	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1
80	0	0	0	1	1	1	0	1	0	0	0	1	1	0	0
90	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
124	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
131	0	1	1	0	0	0	0	0	0	0	0	0	1	0	1
139	0	0	0	1	0	1	0	1	0	0	1	0	0	0	0
151	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
170	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
224	1	0	1	0	0	0	0	0	0	0	0	1	1	0	0
241	0	1	0	0	0	0	1	0	0	1	0	0	0	0	1
251	0	0	0	1	0	1	0	1	0	0	1	0	0	0	0
348	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
391	0	0	1	0	0	0	0	1	0	0	1	0	1	0	0
412	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1
423	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
544	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
1409	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
1501	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
1533	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
1566	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0

H= Hakaluki Haor= P2

Appendix 16c. Allelic information of P2 from the primer OPB 05

Situation of Locus	Individuals			Genotype Frequency		Allele Frequency		Hj	Hi
	n	n1	n2	p1	p2	q	p	(1-p2-q2)	
71	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
80	15	6	9	0.4	0.6	0.77459667	0.22540333	0.34919334	
90	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
124	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
131	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
139	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
151	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
170	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
224	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
241	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	0.16467167
251	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
348	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
391	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
412	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
423	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
544	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1409	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1501	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
1533	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1566	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	

Appendix 17a. DNA profiling on the gel of RAPD primer OPB 08 for P1



Appendix 17b. Data scoring from gel DNA of RAPD primer OPB 08 for P1

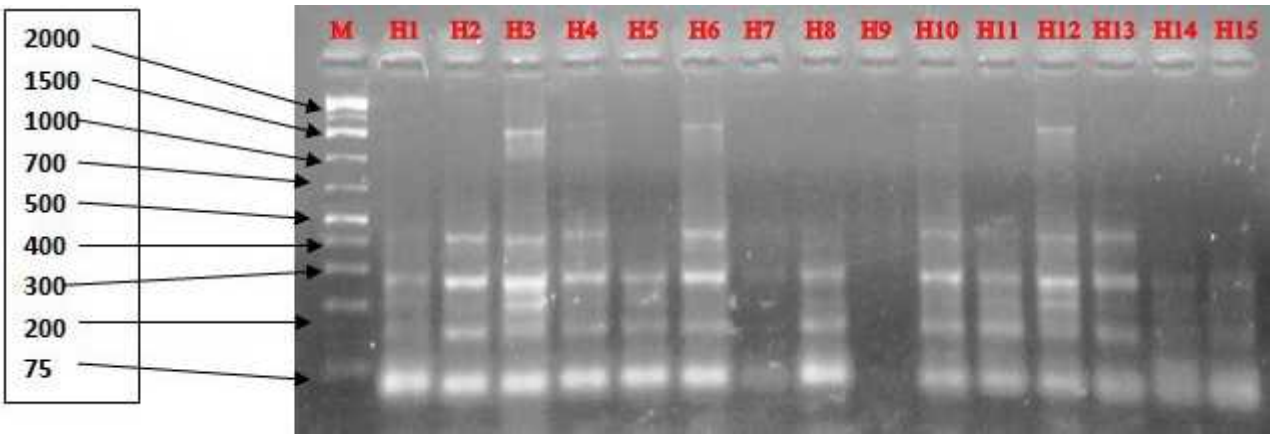
T	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
60	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
115	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
124	1	1	1	1	0	0	1	1	0	0	1	1	0	0	0
139	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
160	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
170	0	0	0	1	0	0	0	1	1	0	1	0	0	0	0
191	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
232	0	1	0	0	0	0	0	0	1	0	1	0	0	1	0
241	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1
261	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
348	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
412	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
1605	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

T= Tanguar Haor= P1

Appendix 17c. Allelic information of P1 from the primer OPB 08

	Individuals No.			Genotype Frequency		Allele Frequency		Hj	Hi
Locus	n	n1	n2	p1	p2	q	p	(1-p2-q2)	
115	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
124	15	8	7	0.53333333	0.46666667	0.68313005	0.31686995	0.43292677	
139	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
160	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
170	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
191	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	0.14494615
232	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
241	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
261	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
348	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
412	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
1605	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	

Appendix 18a. DNA profiling on the gel of RAPD primer OPB 08 for P2



Appendix 18b. Data scoring from gel DNA of RAPD primer OPB 08 for P2

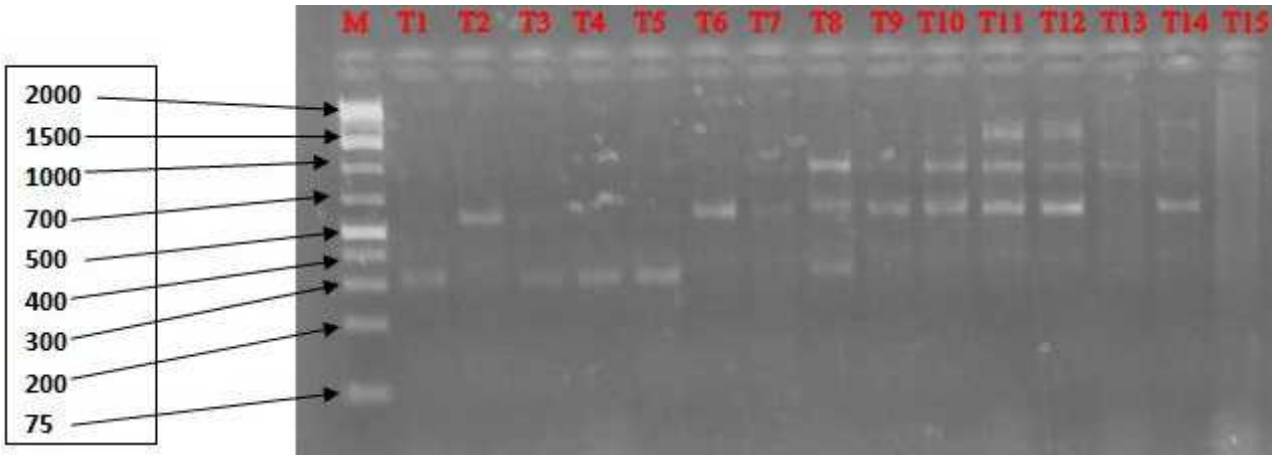
H	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
60	0	1	1	0	0	0	0	0	0	1	0	1	0	1	1
71	1	0	0	1	1	1	1	1	0	0	1	0	1	0	0
115	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
124	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0
139	0	0	0	1	1	1	0	0	0	1	1	0	0	0	0
160	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
191	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
199	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
212	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1
232	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0
241	1	0	1	0	1	0	0	0	0	0	0	1	1	0	0
261	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
270	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
318	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
372	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
391	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0
423	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0
1339	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
1517	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0

H= Hakaluki Haor= P2

Appendix 18c. Allelic information of P2 from the primer OPB 08

Situation of Locus	Individuals			Genotype Frequency		Allele Frequency		Hj	Hi
	n	n1	n2	p1	p2	q	p	(1-p2-q2)	
60	15	6	9	0.4	0.6	0.77459667	0.22540333	0.34919334	
71	15	8	7	0.53333333	0.46666667	0.68313005	0.31686995	0.43292677	
115	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
124	15	5	10	0.33333333	0.66666667	0.81649658	0.18350342	0.29965983	
139	15	5	10	0.33333333	0.66666667	0.81649658	0.18350342	0.29965983	
160	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
191	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
199	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
212	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	0.17282057
232	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
241	15	5	10	0.33333333	0.66666667	0.81649658	0.18350342	0.29965983	
261	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
270	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
318	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
372	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
391	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
423	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
1339	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
1517	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	

Appendix 19a. DNA profiling on the gel of RAPD primer OPB 19 for P1



Appendix 19b. Data scoring from gel DNA of RAPD primer OPB 19 for P1

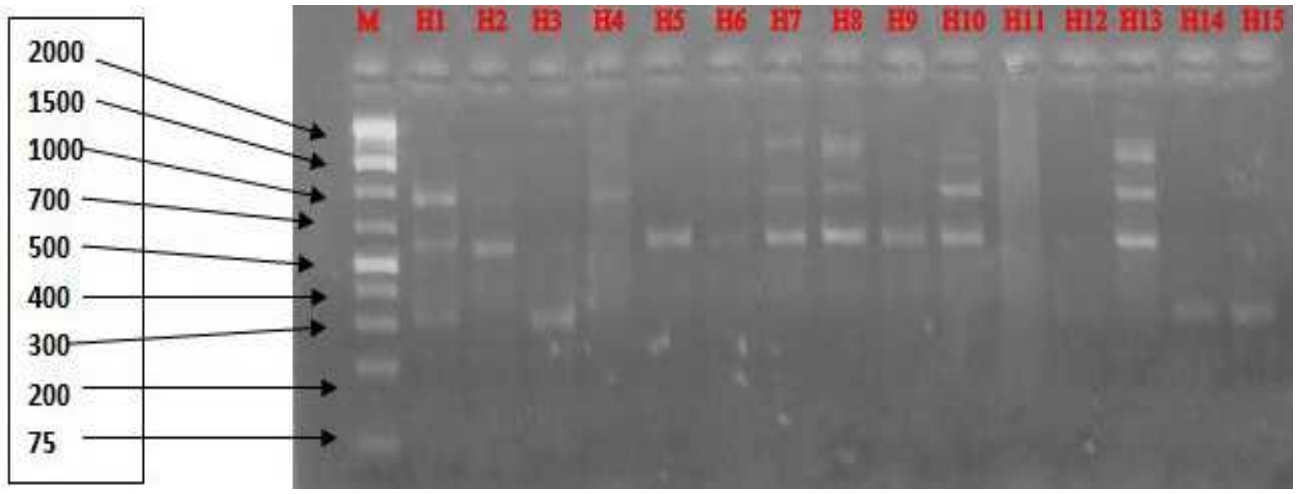
T	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
280	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
310	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
348	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
571	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
650	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
685	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0
705	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0
867	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
1047	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
1109	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
1129	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
1153	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
1181	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
1248	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
1605	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
1675	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
1710	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
1920	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

T= Tanguar Haor= P1

Appendix 19c. Allelic information of P1 from the primer OPB 19

Situation of Locus	Individuals			Genotype Frequency		Allele Frequency		Hj	Hi
	n	n1	n2	p1	p2	q	p	(1-p2-q2)	
280	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
310	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
318	15	0	15	0	1	1	0	0	
348	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
571	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
605	15	0	15	0	1	1	0	0	
618	15	0	15	0	1	1	0	0	
635	15	0	15	0	1	1	0	0	
650	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
685	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
705	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	0.09673452
867	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1047	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1074	15	0	15	0	1	1	0	0	
1109	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1129	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
1153	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1181	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1248	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1605	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1675	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1710	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1920	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	

Appendix 20a. DNA profiling on the gel of RAPD primer OPB 19 for P2



Appendix 20b. Data scoring from gel DNA of RAPD primer OPB 19 for P2

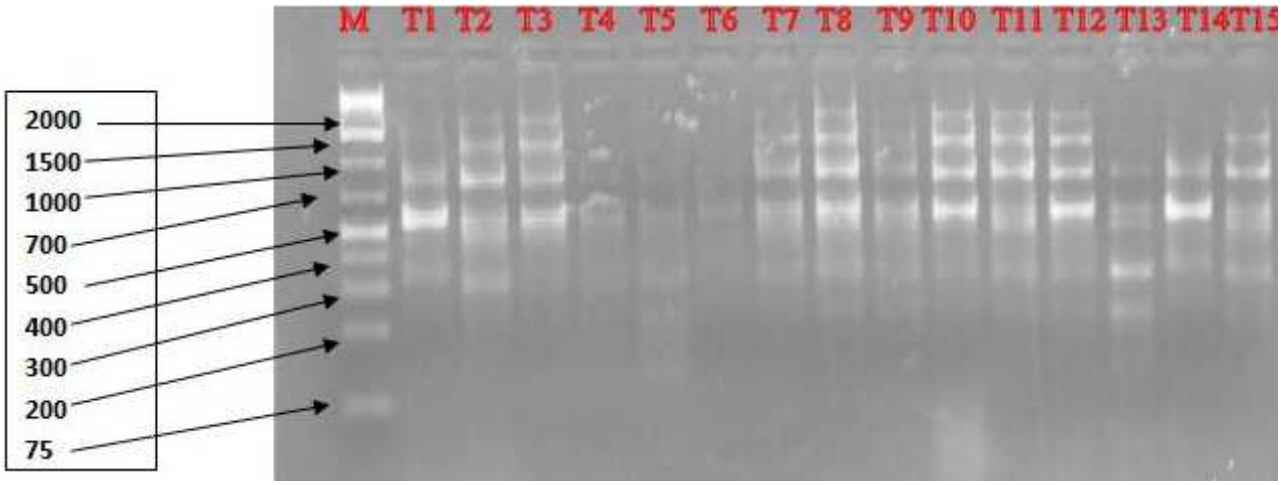
H	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
280	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
318	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1
571	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
605	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
618	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
635	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
650	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
685	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
705	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
926	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
993	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
1015	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
1047	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
1074	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
1129	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
1153	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
1422	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
1581	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
1656	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
1728	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
1766	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
2703	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0

H= Hakaluki Haor= P2

Appendix 20c. Allelic information of P2 from the primer OPB 19

Situation of Locus	Individuals			Genotype Frequency		Allele Frequency		Hj	Hi
	n	n1	n2	p1	p2	q	p	(1-p2-q2)	
280	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
318	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
571	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
605	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
618	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
635	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
650	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
685	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
705	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
926	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
993	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	0.08831818
1015	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1047	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
1074	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1129	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1153	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
1422	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1581	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1656	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1728	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1766	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
2703	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	

Appendix 21a. DNA profiling on the gel of RAPD primer OPB 12 for P1



Appendix 21b. Data scoring from gel DNA of RAPD primer OPB 12 for P1

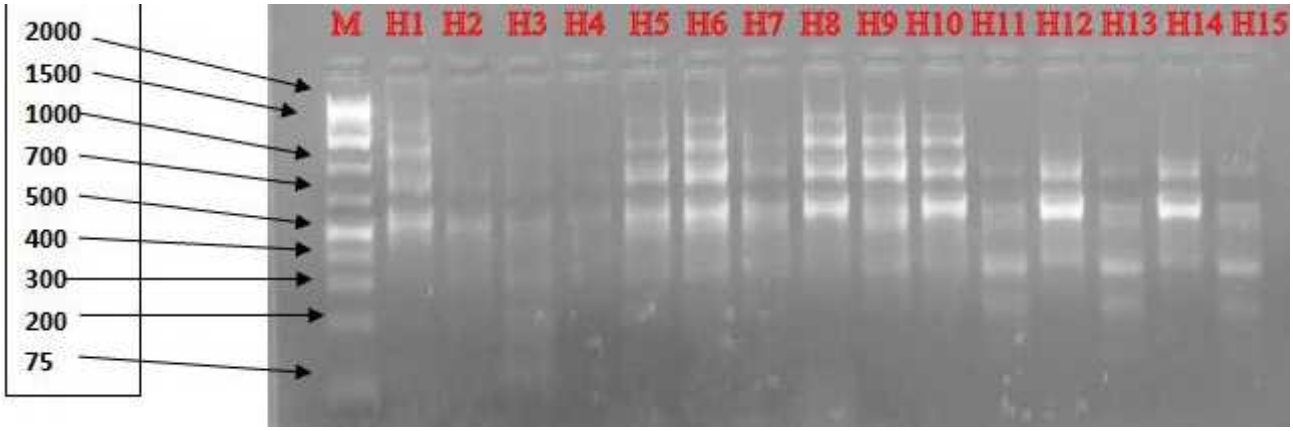
T	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
212	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
232	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
301	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
329	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
348	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0
360	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
383	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
544	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
589	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
605	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0
618	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
635	0	0	0	0	1	1	1	0	0	0	0	1	1	0	1
650	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
890	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
926	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
959	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
976	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0
993	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
1029	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
1047	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
1074	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
1315	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
1339	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0
1362	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
1396	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
1422	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
1742	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
1766	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0
1836	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0

T= Tanguar Haor= P1

Appendix 21c. Allelic information of P1 from the primer OPB 12

Situation of Locus	Individuals			Genotype Frequency		Allele Frequency		Hj	Hi
	n	n1	n2	p1	p2	q	p	(1-p2-q2)	
212	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
232	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
301	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
329	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
348	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
360	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
383	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
544	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
589	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
605	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
618	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
635	15	6	9	0.4	0.6	0.77459667	0.22540333	0.34919334	
650	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
890	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
926	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	0.11613069
959	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
976	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
993	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1029	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1047	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
1074	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1315	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1339	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
1362	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
1396	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1422	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1742	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1766	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
1836	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	

Appendix 22a. DNA profiling on the gel of RAPD primer OPB 12 for P2



Appendix 22b. Data scoring from gel DNA of RAPD primer OPB 12 for P2

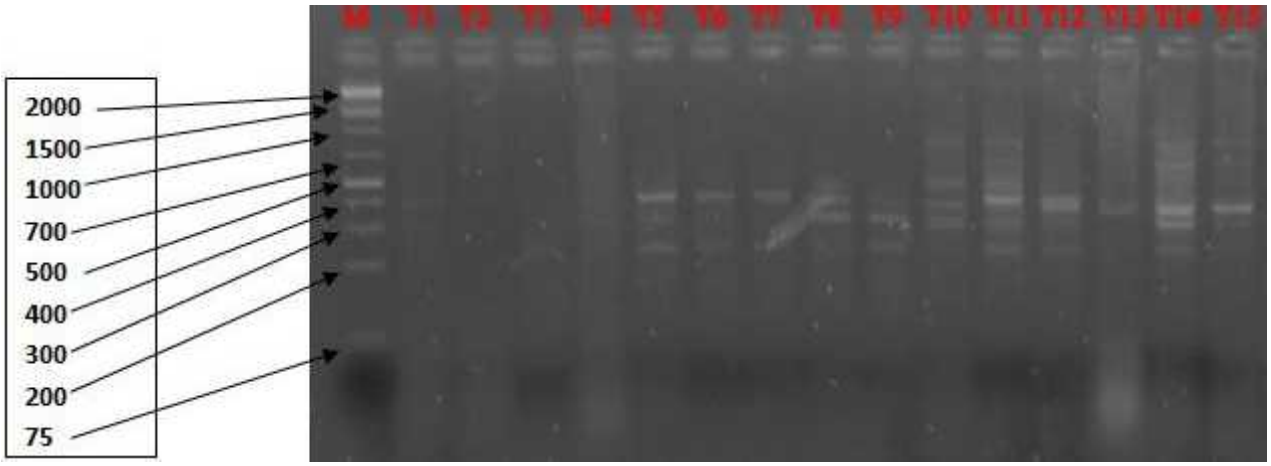
H	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
170	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
212	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
232	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
301	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
329	0	0	0	0	1	1	0	0	1	0	0	0	1	0	1
360	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
404	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
520	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
544	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
571	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1
589	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
618	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
650	0	0	0	0	1	0	0	1	0	0	1	1	0	0	0
685	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
826	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
890	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
959	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0
976	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0
1015	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
1029	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
1047	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
1074	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
1248	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1295	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
1362	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
1396	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
1422	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
1449	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
1728	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1836	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
1884	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
1920	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

H= Hakaluki Haor= P2

Appendix 22c. Allelic information of P2 from the primer OPB 12

Situation of Locus	Individuals			Genotype Frequency		Allele Frequency		Hj	Hi
	n	n1	n2	p1	p2	q	p	(1-p2-q2)	
170	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
212	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
232	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
301	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
329	15	5	10	0.33333333	0.66666667	0.81649658	0.18350342	0.29965983	
360	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
404	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
520	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
544	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
571	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
589	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
618	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
650	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
685	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
826	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
890	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	0.10955696
959	15	4	11	0.26666667	0.73333333	0.85634884	0.14365116	0.24603101	
976	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
1015	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
1029	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1047	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1074	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1248	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1295	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1362	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1396	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
1422	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1449	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1728	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1836	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
1884	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	
1920	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	

Appendix 23a. DNA profiling on the gel of RAPD primer UBC 122 for P1



Appendix 23b. Data scoring from gel DNA of RAPD primer UBC 122 for P1

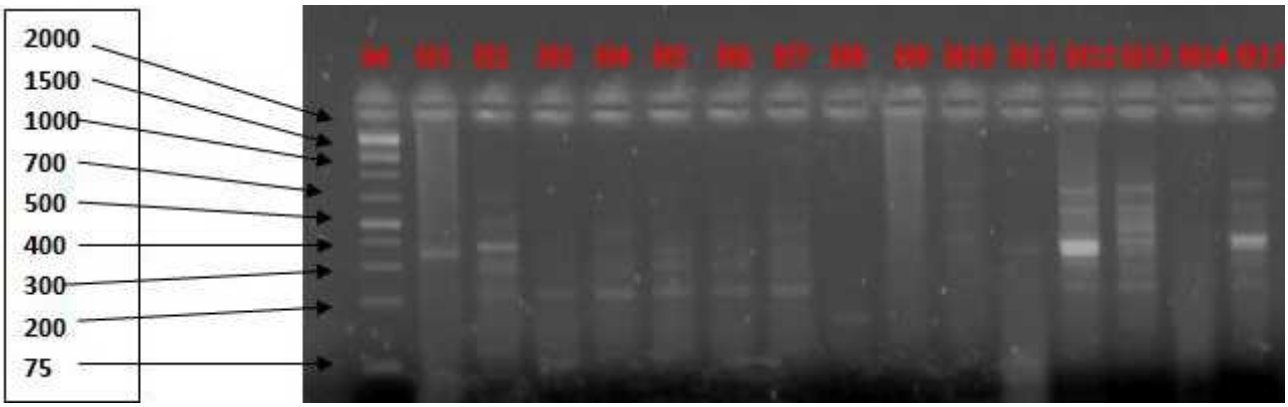
T	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
360	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
520	1	0	0	0	1	0	1	0	0	0	1	0	0	1	0
544	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0
571	0	0	0	0	0	0	0	1	1	1	1	0	0	1	0
589	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1
605	0	0	0	0	1	1	1	1	0	0	1	0	0	0	0
518	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
635	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0

T= Tanguar Haor= P1

Appendix 23c. Allelic information of P1 from the primer UBC 122

Situation of Locus	Individuals			Genotype Frequency		Allele Frequency		Hj	Hi
	n	n1	n2	p1	p2	q	p	(1-p2-q2)	
360	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
520	15	5	10	0.33333333	0.66666667	0.81649658	0.18350342	0.29965983	
544	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	
571	15	5	10	0.33333333	0.66666667	0.81649658	0.18350342	0.29965983	0.20588661
589	15	5	10	0.33333333	0.66666667	0.81649658	0.18350342	0.29965983	
605	15	5	10	0.33333333	0.66666667	0.81649658	0.18350342	0.29965983	
518	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
635	15	2	13	0.13333333	0.86666667	0.93094934	0.06905066	0.12856534	

Appendix 24a. DNA profiling on the gel of RAPD primer UBC 122 for P2



Appendix 24b. Data scoring from gel DNA of RAPD primer UBC 122 for P2

H	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
310	0	0	1	1	1	1	1	1	0	0	0	1	1	0	0
318	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
329	1	1	0	0	0	0	0	0	0	0	0	1	1	0	1
348	0	0	0	1	0	0	0	0	0	1	1	1	1	0	0
360	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1

H= Hakaluki Haor= P2

Appendix 24c. Allelic information of P2 from the primer UBC 122

Situation of Locus	Individuals			Genotype Frequency		Allele Frequency		Hj	Hi
	n	n1	n2	p1	p2	q	p	(1-p2-q2)	
310	15	8	7	0.53333333	0.46666667	0.68313005	0.31686995	0.43292677	0.25732354
318	15	1	14	0.06666667	0.93333333	0.96609178	0.03390822	0.0655169	
329	15	5	10	0.33333333	0.66666667	0.81649658	0.18350342	0.29965983	
348	15	5	10	0.33333333	0.66666667	0.81649658	0.18350342	0.29965983	
360	15	3	12	0.2	0.8	0.89442719	0.10557281	0.18885438	

RFLP Analysis

Appendix 25. Number of total bands revealed from 30 individuals of freshwater mud eel, *Monopterus cuchia* after digestion by +Hpy178III and +CfrI

Individuals	+Hpy178III	+CfrI	Total bands
1	2	2	4
2	2	2	4
3	2	2	4
4	2	2	4
5	2	2	4
6	2	2	4
7	2	2	4
8	2	2	4
9	2	2	4
10	2	2	4
11	2	2	4
12	2	2	4
13	2	1	3
14	2	1	3
15	2	2	4
16	1	2	3
17	2	2	4
18	2	2	4
19	3	2	5
20	1	2	3
21	2	2	4
22	3	2	5
23	1	2	3
24	3	2	5
25	3	2	5
26	1	2	3
27	3	2	5
28	2	1	3
29	1	1	2
30	1	1	2

Appendix 26. Analysis of RFLP banding patterns and calculated F-value for 30 individuals of *Monopterus cuchia* [above the diagonale: the shared amount of fragments; on the diagonale: The amount of bands in each individual; below the diagonale: Calculated F-value, where F=2 N_{XY} / (Nx + Ny)]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	**4**	4	4	4	4	4	4	4	4	4	4	4	2	2	4	2	4	4	2	2	4	2	2	2	2	2	2	2	0	0
2	1	**4**	4	4	4	4	4	4	4	4	4	4	2	2	4	2	4	4	2	2	4	2	2	2	2	2	2	2	0	0
3	1	1	**4**	4	4	4	4	4	4	4	4	4	2	2	4	2	4	4	2	2	4	2	2	2	2	2	2	2	0	0
4	1	1	1	**4**	4	4	4	4	4	4	4	4	2	2	4	2	4	4	2	2	4	2	2	2	2	2	2	2	0	0
5	1	1	1	1	**4**	4	4	4	4	4	4	4	2	2	4	2	4	4	2	2	4	2	2	2	2	2	2	2	0	0
6	1	1	1	1	1	**4**	4	4	4	4	4	4	2	2	4	2	4	4	2	2	4	2	2	2	2	2	2	2	0	0
7	1	1	1	1	1	1	**4**	4	4	4	4	4	2	2	4	2	4	4	2	2	4	2	2	2	2	2	2	2	0	0
8	1	1	1	1	1	1	1	**4**	4	4	4	4	2	2	4	2	4	4	2	2	4	2	2	2	2	2	2	2	0	0
9	1	1	1	1	1	1	1	1	**4**	4	4	4	2	2	4	2	4	4	2	2	4	2	2	2	2	2	2	2	0	0
10	1	1	1	1	1	1	1	1	1	**4**	4	4	2	2	4	2	4	4	2	2	4	2	2	2	2	2	2	2	0	0
11	1	1	1	1	1	1	1	1	1	1	**4**	4	2	2	4	2	4	4	2	2	4	2	2	2	2	2	2	2	0	0
12	1	1	1	1	1	1	1	1	1	1	1	**4**	2	2	4	2	4	4	2	2	4	2	2	2	2	2	2	2	0	0
13	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	**3**	2	2	0	2	2	0	0	2	0	0	0	0	0	0	3	1	1
14	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.67	**3**	2	0	2	2	0	0	2	0	0	0	0	0	0	3	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	0.57	0.57	**4**	2	4	4	2	2	4	2	2	2	2	2	2	2	0	0
16	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0	0	0.57	**3**	2	2	2	3	2	2	3	2	2	3	2	0	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1	0.57	0.57	1	0.57	**4**	4	2	2	4	2	2	2	2	2	2	2	0	0
18	1	1	1	1	1	1	1	1	1	1	1	1	0.57	0.57	1	0.57	1	**4**	2	2	4	2	2	2	2	2	2	2	0	0
19	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0	0	0.45	0.5	0.45	0.45	**5**	2	2	5	2	5	5	2	5	0	0	0
20	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0	0	0.57	1	0.57	0.57	0.5	**3**	2	2	2	2	2	2	2	0	1	1
21	1	1	1	1	1	1	1	1	1	1	1	1	0.57	0.57	1	0.57	1	1	0.45	0.57	**4**	2	2	2	2	2	2	2	0	0
22	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0	0	0.45	0.5	0.45	0.45	1	0.5	0.45	**5**	2	5	5	2	5	0	0	0
23	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0	0	0.57	1	0.57	0.57	0.5	0.67	0.5	0.5	**3**	2	2	2	2	0	1	1
24	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0	0	0.45	0.5	0.45	0.45	1	0.5	0.45	1	0.5	**5**	5	2	5	0	0	0
25	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0	0	0.45	0.5	0.45	0.45	1	0.5	0.45	1	0.5	1	**5**	2	5	0	0	0
26	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0	0	0.57	1	0.57	0.57	0.5	0.67	0.57	0.5	0.67	0.5	0.5	**3**	2	0	1	1
27	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0	0	0.45	0.5	0.45	0.45	1	0.5	0.45	1	0.5	1	1	0.5	**5**	0	0	0
28	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	1	1	0.57	0	0.57	0.57	0	0	0.57	0	0	0	0	0	0	**3**	1	1
29	0	0	0	0	0	0	0	0	0	0	0	0	0.4	0.4	0	0.4	0	0	0	0.4	0	0	0.4	0	0	0.4	0	0.4	**2**	2
30	0	0	0	0	0	0	0	0	0	0	0	0	0.4	0.4	0	0.4	0	0	0	0.4	0	0	0.4	0	0	0.4	0	0.4	1	**2**

Appendix 27. Number of total bands revealed from P1of *Monopterus cuchia* after digestion by +Hpy178III and +CfrI

Individuals (T)	+Hpy178III	+CfrI	Total bands
1	2	2	4
2	2	2	4
3	2	2	4
4	2	2	4
5	2	2	4
6	2	2	4
7	2	2	4
8	2	2	4
9	2	2	4
10	2	2	4
11	2	2	4
12	2	2	4
13	2	1	3
14	2	1	3
15	2	2	4

Appendix 28. Number of total bands revealed from P2 of *Monopterus cuchia* after digestion by +Hpy178III and +CfrI

Individuals (H)	+Hpy178III	+CfrI	Total bands
1	1	2	3
2	2	2	4
3	2	2	4
4	3	2	5
5	1	2	3
6	2	2	4
7	3	2	5
8	1	2	3
9	3	2	5
10	3	2	5
11	1	2	3
12	3	2	5
13	2	1	3
14	1	1	2
15	1	1	2

Appendix 29. Analysis of RFLP banding patterns and calculated F-value of P1 [above the diagonale: the shared amount of fragments; on the diagonale: The amount of bands in each individual; below the diagonale: Calculated F-value, where $F=2 N_{XY} / (Nx + Ny)$]

T	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	**4**	4	4	4	4	4	4	4	4	4	4	4	2	2	4
2	1	**4**	4	4	4	4	4	4	4	4	4	4	2	2	4
3	1	1	**4**	4	4	4	4	4	4	4	4	4	2	2	4
4	1	1	1	**4**	4	4	4	4	4	4	4	4	2	2	4
5	1	1	1	1	**4**	4	4	4	4	4	4	4	2	2	4
6	1	1	1	1	1	**4**	4	4	4	4	4	4	2	2	4
7	1	1	1	1	1	1	**4**	4	4	4	4	4	2	2	4
8	1	1	1	1	1	1	1	**4**	4	4	4	4	2	2	4
9	1	1	1	1	1	1	1	1	**4**	4	4	4	2	2	4
10	1	1	1	1	1	1	1	1	1	**4**	4	4	2	2	4
11	1	1	1	1	1	1	1	1	1	1	**4**	4	2	2	4
12	1	1	1	1	1	1	1	1	1	1	1	**4**	2	2	4
13	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	**3**	2	2
14	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.67	**3**	2
15	1	1	1	1	1	1	1	1	1	1	1	1	0.57	0.57	**4**

T= Tanguar Haor= P1

Appendix 30. Analysis of RFLP banding patterns and calculated F-value of P2 [above the diagonale: the shared amount of fragments; on the diagonale: The amount of bands in each individual; below the diagonale: Calculated F-value, where $F=2 N_{XY} / (Nx + Ny)$]

H	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	**3**	2	2	2	3	2	2	3	2	2	3	2	0	1	1
2	0.57	**4**	4	2	2	4	2	2	2	2	2	2	2	0	0
3	0.57	1	**4**	2	2	4	2	2	2	2	2	2	2	0	0
4	0.5	0.45	0.45	**5**	2	2	5	2	5	5	2	5	0	0	0
5	1	0.57	0.57	0.5	**3**	2	2	2	2	2	2	2	0	1	1
6	0.57	1	1	0.45	0.57	**4**	2	2	2	2	2	2	2	0	0
7	0.5	0.45	0.45	1	0.5	0.45	**5**	2	5	5	2	5	0	0	0
8	1	0.57	0.57	0.5	0.67	0.5	0.5	**3**	2	2	2	2	0	1	1
9	0.5	0.45	0.45	1	0.5	0.45	1	0.5	**5**	5	2	5	0	0	0
10	0.5	0.45	0.45	1	0.5	0.45	1	0.5	1	**5**	2	5	0	0	0
11	1	0.57	0.57	0.5	0.67	0.57	0.5	0.67	0.5	0.5	**3**	2	0	1	1
12	0.5	0.45	0.45	1	0.5	0.45	1	0.5	1	1	0.5	**5**	0	0	0
13	0	0.57	0.57	0	0	0.57	0	0	0	0	0	0	**3**	1	1
14	0.4	0	0	0	0.4	0	0	0.4	0	0	0.4	0	0.4	**2**	2
15	0.4	0	0	0	0.4	0	0	0.4	0	0	0.4	0	0.4	1	**2**

H= Hakaluki Haor= P2

Appendix 31. Genotype and allele frequencies of P1 by the enzyme +Hpy178III

	Genotypes			Total
	p/p	p/q	q/q	
No. of individuals	0	0	15	15
No. of p alleles	0	0	0	0
No. of q alleles	0	0	30	30
No. of p + q alleles	0	0	30	30
Alleles p= 0, Alleles q=1				

Appendix 32. Genotype and allele frequencies of P1 by the enzyme +CfrI

	Genotypes			Total
	p/q	p/q	q/q	
No. of individuals	2	0	13	15
No. of p alleles	4	0	0	4
No. of q alleles	0	0	26	26
No. of p + q alleles	4	0	26	30
Alleles frequency p= 0.133, Alleles frequency q = 0.867				

Appendix 33. Genotype and allele frequencies of P2 by the enzyme +Hpy178III

	Genotypes			Total
	p/p	p/q	q/q	
No. of individuals	6	5	4	15
No. of p alleles	12	5	0	17
No. of q alleles	0	5	8	13
No. of p + q alleles	12	10	8	30
Alleles frequency p = 0.567, Alleles frequency= 0.433				

Appendix 34. Genotype and allele frequencies of P2 by the enzyme +CfrI

	Genotypes			Total
	p/q	p/q	q/q	
No. of individuals	3	0	12	15
No. of p alleles	6	0	0	6
No. of q alleles	0	0	24	24
No. of p + q alleles	6	0	24	30
Alleles frequency p=0.2 , Alleles frequency q= 0.8				

Appendix 35. Inter population differentiation (g_{st})

Genotypes	A_1A_1	A_1A_2	A_2A_2	p	q	P^2+q^2
Population 1	4	0	56	0.0665	0.9335	0.876
Population 2	18	10	32	0.3835	0.6165	0.527
H_0=1/2(0+0.17)=0.085				s=2		$\sum(P^2+q^2)$=1.403
$1/\tilde{n}= 1/n_1+1/n_2$= 1/60+ 1/60=0.033						$\tilde{n}$ = 30
h_s = (30/30 - 1)[1-1/2(1.403)-(0.085/2(30))] = 0.3073						
$\sum 1/2\sum x_{ij}]^2 = (1/2(0.0665))^2 +(1/2(0.9335))^2 +(1/2(0.3835))^2 +(1/2(0.6165))^2$ = 0.3507						
ht = 1-- 0.3507+ [0.3073/(30x2)] – [0.085/(2x30x2)] = 0.6537						
$g_{st} = 1-(h_s/h_t)$ = 1-(0.3073/0.6537) = 0.5299						

Appendix 36. F statistics

Population	Genotype frequency			Allele frequency			F
	A_1A_1	A_1A_2	A_2A_2	p	q	2pq	
Population 1	0.067	00	0.933	0.0665	0.9335	0.1242	1
Population 2	0.3	0.167	0.533	0.3835	0.6165	0.4729	0.647
H_T	2(0.225)(0.775) =0.3488			p_0 = (0.0665 + 0.3835)/2= 0.225			
H_I	(00+0.167)/2 = 0.0835			q_0 = (0.9335 + 0.6165)/2 =0.775			
H_S	(0.1242 + 0.4729)/2 = 0.2986						
F_{IT} =1-(0.0835/0.3488) = 0.7606							
F_{IS} = 1- (0.0835/0.2986) = 0.7204							
F_{ST} = 1- (0.2986/0.3488) = 0.1439							