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Research Article

Phylogenetic analysis of five populations of rice eel in south china based on mtDNA D-loops

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Abstract: Phylogenetic inference was conducted to analyze genetic variation of five *Monopterus albus* populations from south China based on mitochondrial control region of 501 bp in length. Totally 69 individuals were examined and 74 variable sites were detected, in which 12 were singleton variable sites, 62 were parsimony informative sites. These individuals indicated lower level of nucleotide diversity (0.03385±0.00326). Thirty-four haplotypes were analyzed from the 69 individuals, with a haplotype diversity of 0.959±0.013. Phylogenetic analysis revealed three genetic lineages, lineage A, lineage B and lineage C. Among the five populations, only lower degree of gene flow occurred between Hengyang and Guilin populations for relatively short distances departed from each other. However, other three populations (Fuqing, Shantou and Nanjing) were departed from on another by long distances and geographical segregation. Geographic isolation forces all animals to evolve on their own separate paths.
Keywords: *Monopterus albus*, South China, phylogenetic relationships, mtDNA D-loop

INTRODUCTION

Monopterus albus (rice field eel) belongs to the class Osteichthyes (Os-Teichthyes), synbranchiformes (Symbranchiformes), synbranchidae (Synbranchidae) [1]. Eel is a freshwater fish of subtropical regions and has sex change phenomenon with sex reversal from female typical. The female changes gradually to the inter-sex, finally develop into males. In recent years, Chinese scholars such as Yang et al (2005) used enzyme analysis on genetic difference of field eel with different colors from Poyang Lake area [2]. Lu et al (2005) employed common carp microsatellite primers to analyze eel genome DNA polymorphism from different regions [3]. The study of Yin et al (2005) have showed genetic structure between wild population and cultured populations of rice field eel using RAPD technology [4]. In addition, research of polymorphism of *Monopterus albus* mtDNA D-loop was also reported [5].

Mitochondria is an unique cellular organelle in the eukaryotic cells. Mitochondrial DNA is constituted to code and non code regions [6]. The non coding regions is also known as the control area. There are many polymorphism in the D-loop region and near the origin of replication, which are called the hypervariable region I (HVI) and II (HVII). Among the mitochondrial DNA, there are also other polymorphic region some found by scholars [7].

Mitochondrial DNA (mtDNA) has used for animal genetic map construction, germplasm identification and population analysis study, etc. There are many methods about analysis of mtDNA polymorphism used for group or inter-specific genetic research, such as direct sequencing, restriction enzyme digestion, probe method, PCR-RFLP, etc. In this study, we aim to research phylogenetic relationships of five *Monopterus albus* populations from south China based on mtDNA D-loops polymorphisms.

MATERIAL AND METHODS

Animal test and sample collection

We collected a total of 69 specimens of the rice eel from wild populations in five localities (Figure.1, Table.1): Hengyang city, Guilin city, Shantou city, Nanjing city and Fuqing city, all in South China. Swamp eels were found by searching through naturally occurring areas, such as rice field and swamps. In each locality, we selected at least three spots, which were more than 20 km apart from one another. All individuals were captured directly or by means of trapping. From each captured rice eel,we removed approximately 1cm of tail tissue with forceps and Placed it in a sterile1.5-ml microtube containing 75% ethanol. We placed the samples in an ice chest during transport to the laboratory and performed DNA extraction with in 24h [8].

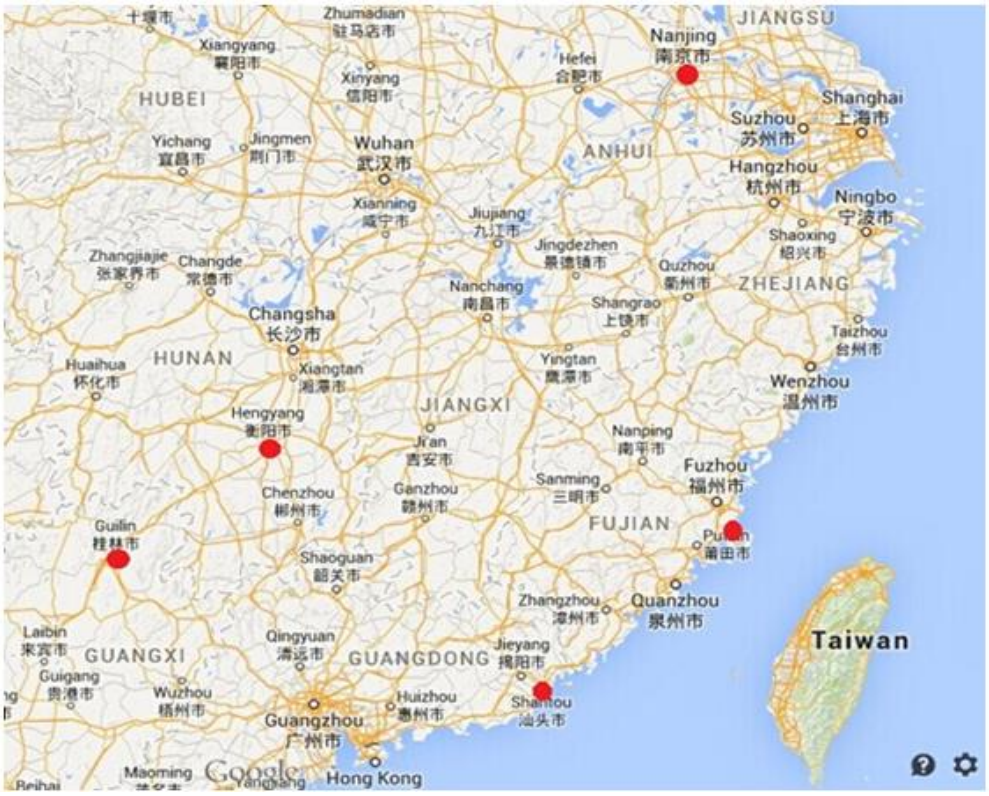

Fig-1: Sampling localities of Chinese swamp eel populations.Sampling localities indicated by red dots; NJ, nanjing; HY ,hengyang; GL, guilin; ST ,shantou; FQ ,fuqing

Table-1: List of M.albus samples used in the study.

Population (Abbrev.)	Locality	Coordinates	Sample size	Haplotypes in each population
Hengyang(HY)	Hengyang,Hunan	112°37′E/26°53′N	15	H19, H20
Nanjing(NJ)	Nanjing,Jiangsu	118°46′E/32°03′N	11	H21,H22,H,H23,H24,H25,H26,H27, H28,H29
Fuqing(FQ)	Fuqing,Fujian	119°23′E/25°42′N	14	H2,H3,H4,H5,H6,H7,H8,H9
Guilin(GL)	Guilin,Guangxi	110°17′E/25°17′N	15	H10,H11,H12,H13,H14,H15,H16,H1 7,H18
Shantou(ST)	Shantou,Guangdong	116°41′E/23°22′N	11	H30,H31,H32,H33,H34
Total			66	

Specific primer design

The mtDNA D-loop sequence (501 bp) of each sample was amplified by PCR, using the primers D1 (5'-GCAGTAAGAGACCACCAACCAGT-3') and D2 (5'-GTGATGGGTGGGAAAAAG AAGTT-3'), designed from the mtDNA genome sequence of *Monopterus albus* (GenBank Accession No.NC_003192) [9].

DNA extraction and PCR amplification

Genomic DNA was isolated from the tissue using the protocols described By Cabe et al. (2007), PCR conditions were as follows: each 50 ul amplification reaction consisted of 400 ng template DNA, 1.5 mM Mg2+, 10 mM Tris-HCl (pH8.3), 50 mM KCl, 0.6 U Taqpolymerase(TaKaRa), 10 uM each primer, and 0.2 mM each dNTP. Thermal cycling was conducted under the following conditions: 4 min denaturation at 94˚C, followed by 30 cycles of 30s at 94˚C, 30s at 56˚C ,1 min at 72˚C, and a final 7 min extension at 72 ˚C, before cooling to 4˚C, for 10 min [9]. The PCR product of each sample was purified using a Qiagen QIAquick PCR purification kit. Each mtDNA D-loop was sequenced using primers D1 and D2 on both directions by an ABI 3730 automated sequencer at Beijing Sunbi006 Ftech Inc.,Beijing,China[6].

Nucleotide and haplotype diversity

All 69 sequences of the mtDNA D-loop were aligned together with the corresponding sequence segment of *Monopterus albus* (GenBank Accession No. NC_003192) in CLUSTAL X [10]. The number of variable sites in the alignment was counted by the software MEGA version4.0 (Tamura et al. 2007). Calculations of nucleotide diversity(Pi) and haplotype

diversity (Hd) and Tajima's test were performed with DNASP version 5.0 [11].

Phylogenetic analysis

Chetia brevis (GenBank Accession No: AY913869) was chosen as out-group for phylogenetic analysis. Although C. brevis is far from monopterus in the phylogeny of acanthomorphfish, yet the identity of mtDNA D-loop of C.brevis to swamp eels (77%) is higher than those of the species belonging to the sister group of the genus Monopterus, such as indostomus paradoxus (NC_004401). In addition, C. brevis was proven to be an optimum out-group in our previous study of phylogeny of *Monopterus albus* from the South China [12].

RESULTS AND DISCUSSION

Totally 69 mtDNA D-loops with the length of 501 bp were examined. Mutations of each haplotype determined from the 69 mtDNA D-loops are scored relative to the reference sequence(acc.no.NC_003192) (Figure 2). Among the 501 sites, 427 were invariable (monomorphic) sites and 74 were variable sites. The 74 variable sites included 12 singleton variable sites and 62 parsimony informative sites (Figure 2). Haplotypes H2 to H8 were observed to contain many mutation sites. Haplotypes H9 to H29 were observed to contain less mutation sites. The intermediate level of mutation were observed among four haplotypes (H30~H33). Due to the high frequency of base substitution, insert or deletion, the phenomenon could lead to the change of mtDNA D-loop site.

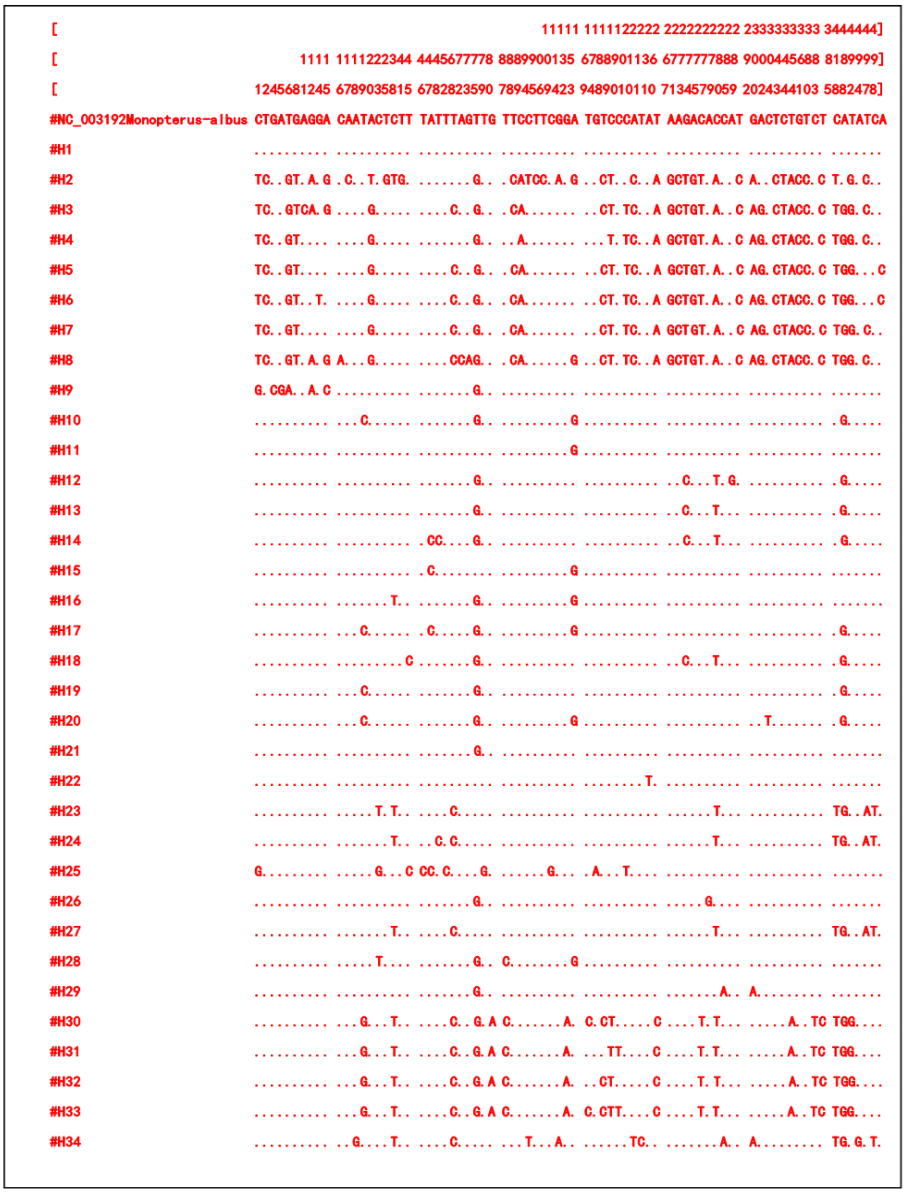

Fig-2: Analysis of wild eel mtDNA D-loop sequence variation site by haplotypes. NC represents a standard sequence (GenBank Accession No.NC_003192); H1-H34 represent different hapotypes. Nucleotide position numbers at the top of the figure correspond to those in NC_003192.

Nucleotide and haplotype diversity were analyzed using software DNASP version 5. The global nucleotide diversity(Pi) of the 69 mtDNA D-loops was 0.03385± 0.00326. The global haplotype diversity for the five populations was 0.959±0.0013 (Table.2). The various populations exhibited diverged Pi and Hd values. Fuqing population indicated highest level of nucleotide diversity (0.02371±0.00757), while HY population presented lowest degree of nucleotide diversity (0.00167±0.00045). NJ population showed the highest level of haplotype diversity (0.936±0.051), while lowest level of haplotype diversity was displayed by HY population (0.419±0.113). The lower Pi(0.00167±0.00045) and Hd (0.419±0.113) of the eel population also suggested a lower genetic heterogeneity in this population.

Table-2: Nucleotide diversity (Pi±SD) and haplotype diversity (Hd ± SD) of each population.

Population(Abbrev.)	Nucleotide diversity(Pi ± SD)	Haplotype diversity(Hd ±SD)
HY	0.00167±0.00045	0.419±0.113
NJ	0.01054±0.00164	0.936±0.051
FQ	0.02371±0.00757	0.934±0.038
GL	0.00768±0.00062	0.924±0.044
ST	0.00254±0.00058	0.709±0.137
Total	0.03385±0.00326	0.959±0.0013

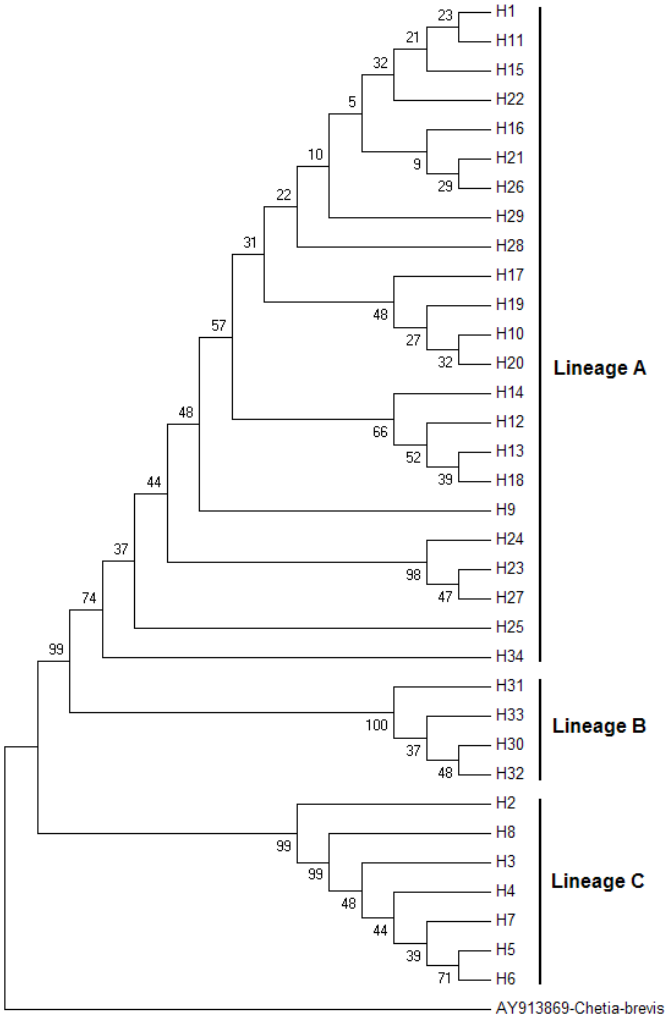

Fig-3: UPGMA tree constructed from 34 mt DNA D-loop haplotypes (501bp) for *Monopterus albus* from South China. Phylogenetic analysis revealed that all the individuals of the five population fell into three genetic lineages, lineage A, lineage B and lineage C.

Mitochondrial DNA is maternally inherited, through which maternal phylogenetic can be analyzed. The tree illustrated that the 34 haplotypes fell into three genetic lineages A, B and C (Figure.3). Firstly, the H2, H8, H3, H4, H7, H5 and H6 were converged into lineage C. Secondly,H31, H32, H33 and H34 was comprised into

lineage B. Lastly, the other was concluded into lineage A. They maintain their identities in nature mainly because of geographic isolation. In further analysis, the individuals from HY, NJ and GL population were mainly include in lineage A. The combination of Limited variation and weak phylogenetic structure revealed that the individuals from the HY and GL exhibited lower level of mtDNA diversity and therefore should probably be treated as a single ancestor in maternal lineages. Individuals from ST population were mainly comprised in lineage B showed little gene exchange with other regional populations. In this sense, ST population might originate from a single sub-group of maternal lineages. Lineage C comprised individuals from FQ population. Fuqing is located in Fujian province and there are fewer rivers, but more mountains in its territory than in other places. Therefore, FQ population should also be a native population and we further referred that Lineage C dominating FQ population may be an indigenous lineage in China South.

CONCLUSIONS

Phylogenetic inference was conducted to analyze genetic variation of five *Monopterus albus* populations from south China based on DNA D-loops of 501 bp in length. The global nucleotide diversity (Pi) of the 69 mt DNA D-loops was 0.03385±0.00326 and the global haplotype diversity was 0.959±0.0013. Phylogenetic analysis revealed that all the individuals of the five population fell into three genetic lineages, lineage A, lineage B and lineage C. Genetic differences among the five population could possibly be attributed to geographical isolation. The result of this work could provide useful information to the research on specific origin, classification, phylogenetic evolution and breeding for rice field eel in the future.

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