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Genetic Diversity of *Silurus asotus* on Mitochondrial DNA *16SrRNA* Gene Partial Sequence

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Abstract: The 600 bp fragments of *16SrRNA* gene were obtained by PCR Method from the mitochondrial DNA of *Silurus asotus* from four species groups and a mitochondrial DNA 16SrRNA of 540 bp sequence was successfully sequenced by Clustal X nomologous sequence analysis. The three upper branch groups are from Yalong Jiang in Sichuan province, Min river in Sichuan province and Wujiang river in Guizhou province, respectively. The middle branch group is from Wuyang river in Guizhou province. The genetic identity from 1~0.9980 among groups and the genetic distance from 0~0.0020 among groups were calculated by PopGen32. The results showed that there was no variation on the gene of the species groups from Wujiang river in Guizhou province. There were some variations on the gene of Yalong Jiang in Sichuan province, Min river in Sichuan province and Wuyang river in Guizhou province. The 4 individuality there were 4 haplotypes in Min river in Sichuan province. The 4 individuality there were 4 haplotypes in Yalong Jiang in Sichuan province. The 4 individuality only 1 haplotype in Wujiang river in Guizhou province. The 6 individuality there were 2 haplotypes in the middle branch group is from Wuyang river in Guizhou province. But there were some variations among the four species groups. The dendrogram of genetic relationship constructed by PopGen32 indicates that they are divided into four branches.

Key words: *Silurus asotus*, mitochondrial DNA, 16SrRNA, genetic diversity, China

INRODUCTION

Silurus asotus belongs to Siluriformes, Siluridae, Silurus which located in the Changjiang river and in the larger rivers of the South Changjiang river (Xiang-Lin, 1977). From the early 1980s, the artificial reproduction, nutrition, etc. have been gradually studied in China (Gang and Huaihui, 1994; Gang *et al*., 2005).

Mitochondrial DNA was widely used in fisheries studies due to its maternal inheritance, faster evolutionary rate than nuclear DNA and so on (Avise, 2000; Brown, 1983; Guo-Qing and Si-Fa, 1998; Khan *et al*., 2010). So far, the researches on fish mtDNA *16SrRNA* gene sequence have been reported while limited studies on analysis of the genetic diversity of different populations of *Silurus asotus* according to the variants of *16SrRNA* gene sequence (Zhou *et al*., 2004; Song *et al*., 2007).

Therefore, in this study, researchers measured the *16S rRNA* gene sequences of 18 individuals from four *Silurus asotus* species and analysed their genetic diversity which can contribute to the protection of fishes resources, genetic breeding and also, provide the basic information for the molecular genetic.

MATERIALS AND METHODS

Silurus asotus were collected from Yalong Jiang (Sichuan, China), Min river (Sichuan, China), Wujiang River (Guizhou, China), Wuyang river (Guizhou, China), respectively (Table 1). Tissue samples, i.e., skeletal muscle, liver, ovary were frozen in liquid nitrogen and then stored at -20°C.

Genomic DNA extraction: About 100 mg liver or 200 mg muscle was digested with the lysis buffer containing proteinase K (30 mmol L^{-1} Tris-HCl, pH 8.0, 0.1 mol L^{-1} EDTA, 0.1 mol L^{-1} NaCl, 1% SDS) at 55°C water bath for 2-4 h then extracted with phenol:chloroform:isoamyl alcohol (25:24:1) twice followed by chloroform:isoamyl alcohol (24:1) extraction and then with cold ethanol (-20°C) precipitation DNA, dried and dissolved in TE, the set stored at -20°C.

Table 1: Material origins, numbers and codes of *Silurus asotus*

Original sources	Number	Codes	Numbers
Yalong Jiang (Sichuan)	4	YLJ	YLJ1 YLJ2 YLJ3 YLJ4
Min river (Sichuan)	4	MJ	MJ1 MJ2 MJ3 MJ4
Wujiang river (Guizhou)	4	WJ	WJ1 WJ2 WJ3 WJ4
Wuyang river (Guizhou)	6	WYH	WYH1 WYH2 WYH3 WYH4 WYH5 WYH6

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Amplification and sequencing of *16SrRNA* gene: Primers P1:5'-GATTAGATACCCTGGTAGTCCAC-3' and P2: 5'-CCCGGGAACGTATTC ACCG-3') which synthesized by Shanghai Biotechnology company were used for PCR amplification of the *16SrRNA* gene. The total reaction volume is 50 μL including template DNA 1 μL, each forward and reverse primer, 1 μL (10 μmol L^{-1}), 10× Buffer 5 μL, 25 mmol L^{-1} $MgCl_2$ 5 μL, 1 unit of Taq DNA polymerase, make up the sterile double-distilled water to a final volume, add 2 drops of its disinfection paraffin oil. Thermocycling conditions of PCR reactions are as follows: denaturation at 92°C for 2 min followed by 35 cycles of denaturation at 92°C for 45 sec, annealing at 55°C for 45 sec, extension at 72°C for 1 min then final extension at 72°C for 10 min. PCR amplification products were electrophoresed 2% agarose gel (containing ethidium bromide 0.5 μg mL^{-1}), observed by the UV lamp observation and took a photograph.

PCR products as templates for DNA sequencing were purified by using UNIQ-10 column PCR purification kit (Shanghai Sangon) and ABI 377 automated sequencer was used for two-way sequenced by using sequencing reagent Bigdye terminator 2.0 (Shanghai Sangon). All the sequencing were done in Shanghai biotechnology company.

Data analysis: The sequencing results were upload into the Clustal X and then manually check and choose 540 bp homologous fragments for further analysis. PopGen32 Software was used to calculate genetic distance (Genetic Distance, GD) and genetic identity (Genetic Identity, GI) and the UPGMA (unweighted pair group method using arithmetic average) dendrogram of genetic relationship was also build.

RESULTS AND DISCUSSION

16SrRNA cloning: About 600 bp fragments of *16SrRNA* gene were amplified from different populations of *Silurus asotus* (Fig. 1).

Sequencing results of *16SrRNA* gene: Take the individual sequence of No. 1 Min river populations as the standard, the rest of the sequences were compared with the standard (Table 2).

Genetic distance and genetic identity: PopGen32 Software was used to calculate genetic distance and genetic identity. Genetic distance varied from 0-0.0020 and genetic identity varied from 1-0.9980 (Table 3).

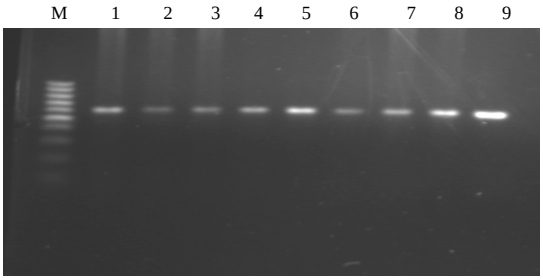

Fig. 1: The *16SrRNA* gene PCR products of *Silurus asotus*. Marker: 100 bp ladder

Cluster analysis: According to the cluster analysis results by using PopGen32 Software, three populations of Yalong Jiang, Min river and Wujiang river clustered into one branch. Individuals 1 and 3 of Wujiang river are together from one branch while individual 2, 4, 5 and 6 are clusted into another branch then together again as one branch.

In this study, *16SrRNA* gene fragments from 18 individual *Silurus asotus* of four populations were measured and the homology of the 540 bp fragment were also analysis.

By comparison (Table 2), among the three populations of the upstream of the Changjiang river, researchers have analysised four individuals of the Min river which have four haplotypes and four individuals of Yalong Jiang which also have four haplotypes were analysised. Toward *16SrRNA* gene, populations in Wujiang river basically have no variation due to the exactly same sequence of four individuals analyzed and the four individuals have only one haplotype. Six individuals in the Wuyang river of upstream the Yuanjiang river were analyed which contains two kinds of haplotypes. In various populations, *16SrRNA* gene sequence of *Silurus asotus* has variation among inter-groups and the variation among populations is more significant than the variation within populations. These results suggested that the genetic diversity of *Silurus asotus* is abundant in the populations from upstream and middle tributaries of the Changjiang river. This results are accordance with the previous publications of the *16SrRNA* gene fragment by RFLP analysis (Wang *et al*., 2007) and cytochrome b gene (Wang and Yu, 2008) sequence analysis.

Table 3, the genetic distance of GD range from 0-0.0020 among the 18 individuals, the largest genetic distance is 0.0020 for the MJ4 and YLJ3 which taken from the Min river and Yalong Jiang, respectively while the lowest genetic distance is 0 for the populations of

Table 2: The 16S rRNA gene sequence of *Silurus astus*

Codes	Sequence					
MJ1	CCGTTGGGAG	CCTTGAGCTC	TTAGTGGCGC	AGCTAACGCA	TTAAGTTGAC	CGCCTGGGGA
YLJ1	-	-	-	-	-	C
YLJ2	-	-	-	-	-	C
YLJ4	-	-	-	-	-	C
MJ1	GTACGGCCGC	AAGGTTAAAA	CTCAAATGAA	TTGACGGGGG	CCCGCACAAG	CGGTGGAGCA
MJ2	-	-	-	-	G	-
YLJ1	T	-	-	-	-	-
YLJ2	T	-	-	-	G	-
YLJ4	T	-	-	-	G	-
MJ1	TGTGGTTTAA	TTCGAAGCAA	CGCGAAAAAC	CTTACCAGGG	CTTGACATCC	AATGAACTTT
YLJ1	-	-	G	C	-	-
YLJ2	-	-	G	C	-	-
YLJ4	-	-	G	C	-	-
WJ	-	-	G	C	-	-
WYH1	-	-	G	C	-	-
WYH4			G	C		
MJ1	CTAGAGATAG	ATTGGTGCCT	TCGGGAACAT	TGAGACAGGT	GCTGCATGGC	TGTCGTCAGC
MJ1	TCGTGTCGTG	AGATGTTGGG	TTAAGTCCCG	TAACGAGCGC	AACCCTTGTC	CTTAGTTACC
MJ3	-	-	-	-	G	-
WYH1	-	-	G	-	-	-
MJ1	TCGTGTCGTG	GGTGGGCACT	CTAAGGAGAC	TG-C-GTGACAA	ACCGGAGGAA	GGTGGGGATG
YLJ1	-	-	-	C G	-	-
YLJ3	-	-	-	C G	-	-
YLJ4	-	-	-	C G	-	-
WYH1	-	-	-	C G	-	-
MJ1	ACGTCAAGTC	ATCATGGCCC	TTACGGCCTG	GGCTACACAC	GTGCTACAAT	GGTCGGTACA
MJ1	GAGGGTTGCC	AAGCCGCGA	GTGGAGCTAA	TCCCACAAAA	CCGATCGTAG	TCCGGATCGC
WJ1	-	A	-	-	-	-
MJ1	AGTCTGCACT	TCGACTGCGT	GAAGTCGGAA	TCGCTAGTAA	TCGCGAATCA	GAATGTCGCG
MJ4	-	-	-	-	-	T
YLJ1	-	-	-	-	-	-
YLJ1	AC	-	-	-	-	-
YLJ2	AC	-	-	-	-	-
YLJ4	AC	-	-	-	-	T
WJ1	AC	-	-	-	-	-
WYH1	AC	-	-	-	-	-
WYH4	AC	-	-	-	-	T

-: The nucleotide absense

Wujiang river and Wuyang river. Genetic Identity (GI) range from 1-0.9980. The biggest is 1 for the two populations of the Wujiang river and Wuyang river and the minimum is 0.9980 for the MJ4 and YLJ3 which were picked up from the Min river and Wuyang river. It can be seen from Fig. 2 in the genetic relationship dendrogram, three populations of the Yalong Jiang, Min river and Wujiang river were clustered into a branch. Samples 1 and 3 from Wuyang river of Yuanjiang together for a small branch while samples 2, 4, 5 and 6 clustered into another branch, two small sticks together again for a general branch and then together with other samples.

Silurus asotus is the indigenous species usually live in some certain section of river and the individual normally is small, weak migration trends and geographic isolation. Each group is maintaining a relatively independent of breeding groups and the gene flow is relatively small for other inter-population. Therefore, the genetic composition of *Silurus asotus* is variated. Thus,

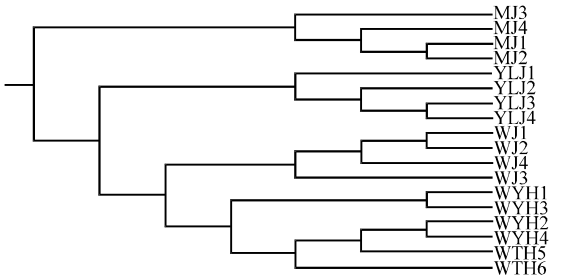

Fig. 2: Dendrogram of genetic relationship among the 18 isolates of *Silurus astus*

Silurus asotus 16SrRNA gene sequences have variations in the populations from upstream and middle tributaries of Changjiang river. Moreover, variation among populations is significant than the variation within populations and their genetic diversity is abundant. Genetic diversity which is an important component of biological diversity is an essential biological resources. Genetic diversity

Table 3: Nei's genetic identity (above diagonal) and genetic distance (below diagonal) for isolates of *Silurus astus*

pop ID	MJ1	MJ2	MJ3	MJ4	YLJ1	YLJ2	YLJ3	YLJ4	WJ1	WJ2	WJ3	WJ4	WYH1	WYH2	WYH3	WYH4	WYH5	WYH6
MJ1	****	0.9998	0.9998	0.9998	0.9987	0.9987	0.9984	0.9984	0.9989	0.9989	0.9989	0.9989	0.9989	0.9991	0.9989	0.9991	0.9991	0.9991
MJ2	0.0002	****	0.9996	0.9996	0.9984	0.9984	0.9982	0.9982	0.9987	0.9987	0.9987	0.9987	0.9987	0.9989	0.9987	0.9989	0.9989	0.9989
MJ3	0.0002	0.0004	****	0.9996	0.9984	0.9984	0.9982	0.9982	0.9987	0.9987	0.9987	0.9987	0.9987	0.9989	0.9987	0.9989	0.9989	0.9989
MJ4	0.0002	0.0004	0.0004	****	0.9982	0.9982	0.9980	0.9984	0.9984	0.9984	0.9984	0.9984	0.9984	0.9987	0.9984	0.9987	0.9987	0.9987
YLJ1	0.0013	0.0016	0.0016	0.0018	****	0.9996	0.9998	0.9996	0.9993	0.9993	0.9993	0.9993	0.9989	0.9996	0.9989	0.9996	0.9996	0.9996
YLJ2	0.0013	0.0016	0.0016	0.0018	0.0004	****	0.9998	0.9996	0.9993	0.9993	0.9993	0.9993	0.9993	0.9996	0.9993	0.9996	0.9996	0.9996
YLJ3	0.0016	0.0018	0.0018	0.0020	0.0002	0.0002	****	0.9998	0.9991	0.9991	0.9991	0.9991	0.9987	0.9993	0.9987	0.9993	0.9993	0.9993
YJL4	0.0016	0.0018	0.0018	0.0016	0.0004	0.0004	0.0002	****	0.9989	0.9989	0.9989	0.9989	0.9984	0.9991	0.9984	0.9991	0.9991	0.9991
WJ1	0.0011	0.0013	0.0013	0.0016	0.0007	0.0007	0.0009	0.0011	****	1.0000	1.0000	1.0000	0.9996	0.9998	0.9996	0.9998	0.9998	0.9998
WJ2	0.0011	0.0013	0.0013	0.0016	0.0007	0.0007	0.0009	0.0011	0.0000	****	1.0000	1.0000	0.9996	0.9998	0.9996	0.9998	0.9998	0.9998
WJ3	0.0011	0.0013	0.0013	0.0016	0.0007	0.0007	0.0009	0.0011	0.0000	0.0000	****	1.0000	0.9996	0.9998	0.9996	0.9998	0.9998	0.9998
WJ4	0.0011	0.0013	0.0013	0.0016	0.0007	0.0007	0.0009	0.0011	0.0000	0.0000	0.0000	****	0.9996	0.9998	0.9996	0.9998	0.9998	0.9998
WYH1	0.0011	0.0013	0.0013	0.0016	0.0011	0.0007	0.0013	0.0016	0.0004	0.0004	0.0004	0.0004	****	0.9998	1.0000	0.9998	0.9998	0.9998
WYH2	0.0009	0.0011	0.0011	0.0013	0.0004	0.0004	0.0007	0.0009	0.0002	0.0002	0.0002	0.0002	0.0002	****	0.9998	1.0000	1.0000	1.0000
WYH3	0.0011	0.0013	0.0013	0.0016	0.0011	0.0007	0.0013	0.0016	0.0004	0.0004	0.0004	0.0004	0.0000	0.0002	*****	0.9998	0.9998	0.9998
WYH4	0.0009	0.0011	0.0011	0.0013	0.0004	0.0004	0.0007	0.0009	0.0002	0.0002	0.0002	0.0002	0.0002	0.0000	0.0002	****	1.0000	1.0000
WYH5	0.0009	0.0011	0.0011	0.0013	0.0004	0.0004	0.0004	0.0007	0.0009	0.0002	0.0002	0.0002	0.0002	0.0002	0.0000	0.0002	****	1.0000
WYH6	0.0009	0.0011	0.0011	0.0013	0.0004	0.0004	0.0007	0.0009	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0000	0.0000	*****

lossing cause to the permanent loss of human survival resources. With the development and rise of industrialization in the nearly two centuries, the natural environment has been artificially damaged continuously causing to deteriorating of ecological environment and significantly accelerated species extinction. Therefore, studies on the genetic diversity, conservation and sustainable use have become a urgent problem (Yan *et al*., 2004).

CONCLUSION

This study shows that there are high genetic diversity among different wild *Silurus asotus* populations which can contribute to strengthen protection of the *Silurus asotus* wild germplasm resources and prevent to overfishing and the adverse effects of invasive species. Therefore, this study have theoretical meaning for wild resources protection and management of *Silurus asotus*.

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