Genetics and Molecular Research

Complete mitochondrial genome of the Southern catfish (*Silurus meridionalis* Chen) and Chinese catfish (*S. asotus* Linnaeus): Structure, phylogeny, and intraspecific variation

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Genet. Mol. Res. 14 (4): 18198-18209 (2015)
Received August 8, 2015
Accepted October 18, 2015
Published December 23, 2015
DOI http://dx.doi.org/10.4238/2015.December.23.7

ABSTRACT. The complete mitochondrial genome of the Southern catfish (*Silurus meridionalis*) and the Chinese catfish (*S. asotus*), was determined using the long and accurate polymerase chain reaction (LA-PCR) method. The mitochondrial DNA nucleotide sequences of *S. meridionalis* and *S. asotus* were compared with those of 47 other catfish species in the same order. The total length of mitochondrial DNA for *S. meridionalis* and *S. asotus* was 16,526 and 16,525 bp, respectively, and included 13 protein-coding genes, 2 ribosomal RNA genes, 22 transfer RNA genes, and a non-coding control region. This mitochondrial gene arrangement is identical to that observed in other Siluriformes. To determine the relative phylogenetic positions of *S. meridionalis* and *S. asotus*, and to discover phylogenetic relationships among 24 families of Siluriformes, analyses were conducted, based on mitochondrial DNA, 12S ribosomal RNA, 16S ribosomal RNA, and 13 protein-coding gene sequence data sets. Phylogenetic analyses were congruent with a basal split of the order into Clupeiformes, Characiformes, Cypriniformes, and Siluriformes, and supported a closer relationship of the

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Southern catfish (family Siluridae) and the Chinese catfish (family Siluridae) to Pimelodidae than to Bagridae. We concluded that these two species are part of a molecular clade that is different from that proposed in recent studies, in which Amblycipitidae appears as a sister group. Our results showed Amblycipitidae appearing as the most basal extant, and Bagridae appearing as a sister group of Cranoglanididae and Pangasiidae. The Siluriformes showed close phylogenetic relationship to the Characiformes.

Key words: Southern catfish; Chinese catfish; Mitochondrial genome; Phylogeny; Structure; Intraspecific variation

INTRODUCTION

Catfish (Siluriformes) are widely distributed in every continent worldwide. In China, there are 11 families of the order Siluriformes, in which the family Siluridae is the most widely distributed (Chen, 1977). Of the 13 species of Siluridae in China, the most important economic species include the Southern catfish (*Silurus meridionalis*, Chen) and the Chinese catfish (*S. asotus*, Linnaeus) (Chu et al., 1999).

The Southern catfish is endemic to the main channel and tributaries of the Yangtze and Pearl Rivers, whereas the Chinese catfish is distributed worldwide (Chu et al., 1999). To improve the culture of both species, several studies have focused on reproduction (Wei and Luo, 1998), cell biology (Hong and Liu, 1998), and biochemistry (Shirai et al., 2006; Li et al., 2010; Zhang et al., 2010).

Genetic diversity in the wild is crucial for the culture of farmed populations. However, due to overfishing, pollution, and other environmental disturbances, populations of both species have rapidly declined in recent years (Chen, 1977). Therefore, it is important to conserve these species to have a better understanding of the genetic diversity of wild populations.

The random amplified polymorphic DNA (RAPD) method has evaluated the genetic diversity of Chinese catfish (Yoon and Kim, 2004; Li et al., 2009). However, the results based on RAPD are difficult to compare among different studies. By comparison, mitochondrial DNA (mtDNA) is a useful molecular marker for research on population genetics because of its maternal inheritance, relative lack of recombination, and its prompt evolutionary rate, as compared to nuclear DNA (Moore, 1995).

In fish, several mitochondrial genes have been employed for population genetic studies, such as cytochrome *b* (Cytb) and 16S rRNA genes (Peng et al., 2004; Kartavtsev et al., 2007). In studies of the Southern catfish that were based on these two mitochondrial genes, there was lower genetic diversity in the tributaries of the Yangtze River (Wang and Yu, 2008; Wang and Wang, 2009).

Recently, with the improvement of molecular techniques, it has become comparatively easier to obtain complete sequences of mitochondrial genome. The size of the mitochondrial genome ranges from 15 to 16 kb, and consists of 13 protein-coding genes, 2 rRNA genes, 22 tRNA genes, and a non-coding control region (Boore, 1999). The mitochondrial genome of Siluriformes may therefore provide valuable information for population genetics analysis.

In the present study, we report the complete DNA sequence and structure of the mitochondrial genomes of the Southern catfish and Chinese catfish in the Wujiang River, a tributary of the Yangtze River. In combination with the mitochondrial genomes of other fishes (Table 1), phylogenetic relationships in the order Siluriformes were analyzed. To date, nine mitochondrial genomes of four species in the genus *Silurus* have been reported in GenBank, including four of *S. asotus*, and three of *S. meridionalis* (Table 1). Thus, genetic variation can be analyzed among

different geographical populations. Our overall objective was to obtain additional information by evaluating the comparative genetic diversity of Southern and Chinese catfish in the wild.

Table 1. The information of mitochondrial genomes of fishes used in this study.

Species	GenBank accession No.	Source Siluriformes
Leiocassis longirostris	GU596454	Wang et al. (2011)
Leiocassis crassilabris	*JX867257*	Liang et al. (2013)
Pseudobagrus brevicaudatus	*JX867256*	Liang et al. (2013)
Pelteobagrus nitidus	HM746659	Liang et al. (2012)
Pseudobagrus ussuriensis	KC188782	Wan et al. (2013)
Pelteobagrus vachellii	HM746660	Liang et al. (2012)
Pelteobagrus eupogon	KJ001784	Wang et al. (unpublished)
Pseudobagrus truncatus	JX867259	Liang et al. (unpublished)
Pseudobagrus tokiensis	AB054127	Saitoh et al. (2003)
Pseudobagrus brevicorpus	HM355585	Kim et al. (2011)
Pelteobagrus fulvidraco	HM641815	Liang et al. (2012)
Pelteobagrus eupogon	JQ734476	Wang et al. (2013)
Hemibagrus macropterus	JF834542	Zeng et al. (2012)
Glyptothorax fokiensis	JQ917224	Zhou et al. (2012)
Liobagrus kingi	Kc193779	Jia et al. (2013)
Liobagrus nigricauda	KC316116	Jia et al. (2013)
Liobagrus marginatoides	Kc473938	Jia et al. (2013)
Ictalurus punctatus	AF482987	Waldbieser et al. (2003)
Cranoglanis bouderius	AY898626	Peng et al. (2006)
Pangasianodon gigas	AY762971	Jondeung et al. (2007)
Amphilius sp *NM-2010*	AP012002	Nakatani et al. (2011)
Malapterurus electricus	*AP012016*	Nakatani et al. (2011)
Centromochlus perugiae	AP012024	Nakatani et al. (2011)
Tetranematichthys quadrifilis	AP012025	Nakatani et al. (2011)
Amblydoras gonzalezi	AP012001	Nakatani et al. (2011)
Liobagrus reinii	AP012015	Nakatani et al. (2011)
Hara jerdoni	AP012012	Nakatani et al. (2011)
Pangasius larnaudii	AP012018	Nakatani et al. (2011)
Sciades seemanni	AP012003	Nakatani et al. (2011)
Auchenoglanis occidentalis	AP012005	Nakatani et al. (2011)
Pareutropius debauwi	AP012017	Nakatani et al. (2011)
Chrysichthys sp *NM-2010*	AP012009	Nakatani et al. (2011)
Synodontis schoutedeni	AP012023	Nakatani et al. (2011)
Pimelodus pictus	AP012019	Nakatani et al. (2011)
Heteropneustes fossilis	AP012013	Nakatani et al. (2011)
Clarias sp *NM-2010*	AP012010	Nakatani et al. (2011)
Chaca bankanensis	AP012008	Nakatani et al. (2011)
Plotosus japonicus	AP012020	Nakatani et al. (2011)
Bunocephalus coracoideus	AP012006	Nakatani et al. (2011)
Cetopsidium sp *NM-2010*	AP012007	Nakatani et al. (2011)
Helogenes marmoratus	AP012014	Nakatani et al. (2011)
Diplomystes nahuelbutaensis	AP012011	Nakatani et al. (2011)
Trichomycterus areolatus	AP012026	Nakatani et al. (2011)
Astroblepus sp *NM-2010*	AP012004	Nakatani et al. (2011)
Pterygoplichthys disjunctivus	AP012021	Nakatani et al. (2011)
Silurus glanis	AM398435	Vittas et al. (2011)
Silurus meridionalis	JX087350	Wang et al. (this study)
Silurus meridionalis	HQ907992	Zhou et al. (unpublished)
Silurus meridionalis	HM746661	Liang et al. (unpublished)
Silurus asotus	JX087351	Wang et al. (this study)
Silurus asotus	AP012022	Nakatani et al. (2011)
Silurus asotus	JX256247	Wang et al. (unpublished)
Silurus asotus	JN116720	Zeng et al. (unpublished)
Silurus lanzhouensis	JF895472	Wang et al. (2012)
Liobagrus obesus	DQ321752	Kartavtsev et al. (2007)
Corydoras rabauti	AB054128	Saitoh et al. (2003)
Outgroup		
Cypriniformes		
Crossostoma lacustre	M91245	Tzeng et al. (1992)
Clupeiformes		
Sardinops melanostictus	AB032554	Inoue et al. (2000)
Characiformes		
Phenacogrammus interruptus	AB054129	Saitoh et al. (2003)

MATERIAL AND METHODS

Fish samples and DNA extraction

Samples of *S. meridionalis* and *S. asotus* were collected from the Wujiang River in Guizhou Province, China, and were identified according to their morphology, as previously described (Chen, 1977; Wu, 1989). Total genomic DNA was extracted from muscle tissue using a 3S Spin Genomic DNA Miniprep Kit (Shanghai, China) following the manufacturer's protocol.

PCR amplification and sequencing

In a previous study on the mitochondrial genome of *S. lanzhouensis* (Wang Q. R. et al., 2012b) we designed 7 pairs of long and accurate polymerase chain reaction (LA-PCR) primers using primer premier 5.0. These primers were designed based on the complete mitochondrial genome of *S. glanis* (GenBank accession No. AM398435) and worked well for *S. meridionalis*, but not for *S. asotus*. Therefore, we designed eight pairs of LA-PCR primers specific for *S. asotus*. All primers are listed in Table 2. Some short fragments were amplified to link the long fragments, based on the sequences of those long fragments.

Table 2. PCR primers for *S. meridionalis* and *S. asotus* designed according to the complete mitochondrial genome of *S. glanis*.

Primer	Forward sequence	Reverse sequence	Size of fragment (bp)
S. meridionalis			
Phe-ND2	AATCAAAGCATAACACTG	GCTTATGAATGTGAGGGT	4100
Met-CO II	TCTTATCAATGAGCCCCTAC	GCTAATGCTAATCCCT	3900
Ser-ND4L	TAGTTTCAAGCCAGTCA	TTCTGAGCACCATTTGAT	3400
ND4L-ND4	CCGCATTTACTCTGGGACT	TTGGCCTCTTTCGGTGGA	1800
ND4-Cytb	CAGCCTACTCCCTTTACC	TACTACGCCAATGTTTCA	3000
ND6-Thr	TACTACGCCAATGTTTCA	CTCCGATCTCCGGATTACAAGAC	1100
Cytb-12S	GCACTCCTGCTATCCATC	CGTTTCTAGGGTTCGT	1800
S. asotus			
Phe-Val	AATCAAAGCATAACACTG	TTTGCCTTTTCTGTCT	1200
12S-ND2	AAAATAAGTGCCCGAAGG	TTGGATGGGTTAGACAGC	3600
Gln-Lys	TCTTATCAATGAGCCCCTAC	GCTAATGCTAATCCCT	3800
COII-ND4	TAGAAACGGACCACCGAATA	TGGCTAGGCAGAAGAGGG	3700
ND4-ND4	GAGGCTATGGTATAATGCG	ATAAGGTTTGGTAGTGGG	500
ND4-Glu	GCCTTATGAGGAATTGTC	TGAGTCAGCCGTAGTTTA	3800
ND6-Thr	GACTTGAAAACCACCGTTG	CTCCGATCTCCGGATTACAAGAC	1100
CYTB-12S	TTCATTCTACCATTCGCTAT	CTTTACGCCGTGTCTATT	2500

LA-PCR was carried out in a 50-μL reaction volume containing 5 μL 10X LA-PCR buffer II, 8 μL 1.5 mM dNTPs, 2 μL 10 μM of each primer, 0.5 μL LA Taq polymerase (TaKaRa, Japan) and approximately 50 ng of the template DNA. The thermal cycle profile included a pre-denaturation step at 94°C for 4 min, 40 cycles of denaturation at 94°C for 60 s, annealing at 50°C for 60 s, followed by an extension at 72°C for 5 min, and a final extension at 72°C for 10 min. The PCR mixture for the short fragments was the same as that described for the LA-PCR, with the exception that the extension time was changed to 1 min in the PCR program. PCR products were sent to Biosune Biotech Company (Beijing, China) for sequencing using primer walking.

Data analysis

DNA sequences were corrected and analyzed using Sequencing Analysis v3.4.1 (Applied Biosystems, USA) and Seqman v5.05 (DNASTAR Inc., USA). The nucleotide sequences of the mitochondrial 12S and 16S rRNA genes, and the deduced amino acid sequences of each of the 13 complete mitochondrial protein-coding genes were aligned separately, using the default parameters of CLUSTAL X version 1.81 (Thompson et al., 1997). Alignments (available from the authors upon request) were then revised by sight, in an effort to maximize positional homology.

The combined protein data set was constructed after excluding gapped positions. The rRNA data set included both complete and partial sequences, and gaps were treated as missing data. Additionally, tRNA genes were identified by tRNAscan-SE 1.21 (Lowe and Eddy, 1997). The locations of the protein-coding genes and rRNA genes were identified, and compared with the known sequences of other catfish species, including *S. glanis* (GenBank accession No. AM398435) and *Pangasianodon gigas* (GenBank accession No. AY762971) (Jondeung et al., 2007).

We reconstructed phylogenetic relationships of the order Siluriformes with the maximum likelihood method using RAxML 8.0X (Stamatakis, 2006) and MEGA 5 (Tamura and Peterson, 2011), respectively. The DNA sequences of all 13 mitochondrial protein-coding genes were used for the analysis. The species used in the analysis are listed in Table 1.

RESULTS AND DISCUSSION

Structure of the mitochondrial genome

The complete mitochondrial genomes of *S. meridionalis* and *S. asotus* were obtained and are deposited in GenBank with the accession Nos. JX087350 and JX087351, respectively. The total length of the complete mitochondrial genome of *S. meridionalis* and *S. asotus* was 16,526 and 16,525 bp, respectively. The gene order and gene coding strand of *S. meridionalis* and *S. asotus* were identical to those of the Atlantic cod, the Javeline goby, and the Japanese flying fish (Johansen and Bakke, 1996; Kim et al., 2004; Nagase et al., 2005), consisting of 2 rRNAs, 22 tRNAs, 13 protein-coding genes, and a control region (Figure 1 and Table 3). The overall base composition of the H-strand was A: 30.3%, C: 28.7%, G: 16.0%, and T: 25.0% in *S. meridionalis*, and A: 30.5%, C: 27.9%, G: 15.8%, and T: 25.8% in *S. asotus*.

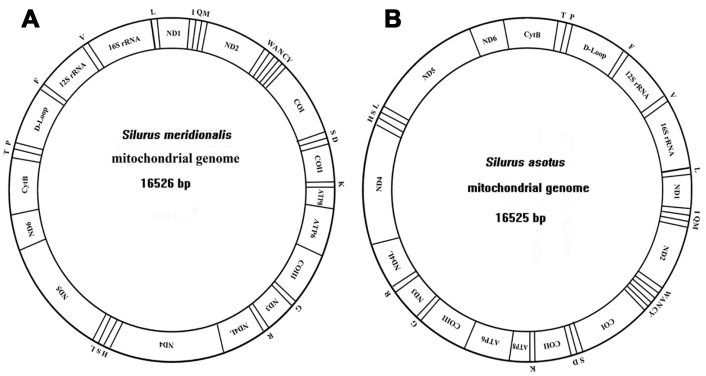

Figure 1. Gene organization for the mitochondrial genomes of *S. meridionalis* (**A**) and *S. asotus* (**B**).

Table 3. Organization of the *Silurus meridionalis* and *Silurus asotus* mitochondrial genome.

Gene/element	*S. asotus*				*S. meridionalis*			
	Abbreviation	Strand	Position	Size	Position	Size	Start codon	Stop codon
$tRNA^{Phe}$	F	H	1-70	70	1-70	70		
12S ribosomal RNA	12S	H	71-1022	952	71-1023	953		
$tRNA^{Val}$	V	H	1023-1094	72	1024-1095	72		
16S ribosomal RNA	16S	H	1095-2769	1675	1096-2771	1676		
$tRNA^{Leu}$	L	H	2770-2844	75	2772-2846	75		
NADH dehydrogenase subunit 1	ND1	H	2845-3819	975	2847-3821	975	ATG	TAA
$tRNA^{Ile}$	I	H	3821-3892	72	3823-3894	72		
$tRNA^{Gln}$	Q	L	3962-3892	71	3894-3964	71		
$tRNA^{Met}$	M	H	3962-4030	69	3964-4032	69		
NADH dehydrogenase subunit 2	ND2	H	4031-5077	1047	4033-5079	1047	ATG	TAG
$tRNA^{Trp}$	W	H	5076-5146	71	5078-5148	71		
$tRNA^{Ala}$	A	L	5149-5217	69	5151-5219	69		
$tRNA^{Asn}$	N	L	5219-5291	73	5221-5293	73		
$tRNA^{Cys}$	C	L	5326-5392	67	5328-5393	66		
$tRNA^{Tyr}$	Y	L	5397-5466	70	5398-5467	70		
Cytochrome c oxidase subunit 1	COI	H	5468-7018	1551	5469-7019	1551	GTG	TAG(TAA)
$tRNA^{Ser}$	S	L	7019-7089	71	7020-7090	71		
$tRNA^{Asp}$	D	H	7094-7165	72	7095-7166	72		
Cytochrome c oxidase subunit 2	COII	H	7179-7869	691	7180-7870	691	ATG	T-
$tRNA^{Lys}$	K	H	7870-7943	74	7871-7944	74		
ATP synthase F0 subunit 8	ATP8	H	7945-8112	168	7946-8113	168	ATG	TAA
ATP synthase F0 subunit 6	ATP6	H	8103-8786	684	8104-8787	684	ATG	TAA
Cytochrome c oxidase subunit 3	COIII	H	8786-9569	784	8787-9570	784	ATG	T-
$tRNA^{Gly}$	G	H	9570-9642	73	9571-9643	73		
NADH dehydrogenase subunit 3	ND3	H	9643-9993	351	9644-9994	351	ATG	TAG
$tRNA^{Arg}$	R	H	9992-10062	71	9993-10063	71		
NADH dehydrogenase subunit 4L	ND4L	H	10063-10359	297	10064-10360	297	ATG	TAA
NADH dehydrogenase subunit 4	ND4	H	10353-11733	1381	10354-11734	1381	ATG	T-
$tRNA^{His}$	H	H	11734-11803	70	11735-11804	70		
$tRNA^{Ser}$	S	H	11804-11869	66	11805-11870	66		
$tRNA^{Leu}$	L	H	11873-11945	73	11874-11946	73		
NADH dehydrogenase subunit 5	ND5	H	11946-13772	1827	11947-13773	1827	ATG	TAA
NADH dehydrogenase subunit 6	ND6	L	13768-14287	519	13769-14288	520	ATG	TAA
$tRNA^{Glu}$	E	L	14288-14356	69	14289-14357	69		
Cytochrome b	cyt b	H	14358-15495	1138	14359-15496	1138	ATG	T-
$tRNA^{Thr}$	T	H	15496-15566	71	15497-15567	71		
$tRNA^{Pro}$	P	L	15565-15634	70	15566-15635	70		
Displacement loop (control region)	D-loop	H	15635-16525	891	15636-16526	891		

The protein-coding genes in the mitochondrial genomes of *S. meridionalis* and *S. asotus* utilized ATG as the start codon, except for COI, which initiated with GTG (Table 3). These findings are similar to those for the dogfish, the Chinese longsnout catfish, and the black carp (Delarbre et al., 1998; Wang et al., 2011, 2012a). Nine open reading frames ended with TAA or TAG, while each of the others had an incomplete stop codon T (Table 3). The stop codon of the protein-coding genes was the same between the two species, except for COI, which ended with TAA in *S. meridionalis*, and TAG in *S. asotus*. In addition, overlapping sequences were found in ATP8-ATP6 (10 bp) and ND4-ND4L (7 bp), which occurred on the same strand, and in ND5-ND6 (5 bp), which occurred on different strands, in both *S. meridionalis* and *S. asotus*.

The mitochondrial genome of *S. meridionalis* and *S. asotus* contained 22 tRNA genes, which ranged from 66 to 75 bp in size. All tRNA genes, with the exception of $tRNA^{Ser}$ (AGY), were predicted to fold into the typical cloverleaf secondary structure with normal base pairing. Among these tRNA genes, eight genes were encoded on the L-strand, while the others were encoded on the H-strand. The 12S rRNA genes in *S. meridionalis* and *S. asotus* were 953 and 952 bp, respectively, and the 16S rRNA genes were 1676 and 1675 bp, respectively. As in other vertebrates

(Inoue et al., 2000; Wang et al., 2011, 2012a), 12S and 16S rRNA genes were located between the $tRNA^{Phe}$ and $tRNA^{Leu}$ genes, and separated by the $tRNA^{Val}$ gene.

The control region, which is the major non-coding region in the mitochondrial genome, regulates replication and transcription (Thompson et al., 1997; Saitoh et al., 2003). The length of the control region was 891 bp in both *S. meridionalis* and *S. asotus*. In comparison to other species of fish (Xu et al., 2011; Kim et al., 2005), three conserved sequence block (CSB) domains were found in the control region of *S. meridionalis* and *S. asotus*: CSB-1 at the 5′ end; and CSB-2 and CSB-3 at the 3′ end.

Phylogenetic analysis

Phylogenetic relationships of *S. meridionalis* and *S. asotus* in the order Siluriformes are shown in Figure 2.

Figure 2. Phylogenetic relationships among Siluriformes by RAxML. Majority rule consensus of maximum likelihood bootstrap measure trees from RAxML inference analysis based on rRNA and protein code data set under the GTR+I+G (General Time Reversible; I: invariable sites; G: gamma distribution) model is shown. Branch lengths are mean estimates. Asterisks indicate specimens used in the present study. Numbers in the nodes are from highest to lowest bootstrap value. The scale in the left lower corner indicates relative branch lengths.

Four species of catfish in the family Siluridae formed a distinct clade with a high bootstrap value (100). This is consistent with the previously determined morphological classification of the family Siluridae and its significant differentiation from other families in the order Siluriformes (Chen, 1977). As expected, the monophyly of Siluriformes, and the inclusion of *S. meridionalis* and *S. asotus* within this order was highly supported (bootstrap value of 100).

Siluridae was recovered with maximal support as a sister group of *Pimelodus pictus* (Pimelodidae), which is consistent with the previous report (Nakatani et al., 2011). The families of Plotosidae, Chacidae, Heteropneustidae and Clariidae, were also recovered as sister groups; however, these relationships were not as well supported (bootstrap value of 9).

In other branches, the families of Schilbeidae, Malapteruridae, Amphiliidae, Cranoglanididae, Pangasiidae, Ariidae, and Ictaluridae formed a distinct group. The families of Bagridae and Amblycipitidae were strongly supported (bootstrap value of 96) as a sister group. However, *Chrysichthys* sp NM2010 and *Auchenoglanis occidentalis* are excluded from Bagridae.

Even though phylogenetic interrelationships of highly diversified families of the order Siluriformes, such as Loricariidae, Trichomycteridae, Astroblepidae, and Callichthyidae, are well studied at the molecular level, the main phylogenetic interrelationships of siluriform families remain largely unresolved. *Corydoras rabauti* (Callichthyidae), which is assigned to the suborder Loricarioidei, was placed as a sister group of all other analyzed siluriforms. The family Callichthyidae is an independent clade in the Siluriformes.

The phylogenetic relationship of the families in the Siluriformes obtained from this study was similar to another study based on the entire mitochondrial genome excluding the control region (Wang et al., 2011). At a higher taxonomic level, the monophyly of Otophysi (Cypriniformes, Gymnotiformes, Characiformes, and Siluriformes) was highly supported (bootstrap value of 100) by all methods of phylogenetic inference. We inferred therefore that Siluriformes originate from Characiformes. The monophyly of the different orders within Ostariophysi is well supported based on morphological grounds.

In the phylogenetic tree reestablished with MEGA 5 (Figure 3), four species of catfish in the family Siluridae formed a distinct clade with a high posterior probability (bootstrap value of 100). The families of Mochokidae, Schibeidae, and Bagridae (*Chrysichthy* sp NM2010 and Auchenoglanis) were recovered as sister groups. However, their relationship was not very well supported (bootstrap value of 7). This differs from our analysis based on the RAxML tree (Figure 2).

The families Loricariidae, Trichomycteridae, and Astroblepidae constituted a group. Consistent with our results based on the RAxML tree, *Corydoras rabauti* (Callichthyidae), which is assigned to the suborder Loricarioidei, was placed as a sister group of all other analyzed siluriforms. The family Callichthyidae was an independent clade in the Siluriformes (bootstrap value of 100).

In general, both trees formed with RAxML and MEGA support the fact that the family Callichthyidae was an independent clade in the Siluriformes. At a higher taxonomic level, the monophyly of Otophysi (Cypriniformes, Gymnotiformes, Characiformes, and Siluriformes) was highly supported (bootstrap value of 100), by all methods of phylogenetic inference. Siluriformes was found to originate from Characiformes.

Nucleotide variation

When the mitochondrial genome of *S. meridionalis* obtained in the present study was compared with two others submitted to GenBank (Table 4), among the 16536 bp aligned sequences, 1.5% of the sites were variable. The genes of rRNA (1.0%) and tRNA (0.5%) were more conserved, whereas the control region was more variable (6.2%). The percentage of variable sites in all 13 protein-coding genes was 1.4%, and this value ranged from 0.3% (COII) to 2.2% (ND2) in one of 13 protein-coding genes.

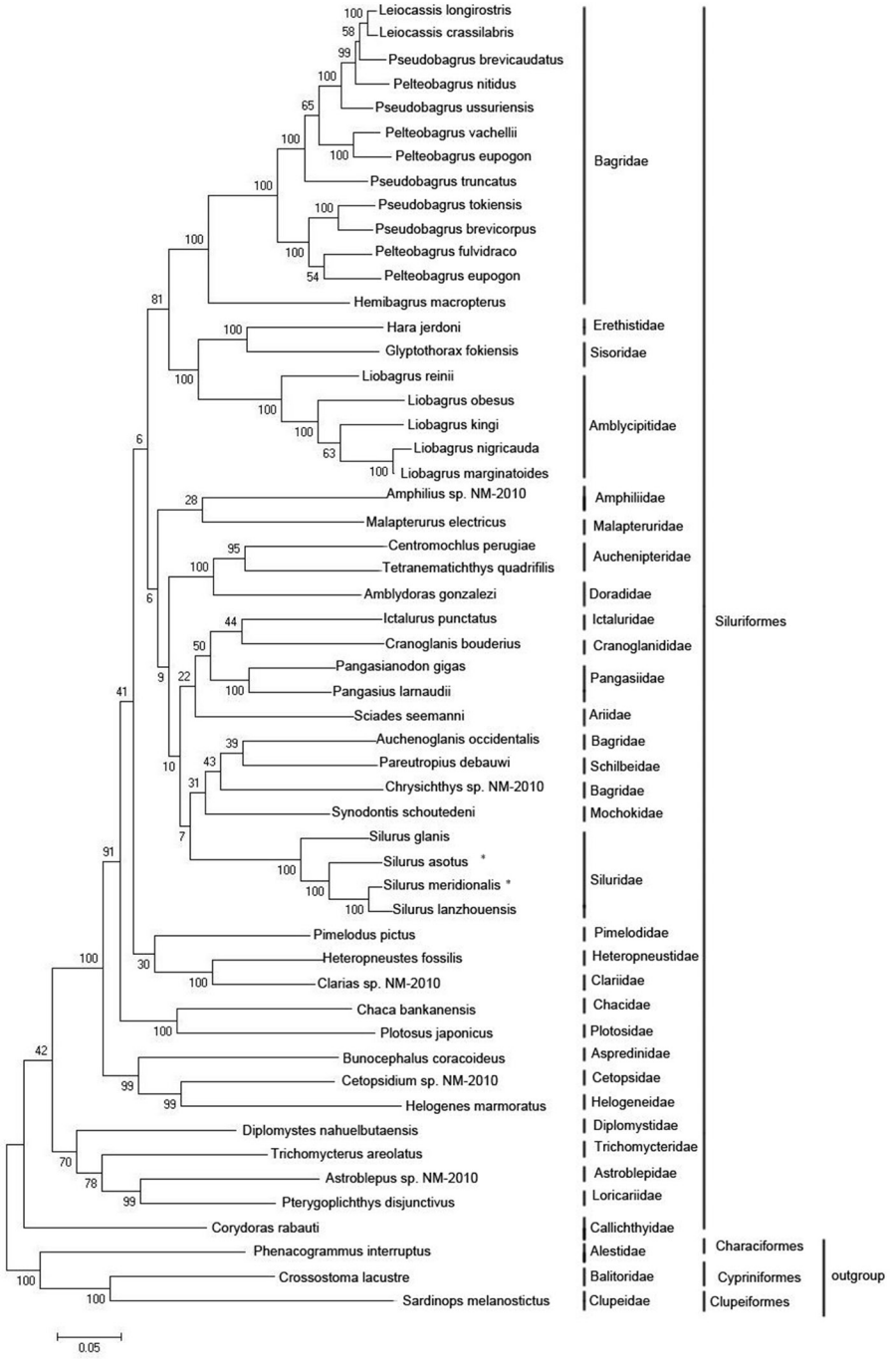

Figure 3. Phylogenetic relationships of fishes in the order Siluriformes based on 13 mitochondrial protein-coding genes and analyzed with the maximum likelihood method in MEGA 5. Repetition frequencies (posterior probabilities) for N = 500 simulated generations are shown in the nodes. The scale in the lower left corner indicates relative branch lengths.

Table 4. Intraspecific variation of mitochondrial genomes of 3 individuals of *Silurus asotus and Silurus meridionalis*.

	S. asotus			*S. meridionalis*		
	Length of aligned sequence	Number of variable sites	Percentage of variable sites (%)	Length of aligned sequence	Number of variable sites	Percentage of variable sites (%)
All sites	16533	873	5.3	16536	250	1.5
tRNAs	1665	49	2.9	1568	8	0.5
rRNAs	2631	67	2.5	2630	27	1.0
Control region	892	84	9.4	900	56	6.2
Protein-coding genes	11391	673	5.9	11391	159	1.4
ND1	975	65	6.7	975	18	1.8
ND2	1047	65	6.2	1047	23	2.2
COI	1551	87	5.6	1551	17	1.1
COII	691	24	3.5	691	2	0.3
ATP8	168	5	3.0	168	1	0.6
ATP6	684	41	6.0	684	11	1.6
COIII	784	38	4.8	784	9	1.1
ND3	351	17	4.8	351	4	1.1
ND4L	297	12	4.0	297	2	0.7
ND4	1381	100	7.2	1381	23	1.7

The level of intraspecific variation in *S. asotus* was much higher than that found in *S. meridionalis*. The percentage of variable sites, out of the 16,533 bp aligned sequences, was 5.3% in *S. asotus*. The levels of variation in the rRNA and tRNA genes were 2.5 and 2.9%, respectively. The level of variation in the control region was 9.4%. The mean percentage of variable sites in all 13 protein-coding genes was 5.9%, and ranged from 3.0% (ATP8) to 7.3% (ND6).

The great differences in intraspecific variation between *S. meridionalis* and *S. asotus* can be attributed to the source of the specimens. Although the source of specimens of two individuals of *S. meridionalis* (GenBank accession Nos. HM746661 and HQ907992) that have been deposited in GenBank is not described, it is reasonable to assume that these specimens were both collected in the Yangtze River. This assumption is based on the fact that the institutes of the respective authors are both located near the Yangtze River.

The specimen of *S. meridionalis* in this study was collected from the Wujiang River, which is a branch of the Yangtze River. Hence, all three individuals of *S. meridionalis* under study were probably sampled from the Yangtze River. In contrast, the specimen of *S. asotus* in the present study was sampled from the Wujiang River. GenBank accession No. JX256427 was sampled from the Sichuan River, and GenBank accession No. JN116720 was sampled from the Hechuan district. The Wujiang River, the Sichuan, and the Hechuan are drainage basins for the Yangtze River. Another mitochondrial genome of *S. asotus* (GenBank accession No. AP012022) was submitted by Japanese researchers (Nakatani et al., 2011). Thus, we believe that catfish species should be collected in rivers of Japan. Higher levels of geographical isolation will probably be sufficient to explain the higher level of genetic divergence of both specimens of *S. asotus* in the present study.

ACKNOWLEDGMENTS

We thank Ms. Yang Xiurong for collecting samples in the field. This work is supported by the key project of Science & Technology Department of Guizhou Province [Qiankehe NZ(2013)3027], and the important scientific project in ‘‘125’’ Program of Guizhou Province [Qianjiaohe (2013)025].

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