CONTENTS

Acknowledgements I-II
Abbreviations III-IV
List of Figures V
List of Photographs VI
List of Tables VII

1. Introduction 1-8
2. Review of Literature 9-39
   2.1 Types of molecular markers 11-21
      2.1.1 Allozyme markers 11-12
      2.1.2 Mitochondrial DNA (mtDNA) markers 12-13
      2.1.3 Random amplified polymorphic DNA (RAPD) markers 13-14
      2.1.4 Amplified fragment length polymorphism (AFLP) 14-15
      2.1.5 Single nucleotide polymorphism (SNP) markers 15-16
      2.1.6 Expressed sequence tags (ESTs) markers 16
      2.1.7 Tandemly Repeated DNA 17-21
         2.1.7.1 Major Satellite arrays 17
         2.1.7.2 Minisatellite markers 17-18
         2.1.7.3 Microsatellite markers 18-21
   2.2 Available methods for microsatellite isolation 21-26
      2.2.1 Traditional Method 21-22
      2.2.2 Primer extension reaction 22-23
      2.2.3 Selective hybridization 23-24
      2.2.4 FIASCO (Fast isolation by AFLP of sequences containing 24-25
              repeats)
      2.2.5 EST based Type I microsatellite markers 25-26
   2.3 Applications of microsatellites 26-39
      2.3.1 Genetic mapping 26-28
3. Materials and Methods

3.1 Collection of fish samples

3.1.1 Fish samples and sites of collection

3.1.2 Blood and muscle samples

3.2 Isolation and quantification of genomic DNA

3.3 Construction of microsatellite enriched genomic library

3.3.1 Digestion and dephosphorylation of genomic DNA

3.3.2 Size selection and extraction of DNA fragments

3.3.3 Preparation of SAU linkers

3.3.4 Ligation of linkers to size selected genomic DNA fragments

3.3.5 Amplification and purification of linker ligated inserts

3.3.6 Construction of microsatellite repeats (concatmers) by ligation and amplification

3.3.7 Hybridization of amplified inserts to amplified repeats

3.3.8 Extraction and amplification of bound DNA from membrane

3.3.9 Digestion, purification and phosphorylation of amplified DNA fragments

3.3.10 Preparation of Vector (pUC18)

3.3.11 Ligation of Vector and inserts

3.3.12 Preparation of competent E. coli (DH5α) cells

3.3.13 Transformation of competent cells with ligation mixture

3.4 Screening of microsatellite enriched genomic library

3.4.1 Labeling of Probes

3.4.2 Screening of colonies with labeled probes

3.4.3 Hybridization and detection positive clones on nylon membranes
3.5 Isolation of microsatellite sequences and primer designing 54-55
3.5.1 Plasmid isolation 54
3.5.2 Sequencing of plasmids 54
3.5.3 Primer Designing 55
3.6 Characterization of isolated microsatellite loci 56-57
3.6.1 Amplification Reaction 56
3.6.2 Polyacrylamide gel electrophoresis (PAGE) 56
and detection of PCR products 56
3.6.3 Assigning of alleles and genotyping 56
3.7 Statistical analysis of data 58

4. Results 59-84
4.1 Microsatellite enriched genomic library construction 59
and isolation of repeat motifs 59
4.2 Primer designing and characterization of microsatellite loci 64
4.3 Polymorphic microsatellite loci in *Chitala chitala* 64
4.4 Genetic variability and population structure analysis 64-84

5. Discussion 85-88
5.1 Microsatellite enriched genomic library construction, isolation 85-86
and characterization of polymorphic loci.
5.2 Genetic variability and population structure analysis 86-88
5.2.1. Genetic variability parameters in *C. chitala* 86-87
5.2.2. Genetic divergence studies in *C. chitala* 87-88

6. Summary 89-91
6.1 Construction of microsatellite enriched genomic library 89-90
and characterization of polymorphic loci.
6.2 Genetic variability and population structure of *Chitala chitala* 90-91

7. References 92-133
Appendix 134-143
List of publications 144
Reprints attached