CHAPTER-IV
DNA FINGERPRINTING
DNA FINGERPRINTING

INTRODUCTION:

DNA is the molecule of hereditary; it is an integral component of all living matter with the exception of RNA viruses. Any living or non-living organic matter containing relatively intact DNA fragment is used for DNA typing analysis. A common source includes human or other animal blood, semen and solid tissues and plant foliage and seeds.

DNA fingerprinting has become an indelible part of society helping to prove innocence or guilt in criminal cases resolving immigration arguments and clarifying paternity. “DNA fingerprinting started at the headquarters of the British Antarctic Survey in Cambridge,” says Professor Jeffrey, “I collected a big lump of seal meat from their lock up freezer and we got the seal myoglobin gene, had a look at human myoglobin gene and there inside an intron in that gene was tandem repeat DNA.

DNA fingerprinting has proved to be powerful in resolving genetic identity or relationship and is applied in many diverse areas of biological sciences including forensic science, paternity testing, animal breeding and population genetics (Gill et al., 1985; Jeffrey et al., 1985). One attractive DNA fingerprinting method is the detection of hyper variable simple repetitive DNA by means of oligonucleotide probes, which make it possible to establish highly informative DNA fingerprints for any eukaryotic organisms. It is useful for identifying genetic relationship in domestic animals and wild birds (Buitkamp et al., 1991). DNA fingerprinting use of genetic analyses, human percentage, rape and homicide case in animal poaching and medical analysis. The development of the molecular tools in the past decades has stimulated systematic researches, nevertheless, work concerning the parasite is far from being at the level of that of the hosts, especially as the use of molecular data for the study of the phylogenetic relationship and the genetic characterization of the
population of parasites were largely limited to the species being of medical or economic interest (Poulin and Morand, 2000). Although a pair number of genes has been used to study parasitic phylogeny (Olsen and Tkach, 2005). The parasite is more commonly encountered and sampled in the larval stage and hence; morphological studies are not possible, as they are done on adult reproductive complexes or fully developed plerocercoid moreover, few number of morphological characteristics are available for species identification (Dubinina, 1980). Many of the conventional biochemical identification techniques such as, isoenzyme electrophoresis are being by DNA typing.

Okamoto et al., (1995) applied DNA fingerprinting with C (Ac)5 to analysis of genetic variation within *Taenia taeniformis* and reported that C (Ac)5 was a highly resoluble and informative probe for cestodes.

Morphological study has shown that there can be significant variation in parasite length of the adult of *Oesophagostomum bifurcum* (Nematode) among species of primate host (Blotkamp et al., 1993). These observation have suggested the existence of population variation within *Oesophagostomum bifurcum* from human and non-primates, while previous investigations of ribosomal and mitochondrial DNA did not reveal clear evidence of genetic sub structuring within *Oesophagostomum bifurcum* from human and different species of non-human primates (Gasser et al., 1999; De Gruijter et al., 2002), a recent study using random amplification of polymorphic DNA (RADP) analysis provided support for the existence of different genetic groups of *Oesophagostomum bifurcum* according to host species (de Gruijter et al., 2004). The fingerprinting of parasitic nematode *Haemonchus contortus* (Otsen et al., 2001) and bovine lungworm, *Dictyocaulus viviparous* (Huglund et al., 2004) and no investigation of parasitic nematodes of human health importance.

The polymerase chain reaction (PCR) ISSR is a relatively novel technique used to screen a large part of the genome without prior knowledge of sequences. The method provides highly reproducible results and generates abundant
polymorphism in many systems. It is proven to be efficient in distinguishing between populations and closely related species (Zietkiewicz et al., 1994; Robinson et al., 1997; Wolfe et al., 1998 a, b; Hundsdoerfer and Wink, 2006; Maltgiati et al., 2006). The largest regions by ISSR yield higher polymorphism and reducibility (Fang and Roose, 1997; Luque et al., 2002; Wu et al., 2005). According to Behura, (2006) the ISSR technique represents one of the most promising tools in population genetics and deserves increased attention (Esselman et al., 1999). However the relevance of this approach for phylogenetic studies and particularly when comparing genera, tribes or families (Simmons et al., 2007).

The PCR method is commonly used to identify parasitic infection and species diversity for helminthes. The application of PCR method were reported to investigate the genetic variations of the global cestode, *Taenia solium* (Okamoto et al., 2001), to detect the liver fluke, *Ophisthorchis viverrini* from bithynid snail and cyprinid fishes (Maleewong et al., 2003) and to identify the minute intestinal trematodes, *Stellantchasmus falcatus*, infecting half-beaked fish (Dermogenus pusillus) (Sripalwit et al., 2003). Shiff et al., (2000) estimated the quantity of genomic DNA of the cercarial-stage blood fluke, *Schistosoma haematobium* infected snail found that 2.5 ng DNA was optimal for the RAPD method.

DNA fingerprinting of *Ophisthochis viverrini* detected by specific primer (OV-61 and OV-6R), the lowest DNA quantity was 2× 10-17 ng extracted from only one eggs, which was taken directly from the fluke from patient faces (Wongratanachewin et al., 2001). DNA fingerprinting of the lungs fluke, *Paragonimum heterotremus* genomic DNA from 5 eggs taken from the faces of an infected cat was used pPH-13 specific DNA probe and produced 100% sensitivity (Intapal et al., 2005). The differences in species size and stages of parasite may effect the quantities of genomic DNA used in PCR method, the
HAT-RAPD method (Anuatalabhochai et al., 2000) was used effectively to identify *Stellantchasmus falcatus* in half-beaked fish by (Sripalwit et al., 2003).

The present study deals with the investigation on the *Senga rupchandensis* n. sp., *Circumoncobothrium jadhavae* n.sp., *Genarchopsis paithanensis* n.sp., *Allocreadium khami* n sp., *Orientocreadium striatusae* n. sp. collected from freshwater fishes namely *Channa striatus* (Bloch, 1793) and *Mastacembelus armatus* (Lecepede, 1800).
MATERIAL AND METHODS:

Buffer and chemicals:

Extraction buffer:

50 mM Tris-HCl, pH 8.0, containing 1% CTAB (hexadecyl trimethyl ammonium bromide), 0.75 M NaCl, 10 mM EDTA and 100 mg/ml proteinase K.

TAE (Tris-Acetate-EDTA)

- Tris base 48.4 gm
- Acetic Acid 11.42 ml
- 0.5 M EDTA 20 ml

Adjust pH 8.5 using KOH and make final volume to 1 liter.

TBE (Tris-Borate-EDTA) (10X)

- Tris base 108 gm
- Boric Acid 55 gm
- Na<sub>4</sub>EDTA 9.3 gm

Adjust pH 8.3 using KOH and make final volume to 1 Liter.

DNA extraction method:

DNA was extracted from 05 different parasites according to standard protocol Waldschmidt et al., (1997).

Five parasite such as Circumoncobothrium jadhavae n.sp., Genarchopsis paithanensis n. sp., Allocreadium khami n.sp. from Mastacembelus armatus (Lecepede, 1800) and Senga rupchandensis n. sp., Orientocreadium striatusae n.sp. collected from Channa striatus (Bloch, 1793). Each parasite was ground individually in a mortar and pestle containing liquid N2 and homogenized with extraction buffer. Each parasite was ground in a micro centrifuge tube with a plastic pestle in the presence of extraction buffer. The samples were then
incubated at 65°C for 30 min. Samples containing adult individuals were deproteinized twice with one volume each of chloroform. Parasite homogenates were then deproteinized once with one volume Phenol: Chloroform (1:1) and once with one volume chloroform. After deproteinization the samples were centrifuged at 11,750 g for 5 min. Nucleic acid was precipitated by addition of one volume of cold isopropanol and incubation at -20°C for 2 to 24 h. After centrifugation at 16,000 g for 30 min the pellets were washed twice with 70% (v/v) ethanol and vacuum dried for approximately 15 min or dried in the air for 30 min. The pellet was resuspended in 200 ml TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) and incubated with RNAse (100 mg/ml) for 30 min at 37°C. Dry the DNA pellet and dissolve in TE buffer and dissolve the pellet. Store at 4°C.

A small aliquot of isolated DNA was run on a 1% (w/v) TAE gel to check the quality of DNA Sample.

**PCR amplification of DNA with ISSR primers:**

ISSR primer sets were ordered from University of British Columbia (UBC). 10 ISSR primers were used for initial screening. Out of 10 primers, 4 primers gave the amplification (in terms of repeatability, scorability and ability) were selected for identification. The selected 4 primers were 14- 23 mers based with various di-, tri-, nucleotide ISSR repeats. Following primers gave the amplification. The primer sequences such as, Primer 811 – GAG AGA GAG AGA GAG AC; Primer 812 –GAG AGA GAG AGA GAG AA; Primer 814 - CTC TCT CTC TCT CTC TA and Primer 816 – CAC ACA CAC ACA CAC AT.

PCR technique has promoted the development of a range of molecular assay systems which detect polymorphism at molecular level. In this study we used the most widely adopted PCR based ISSR marker technology for characterizing the natural variation amongst the parasite isolates. PCR reactions were carried out in a thermal cycler.
Preparation of master mix for PCR:

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<td>Taq buffer (10X)</td>
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<tr>
<td>DNTP’s (10Mm)</td>
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<tr>
<td>Taq polymerase (3U/ul)</td>
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<td>Primer (3pmol/ul)</td>
<td>2.0µl</td>
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<tr>
<td>Template DNA</td>
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</tr>
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</table>

Setting up a PCR thermal cycler:

- 94 degree for 3 min. - hot start Denaturation
- 94 degree for 15 sec. – Denaturation (38 cycles)
- Annealing for 30 sec. – temp varies according to primer
- Set the reaction for 30 to 38 cycles.
- 72 degree for 1 minute – Extension (38 cycles)
- Last 72 degree for 5 minutes – final extension.
- Proceed 38 reactions
- After completing 38 cycles, load the sample on 1X TBE gel.

Post PCR processes:

PCR products were separated by electrophoresis using 1% Agarose gel in 1X TBE buffer.

ELECTROPHORESIS:

Principle:

Electrophoresis is the movement of charged molecules under the electric field. Electrophoresis is carried out by using agarose gels which are more porous.
It is used to separate large sized macromolecules like DNA or RNA. DNA is negatively charged and move towards anode when an electric field is applied.

**Requirement:**
Agarose powder, boric acid, tris, EDTA, Distilled water, gel loading stock (bromophenol blue), Running buffer commonly used are Tris –borate EDTA (TBE) and Tris- acetate- EDTA (TAE)

**Equipments:**
Electrophoresis apparatus with power supply, magnetic stirrer, micro-pipettes, eppendorf tube etc.

**Procedure:**
1. Dissolve 0.8 agarose in 100ml of 1X TBE by gentle heating on magnetic stirrer with hot plate. This will result in 0.8% agarose gel. Cool the contents.
2. Using a tape seal both the open sides of gel tray.
3. Insert the comb in such a way that 1mm gap between the teeth and surface of tray could be made.
4. To get the thickness of gel to about 4-5 mm, pour agarose solution into the tray. Now hold this for about 30-40 minutes.
5. Gently remove the comb when gel has been solidified. Remove the tapes and keep gel tray into electrophoresis tank.
6. Pour TBE into the tank so that gel can be immersed by about 5mm.
7. Carefully load DNA samples into slots of submerged gel. The samples must be settled at the bottom of the slot.
8. Connect the electric lead in such a way that a negative terminal should be at the end where sample has been loaded.
9. Run electrophoresis at 60 V until loading dye bromophenol blue migrate to the other end of the gel.

10. Turn the button off and disconnect the electric leads. Take out agarose gel from the electrophoresis tank.

**Gel staining and visualization:**

A dye called Ethidium bromide is used to detect the DNA. It intercalates with the DNA. Therefore location of DNA can be detected and visualized when fluoresces under U.V light. From Ethidium bromide stock solution, 1mg/ ml is dissolved in water the agarose gel taken out from electrophoresis tank and dipped in Etbr solution for some time. PCR products on separation by 1% agarose gel using TBE gel buffer and staining are visualized by ultraviolet illumination. The separated genomic DNA as bands fluoresces under U.V light which makes its presence positive.

**Band scoring and data analysis:**

For each sample, each fragment / band that was amplified using ISSR primers was treated as a unit rearrangement in genome. The primers which were given scorable and consistently reproducible amplicons were considered. The gel pictures were taken and documented to computer by using Alpha Imager gel documentation system and size of each amplicon was measured by using Alpha Imager Software with respect to standard molecular weight DNA ladder and molecular weight of each of the potential specific bands was calculated using the software program Alpha Imager.

**Phylogeny analysis:**

The Dendrogram was plotted by using bioinformatics phylogeny Free Tree and Tree View of DNA fingerprint analysis tool.
The phylogenetic analysis carried out by using Free Tree bioinformatics phylogeny tool of DNA fingerprint analysis based on amplicons sizes given by primers showed that isolate number 5 is the basic original sample. Bootstrapping analysis was carried out to find the original an-sister progeny analysis amongst these samples by using Free Tree bioinformatics tool of phylogeny analysis.

**Dendogram analysis**

Dendrogram was plotted y using the Free Tree bioinformatics software to find out the evolution pattern.

**RESULT AND DISCUSSION:**

The result from PCR based ISSR-RAPD method revealed that of the four primers tested such as, Primer 811 – GAG AGA GAG AGA GAG AC; Primer 812 –GAG AGA GAG AGA GAG AA; Primer 814 - CTC TCT CTC TCT CTC TA and Primer 816 – CAC ACA CAC ACA CAC AT produced DNA banding in parasite, total genomic DNA extraction from cestode and trematode parasite such as, *Circumoncobothrium jadhavae* n.sp., *Genarchopsis paithanensis* n.sp., *Allocreadium khami* n.sp. from *Mastacembelus armatus* (Lecèpède, 1800) and *Senga rupchandensis* n.sp., *Orientocreadium striatusae* n.sp. from the host, *channa striatus* (Bloch, 1793).

Amplification of genomic DNA of five parasite sample using ISSR marker analysis, yielded total amplified 50 fragments. ISSR analysis yielded fragments that could be scored, out of which 26 were polymorphic while the remaining were 24 monomorphic in nature.

In ISSR analysis number of amplified fragments ranged from 05 to 15 and which varied in size from 170 bp. to 2230 bp. UBC (University of British Columbia) Primer No. 811, 812, and 816 showed the lowest number of bands while the Primer No. 814 produced the highest number of bands. Out of the 50
amplified 26 bands produced 52% were polymorphic in nature with an average of 10.04 % polymorphic fragments per primer.

The primer based poly (AC at 3’) produced maximum number of bands (15 bands) while the primers having poly (AA at 3’) produced minimum number of bands (5 bands). The PCR amplification products. The complete data was based on a total of 50 bands.

A dendogram analysis was carried out by analysis bioinformatics phylogeny tool Free Tree and Free view of DNA fingerprints anlysis. Distance matrix was calculated by using Neighbour-joining tree construction method of Nei and Li, 1979 which ranged from 0.03797 to 0.30556. five parasites were clustered into four major clusters. Cluster-I comprises of sample Sena rupchandensis n.sp., Genarchopsis paithanensis n.sp.; Cluster-II of single parasite, Circumoncobothrium jadhavae n.sp.; Cluster-III of single parasite namely Orientocreadium striatusae n.sp., Cluster-IV of single parasite namely Allocreadium khami n.sp. thus, all five parasites are grouped together in Cluster I, II, III, and IV. In present molecular analysis, all samples which are significantly different from each other showed genetic variability.

The result of the present study are matching in accordance with the studied carried out by the previous report also used Primer OPA-O9 out of the four tested Primer to identify the hetrophyid trematodes and Ophisthorchis viverrini (Sripalwit et al., 2003) and Metraginimus spp. (Yu et al., 1997 a, b). The lowest DNA concentration and produced distinct bands for DNA analysis between -1×10 -11 ng.

Li and Liao, (2003 ) after suggested for Digrama (a sister genus of Ligula), for this low genetic variability is the migration of the definitive hosts of Ligula (e.g. Larus risidibundus, Ardes cinnerca and Phalacrocorax carbo). Indeed, it is was recently accepted that migratory birds transport organisms across vast distances (McCoy et al., 2003; Gittenberger et al., 2006).
Chalbol Wongswad et al., (2006) observed the banding pattern of some trematode used PCR based HAT- RAPD method revealed that, four primer tested, only used OPA-04, OPA-08, OPA-09 Primer, produced DNA banding in every trematode. OPA-09 was selected for further investigation, since its banding pattern were most distinct.

Pheravut Wongsawad and Chalolb Wongsawad (2007) studied the DNA fingerprinting of some trematodes ie., *Stellantchasmus falcatus* and *Haplorchis taichui* and metacercarial stages used HAT-RAPD method with eight artibitory primers OPA-02, OPA-08, OPA-9, OPN-02, OPN-03, OPN-09, OPX-13, OPH-19 and observed the DNA banding pattern of both adult and metacercarial stage of the trematodes. A total of 180 bands were examined as molecular weight by Kodak ID Image between 160-2,865 bps and three bands were observed in all trematodes of the both stages by OPA-02, OPA-08, and OPX-13 with molecular weights of 580, 560 and 650 bps. The OPN-02 primer had the highest band number (36) and produced the high specific band number (32) in all trematodes.

McGarry et al., (2007) used PCR based two set such as (set-I, set-II). In primer-I, a product of 391 bp. was generated from the genomic DNA of *Fasciola hepatica* whereas no product generated from DNA of *Fasciola gigantica*. PCR based two primer set consistently amplified a 235 bp. product from the DNA of both trematode parasite. *Fasciola hepatica* can be identified by the generation of a 391 bp. by using primer set I and *Fasciola gigantica* can be identified by the lack of a 391- bp product with Primer set-I and the presence of a 235 bp product with primer set-II.

Bouzid W. et al., (2008) are observed the DNA banding pattern of 159 *Ligula intestinalis* sample from the different fishes, PCR based ISSR analyses was performed using nine Primers, use only four Primers such as AUS1-AUS9, TU1, CN1-CN3, FR1- FR3. the size of the bands displayed ranged from 250 to 1500 bp. This four Primers provided different patterns and number of bands but gave almost the same percentage of polymorphism. The total polymorphism (P)
scored between population from different geographical regions was 100% whereas less polymorphism was detected among inmdividuals within each groups. Individuals from China showed the highest polymorphism (55.45%), Germany showed the lowest rate (4055%) the total gene diversity (Ht) was 0.293±0.019, gene diversity within populations (Hs) was 0.065±0.003 and the global coefficient of gene differenciation (Gst) was 0.776. European group gene diversity nwas 0.230. Nei’s genetic indentity (I) ranged from 0.525 to 0.999. the distances in the European-Tunisian group varied from 0.0002 between French and Czech population to 0.0294 between German and Russian ones. Genetic distances between European, Chinese, Algerian, Australian and Canadian. Five main Cluster were defined fro 10 population. The MP tree showed a divergence of groups, with a clear differenciacion between analysed population from China, Australia, Canada and Algeria.

CONCLUSION:

It is concluded that, today the study of DNA fingerprinting method is a very important, this technique applicable for identification of individual, genetic variation, identification of rape suspects, unknown murder, identify of criminals, identify of burnt or unidentified dead body, it also helps in reuniting the lost children with their respective parent or vise versa. DNA fingerprinting method has been patent and being used in Europe and America and accepted in most courts in the United States.
SUMMARY

CHAPTER-I:

The taxonomical study of the cestode and trematode parasites collected from the freshwater fishes, namely Mastacembelus armatus (Lecepede, 1800) and Channa striatus (Bloch, 1793). The cestode parasite belongs to the Eucestoda, Order- Pseudophyllidea, Family- Ptychobothridae. From Order-Pseudophyllidae one genera Circumoncobothrium as one new species described Circumoncobothrium jadhavae n.sp. from Mastacembelus armatus and one genera Senga as two new species are described Senga rambaei n.sp. from Mastacembelus armatus and Senga rupchandensis n.sp. from Channa striatus (Bloch, 1793).

The trematode belongs to the family-Azygiidae belongs one genera Azygia described one redescribed species Azygia stunkardi Rai, 1964 from Channa striatus; family Hemiuridae belongs one genera Genarchopsis described one new species Genarchopsis paithanensis n.sp. from Mastacembelus armatus; family Gorgoderidae, one genera Phyllodistomum described one species Phyllodistomum aurangabadensis n.sp. from Channa striatus; family Allocreadiidae, one genera Allocreadium described two new species, one species Allocreadium khami n.sp. from Mastacembelus armatus and one species Orientocreadium striatusae n.sp. from Channa striatus.

CHAPTER-II:

In this chapter deals with the study of histochemical analysis of acid and alkaline phosphatase of cestode and trematode parasite from freshwater fishes namely Mastacembelus armatus (Lecepede, 1800) and Channa striatus (Bloch, 1793).

Senga rupchandensis n.sp., Orientocreadium striatusae n.sp. collected from Channa striatus and Circumoncobothrium jadhavae n.sp.; Genarchopsis
paithanensis n.sp.; Allocreadium khami n.sp. collected from Mastacembelus armatus.

From histochemically observations of both acid and alkaline phosphatase enzyme activity in Senga rupchandensis n.sp. and Circumoncobothrium jadhavae n.sp. The enzyme activity are high in reproductive organ such as, testes, cirrus pouch, ovary, vitellaria and egg shell and less activity in musculature. In trematode, Genarchopsis paithanensis n.sp. high alkaline phosphatase enzyme activity in sucker, reproductive organ and less in cirrus pouch, musculature and acid phosphatase enzyme activity was observe in reproductive organ except cirrus pouch; Allocreadium khami n.sp. high alkaline phosphatase enzyme activity in intestinal caeca, uterus, vitellaria, testes; moderate in ovary and acid phosphatase enzyme activity in reproductive organ; less in cirrus pouch, sucker and musculature; Orientocreadium striatusae n.sp., the high alkaline phosphatase activity in sucker, reproductive organ and acid phosphatase enzyme activity high in reproductive organ, moderate in intestinal caeca.

CHAPTER-III:

The histopathological study of the cestode and trematode parasites collected from the freshwater fishes, namely Channa striatus (Bloch, 1793) and Mastacembelus armatus (Lecepede, 1800)

Transverse section of the healthy intestine of host, Channa striatus showed the healthy structure whereas in infected intestine with Senga rupchandensis n.sp. is having penetrative type of scolex and they cause heavy mechanical damage to the mucosa and sub mucosa layer.

Transverse section of the healthy liver of Mastacembelus armatus was clearly observed whereas histopathological examination of the infected liver with Circumoncobothrium jadhavae n.sp. cysts is attached into the middle portion of the host liver, the necrosis of parenchyma cells immediately around the cysts is
evident and also large number of inflammatory cells around the cyst, blockage of bile passages, enlargement of hepatocytes and liver vacuolation from the cyst, the sinusoid were ruptured and filled with blood.

Transverse section of the healthy intestine of Mastacembelus armatus was clearly observed whereas in the infected intestine with trematode parasite, Genarchopsis paithanensis n. sp. causing damaged the epithelial layer and approaching the intestinal villi, embedded in the fibroblast, lymphocytes, plasma cells and attached to the intestinal villi, therefore, causing inflammation, vacuolation and damage the intestinal villi.

Transverse section of the healthy liver of Mastacembelus armatus was clearly observed whereas histopathological examination of the infected liver with Allocreadium khami n.sp. cysts attached to the serosal coat of liver therefore, vacuolization, change the shape and size of liver and damage the liver tissue.

Histological structure of healthy buccopharyngeal tissue of the host, Channa striatus (Bloch, 1793) was clearly observed whereas histopathological observation of buccopharyngeal tissue infected with the Orientocreadium striatusae n.sp. attached between pharynx and gills causing inflammation, blocks the gill lamellae and blood capillaries.

The worm is not only successful to enter into the intestine forming the ulceration in the intestinal wall causing damage to the host tissue but the parasite may affect host physiology in many ways that induce stress in the host. The parasitic infection to the disturb the circulation of sugar levels which in turns effects other metabolic pathways.

CHAPTER-IV:

In this chapter deals with the study of DNA fingerprinting of five parasite sample, Circumoncobothrium jadhavae n.sp., Genarchopsis paithanensis n.sp., Allocreadium khami n.sp. collected from the freshwater fish Mastacembelus armatus (Lecepede, 1800) and Senga rupchandensis n.sp., Orientocreadium
*striatusae* n.sp. collected from *Channa striatus* (Bloch, 1793). For DNA fingerprinting using the four ISSR primers like, Primer 811 – GAG AGA GAG AGA GAG AC; Primer 812 – GAG AGA GAG AGA GAG AA; Primer 814 - CTC TCT CTC TCT CTC TA and Primer 816 – CAC ACA CAC ACA CAC AT.

In ISSR analysis number of amplified fragments ranged from 05 to 15 and which varied in size from 170 bp. to 2230 bp. UBC (University of British Columbia) Primer No. 811, 812, and 816 showed the lowest number of bands while the Primer No. 814 produced the highest number of bands.

Today, this technique is advanced and very important for identification of individuals, genetic analyses, human percentage, rape and homicide case in animal poaching and medical analysis.
### Systematic Position of Cestode Parasites with Their Hosts

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<td>1964 (Redescribed)</td>
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<td><em>Channa striatus</em></td>
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Class Trematoda
Rudolphi, 1808
Order Digenea
van Beneden, 1858
Family Hemiuridae
Luhe, 1901
Sub family Halipeginae
Ejsmont, 1931
Genus Genarchopsis
Ozaki, 1925 syn. Progonus
Looss, 1899; Preoccupied
Genarches Looss, 1902;
Preoccupied Ophiocorchis Mastacembelus
Srivastava, 1933. armatus
Species G. paithanensis n.sp. (Lecepede, 1800)

Class Trematoda
Rudolphi, 1808
Order Digenea
van Beneden, 1858
Family Gorgoderidae
Looss, 1901
Sub family Phyllodistominae
(Nybelin, 1926) Yamaguti, 1958
Genus Phyllodistomum Braun, 1899 Channa Striatus
Species P. aurangabadensis n.sp. (Bloch, 1793)
Class Trematoda
Rudolphi, 1808
Order Digenea
van Beneden, 1858
Family Allocreadiidae
(Looss, 1902) Stossich, 1903
Sub family Allocreadiinae
Looss, 1902 Mastacembelus
Genus Allocreadium Looss, 1900 armatus
Species A. Khami n.sp. (Lecepede, 1800)

Class Trematoda
Rudolphi, 1808
Order Digenea
van Beneden, 1858
Family Allocreadiidae
(Looss, 1902) Stossich, 1903
Sub family Orientocreadiinae
Yamaguti, 1958
Genus Orientocreadium
Tubangui, 1931 Syn. Ganada
Chatterji, 1933; Neoganada Dayal, 1938; Nizamia Dayal, 1938; Ganadotrema Dayal, 1949
Paratormopsolus Dubinina et
Bychowsky in Skrjabin, 1954;
Macrotrema Gupta, 1951. Channa striatus
Species O. striatusae n.sp. (Bloch, 1793)
# SYSTEMATIC POSITION OF THE HOST

## HOSTS

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<td>Mastacembellidae</td>
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<tr>
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