Complete Nucleotide Sequence of the Cytochrome *b* Gene of Channel Catfish *Ictalurus punctatus* and Comparison of Sequence Homology among Channel Catfish and Other Fishes

Taiki MATSUO¹⁾, Yasuki OGAWA¹⁾, Atsuo KUMAMARU²⁾, Kozo OCHI³⁾ and Yoshikazu ADACHI¹⁾*

¹⁾Animal Health Laboratory, School of Agriculture, Ibaraki University, 3-21-1 Ami, Ibaraki 300-0393, ²⁾Ibaraki Prefectural Freshwater Fisheries Experimental Station, 1560 Kou, Tamatsukuri, Ibaraki 311-3512 and ³⁾National Food Research Institute, 2-1-21 Kannondai, Tsukuba, Ibaraki 305-8642

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ABSTRACT. To clarify the phylogenetic relationship of channel catfish with other fishes, the cytochrome *b* (*Cyt b*) gene of the catfish was cloned and sequenced. Channel catfish (*Ictalurus punctatus*) belonging to the family *Ictaluridae* in the order *Siluriformes* showed a 78.4–87.4 % similarity to all but one fish of the family *Cyprinidae* and river loach *Crossostoma lacustre* of the family *Balitoridae* in the order *Cypriniformes* in which genes had already been sequenced, and a 97.2 % similarity to the goldfish (*Carassius auratus*) belonging to the family *Cyprinidae*. Within the family *Cyprinidae*, a 78.8–89.2% similarity to one another was recorded. In addition, the similarity rate between the family *Cyprinidae* and the family *Balitoridae* reached a value of 77.8–79.9% in the order *Cypriniformes*. Furthermore, in an unrooted phylogenetic tree consisting of four branches among eight fishes, channel catfish and goldfish appeared in the same branch. These results suggested that the *Cyt b* gene of the channel catfish in the order *Siluriformes* was closely related to that of a goldfish in the order *Cypriniformes*. The results were not agreement with the morphological classification. Genetic reclassification of the fishes may be necessary to identify the ancestor. This is the first report on the cloning and complete sequencing the *Cyt b* gene of the channel catfish which may contribute to the genetic reclassification of catfishes belonging to the order *Siluriformes*.

KEY WORDS: channel catfish, cytochrome *b* gene.

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Catfishes with various characteristics are known to consist of about 2,200 species and are widely distributed throughout the world. However, the catfishes have been classified only morphologically. On the other hand, although analyses of mitochondrial genes such as cytochrome *b* (*Cyt b*) and 16S ribosomal RNA genes of other fishes have been performed, there has been no report on the analysis of the complete sequences of the genes of the catfishes, unlike those of the genes in other fishes (the family *Cyprinidae*). Therefore, the evolutionary relationship between the catfishes and others fishes has not been determined. To address this problem, nucleotide sequence analyses of mitochondrial genes have been attempted. As a result, the complete nucleotide sequence of the *Cyt b* gene of the channel catfish (*I. punctatus*) were analyzed and the base arrangement between the catfish and other fishes was compared.

Total DNAs were isolated from the channel catfish (*I. punctatus*) [6] propagated at the Ibaraki Prefectural Freshwater Fisheries Experimental Station and used as templates of PCR. Four primers synthesized according to the sequence of the *Cyt b* gene of redfin shiner (*Lythrurus umbratilis*, accession no. U17274) were as follows: primer A^{FW} (5'TGTAAAACGACGGCCAGTACCCGATTCTA ACCGAGA3'), primer C^{RV} (5'CCTCCAATCTTCGGAT-TAC3'), and primer D^{FW} (5'TGTAAAACGACGGCCAG-

TATGGCAAGCCTAGAAA3'). The TGTAACGACGGCCAGT of primers A^{FW}, D^{FW} and E^{FW}, corresponding to -21M13, was also used for direct sequence analyses of PCR fragments. The DNA fragment D^{FW}-C^{RV} of the *Cyt b* gene of the channel catfish was amplified by using PCR consisting of incubation periods of 30 sec at 94°C, 60 sec at 50°C, and 90 sec at 72°C for 30 cycles after preincubation for 3 min at 94°C with primers D^{FW} and C^{RV} and Taq DNA polymerase (Takara-Shuzo Co., Kyoto, Japan). This PCR fragment was sequenced by using -21M13 primer. Based on the sequencing results, primers E^{FW} (5'TGTAAAACGACG-GCCAGTGCATGATGAACTTTGGCTCTC3') and E^{RV} (5'GGACATTTGTCCTCATGGAAG3') were synthesized. The DNA fragments A^{FW}-E^{RV}, E^{FW}-C^{RV}, and E^{FW}-E^{RV} of the *Cyt b* gene of the channel catfish were amplified by using PCR: the fragment A^{FW}-E^{RV} was amplified using incubation periods of 30 sec at 94°C, 30 sec at 50°C, and 30 sec at 74 °C for 25 cycles with primers A^{FW} and E^{RV} and KOD DNA polymerase (Toyobo, Tokyo, Japan); the fragment E^{FW}-C^{RV} was amplified using incubation periods of 30 sec at 94°C, 30 sec at 67 °C, and 45 sec at 74°C for 45 cycles with primers E^{FW} and C^{RV} and KOD DNA polymerase (Toyobo, Tokyo, Japan); and the fragment E^{FW}-E^{RV} was amplified using incubation periods of 30 sec at 94°C, 60 sec at 50°C, and 90 sec at 72°C for 30 cycles after preincubation for 3 min at 94°C with primers E^{FW} and E^{RV} and Taq DNA polymerase (Takara-Shuzo Co., Kyoto, Japan). The resultant fragments were introduced into a pUC19 vector and sequenced on both sides by using a DSQ-1000L DNA sequencer (Shimadzu

*CORRESPONDENCE TO: ADACHI, Y., Animal Health Laboratory, School of Agriculture, Ibaraki University, 3-21-1 Ami, Ibaraki 300-0393, Japan.

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M I T R K T H P L F K I A N N A L I D L
atg atc acc cga aaa acc cac ccg ctc ttc aaa att gcc aac aat gca cta att gac cta

P A P S N I S A W W N F G S L L G L C L
ccc gcc cca tct aat atc tcc gca tga tga aac ttt ggc tct cta cta gga tta tgc tta

I T Q I L T G L F L A M H Y T S D I S T
att act caa att cta acc ggc cta ttt cta gct ata cat tac acc tca gac att tca acc

A F S S V T H I C R D V N Y G W L I R N
gca ttc tca tcc gtt acc cac atc tgc cga gat gla aac tac ggc tga cta att cgt aat

I H A N G A S F F F I C I Y M H I A R G
att cac gcc aat gga gca tca ttc ttc ttc atc tgt att tac ata cac att gcc cga ggc

L Y Y G S Y L N K E T W N I G V V L L L
cta tat tat gga tca tac ctt aac aaa gaa acc tga aac att gga gta gtt ctc cta ctc

L V M M T A F V G Y V L P W G R M S F W
cta gtt ata atg aca gcc ttt gtc ggt tat gtt ctt cca tga gga cga atg tcc ttt tga

G A T V I T N L L S A V P Y M G D M L V
ggc gct aca gla atc aca aac ctt cta tcc gcc gtg cca tat ata gga gat ata tta gtt

Q W I W G G F S V D N A T L T R F F A F
caa tga att tga gga ggg ttc tcc gla gac aat gca aca tta aca cga ttc ttt gca ttc

H F L L P F I I A A A T V I H L L F L H
cac ttc cta cta cca ttc att atc gcc gcc gct act gtc att cac cta ctg ttt ctc cac

E T G S N N P I G L N S D A D K I S F H
gaa aca gga tca aat aac ccc atc gga ctg aac tca gac gca gac aaa att tct ttc cac

P Y F S Y K D L L G F V I M L L A L T L
cca tac ttt tca tac aaa gac ctc ctt ggg ttc gtg att ala cta cta gcc ctc aca ctc

L A L F S P N L L G D P E N F T P A N P
ctg gca tta ttc tcc cca aac ctt tta gga gac cca gaa aac ttc act cca gcc aat ccc

L V T P P H I K P E W Y F L F A Y A I L
ctg gtt act ccc cct cac att aaa cca gag tga tat ttc ctg ttt gcc tac gcc att ctc

R S I P N K L G G V L A L L F S I L V L
cga tca att ccc aac aaa cta gga gga gtt ctt gca cta cta ttc tcc atc ctc gta cta

M V V P L L H T S K Q R G L T F R P I T
ata gtg gla ccc cta tta cat acc tca aaa caa cga gga cta acg ttc cgc ccc atc acc

Q F L F W T L V A D M I I L T W I G G M
caa ttc cta ttt tga act cta gtc gca gac atg att atc ctg aca tga att gga gga ata

P V E H P F I I I G Q I A S V L Y F A L
cca gla gaa cat cca ttc att atc atc gga caa atc gca tcc gtc cta tat ttc gca ctg

F L V L F P L A G W L E N K A L K W A
ttc ctt gtt ctc ttc cca cta gca gga tga tta gaa aat aaa gca ctg aaa tga gct

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Fig. 1. DNA sequence and deduced amino acid sequence of the *Cyt b* gene of the channel catfish. This sequence has been assigned the DDBJ accession number AB045119.

Table 1. Comparison of the similarity of the complete sequence of the *cytochrome b* gene among fishes^{a)}

Fishes ^{b)}	Class <i>Osteichthyes</i>								
	Subclass <i>Actinopterygii</i>								
	Order <i>Siluriformes</i>	Order <i>Cypriniformes</i>							
	Family <i>Ictaluridae</i>	Family <i>Cyprinidae</i>						Family <i>Balitoridae</i>	
	<i>I. punctatus</i>	<i>C. auratus</i>	<i>C. carpio</i>	<i>A. huegelii</i>	<i>R. ocellatus</i>	<i>B. barbus</i>	<i>L. umbratilis</i>	<i>C. lacustre</i>	Accession numbers
<i>I. punctatus</i>	100	97.1	96.5	95.2	94.1	95.7	92.8	92.8	AB045119
<i>C. auratus</i>	97.2	100	96.8	94.5	93.2	95.0	92.6	92.9	AF051858
<i>C. carpio</i>	87.4	87.6	100	95.0	94.7	96.1	93.4	93.4	X61010
<i>A. huegelii</i>	84.9	85.4	87.7	100	92.9	98.2	92.1	93.1	AF112133
<i>R. ocellatus</i>	84.8	84.6	89.2	85.2	100	93.4	91.1	93.1	AF051876
<i>B. barbus</i>	84.0	84.1	86.5	89.0	85.0	100	92.1	93.7	AF112123
<i>L. umbratilis</i>	78.4	78.8	79.5	78.9	80.5	78.9	100	91.3	U17274
<i>C. lacustre</i>	79.6	79.5	81.5	79.0	79.9	79.4	77.8	100	M91245

a) The similarities (%) of *Cyt b* genes at the levels of nucleotides and proteins predicted are shown in the left and right regions to a diagonal 100% line, respectively.

b) *I. punctatus*, *Ictalurus punctatus*; *C. auratus*, *Carassius auratus*; *C. carpio*, *Cyprinus carpio*; *A. huegelii*, *Aulopyge huegelii*; *R. ocellatus*, *Rhodeus ocellatus*; *B. barbus*, *Barbus barbus*; *L. umbratilis*, *Lythrurus umbratilis*; *C. lacustre*, *Crossostoma lacustre*.

Fig. 2. Unrooted phylogenetic tree consisting of eight fishes. This tree was constructed by the neighbor-joining method of Saitou and Nei [7] according to Kimura's modification [3]. Homology rates (%) are indicated for each branch. Knuc value is shown in parentheses.

Co., Kyoto, Japan). The nucleotide sequences determined here were analyzed with GENETYX software (Software Development Co., Tokyo, Japan). The *Cyt b* gene of the channel catfish examined had 1140 nucleotide sequences encoding 380 amino acids (Fig. 1). The complete nucleotide sequence was registered with DDBJ (accession no. AB045119). DNA database search did not include information on the complete nucleotide sequences of *Cyt b* genes of the catfishes. The results of sequencing and search demonstrated that the *Cyt b* base arrangement of the channel catfish

(*I. punctatus*) was closely related to that of the goldfish (*C. auratus*, accession no. AF051858). *I. punctatus* belongs to the family *Ictaluridae* in the order *Siluriformes*, while *C. auratus* belongs to the family *Cyprinidae* in the order *Cypriniformes*. These orders belong to the subclass *Actinopterygii* and the class *Osteichthyes* (Table 1). Although there are many reports on the *Cyt b* genes of the family *Cyprinidae* in terms of molecular phylogeny, there are only a few reports on those of other fishes. The *Cyt b* gene of the channel catfish was compared with that of other fishes in which

complete sequences were analyzed (Table 1). The similarity among the genes in the fishes of the family *Cyprinidae* reached a value of 78.8 to 89.2 %. Similarity between the family *Cyprinidae* and the family *Balitoridae* belonging to the order *Cypriniformes* reached a value of 77.8 to 79.9 %. Channel catfish (*I. punctatus*) belonging to the family *Ictaluridae* in the order *Siluriformes* showed a 79.6 % similarity to the river loach (*Crossostoma lacustre*, accession no. M91245) [9] belonging to the family *Balitoridae* in the order *Cypriniformes*. Channel catfish (*I. punctatus*) also showed a similarity value of 78.4 to 87.4 % to the carp (*Cyprinus carpio*, accession no. X61010) [2], the dalmatian barbelgudgeon (*Aulopyge huegelii*, accession no. AF112133) [8], the rosy bitterling (*Rhodeus ocellatus*, accession no. AF051876) [4], *Barbus barbus* (accession no. AF11-2123) [1], and the redfin shiner (*L. umbratilis*) of the family *Cyprinidae* in the order *Cypriniformes*. However, the channel catfish (*I. punctatus*) belonging to the family *Ictaluridae* in the order *Siluriformes* showed the highest similarity (97.2%) to the goldfish (*C. auratus*) of the family *Cyprinidae* in the order *Cypriniformes*, and the results were compatible with the amino acid sequence homology predicted (Table 1). Furthermore, evolutionary distances expressed by Knuc values were calculated as previously described [3] and an unrooted phylogenetic tree was drawn by the neighbor-joining method [7] (Fig. 2). Both channel catfish (*I. punctatus*) and goldfish (*C. auratus*) appeared in the fourth branch. These fishes were divided into four branches in a tree. The findings revealed that the *Cyt b* gene of the channel catfish (*I. punctatus*) was most closely related to that of the goldfish (*C. auratus*) among those of several fishes previously reported. This fact indicates the existence of a dis-

crepancy between genetic and morphological classifications. Therefore, it is important to identify the ancestor of the goldfish genetically. In general, the classification of fishes should be systematically performed based on information on various aspects. Our findings may provide some insight into the evolutionary relationship between the channel catfish and the other fishes. In particular, molecular analyses of the *Cyt b* gene could be important and more accurate for the classification.

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