

Molecular Phylogeny of Korean Loaches Inferred from Mitochondrial DNA Cytochrome *b* Sequences

So-Young Kim, Ik-Soo Kim, Kwang-Yeop Jahng and Mi-Hee Chang

Division of Biological Sciences, Chonbuk National University, Chonju 561-756, Korea

Phylogenetic relationships between 8 species Korean loaches (Cobitidae) were investigated by comparing mitochondrial cytochrome *b* gene sequences. However our results are in accordance with finding observed using other morphological studies, new interesting interspecific variation in Korean loaches were found. *Orthrias* and *Lefua* appeared to be paraphyletic in Cobitidae observed. Their sequence divergence value was agreed with interfamilic sequence divergences between Cobitidae and Cyprinidae ranged from 0.184 to 0.272. Otherwise, the present results support that two species of *Iksookimia* and *Cobitis melanoleuca* were early diverged respectively. And another remarkable result was sequence divergence between *Misgurnus anguillicaudatus* from China and *M. anguillicaudatus* from Yongdok, Korea. That was 0.099, which was interspecific value. Also the phylogenetic location of some *Iksookimia* species was suggested as the cobitid intergeneric hybrid origin.

Key words : Cobitidae, mitochondrial cytochrome *b* gene, phylogenetic relationship

Introduction

The family Cobitidae is composed of 16 genera and about 100 species of freshwater fish which are confined to Eurasia and northern Africa (Nalbant, 1994). Despite the intensive study of cobitid taxonomy, hypotheses on the relationship among the cobitid species based on morphology, cytogenetic, reproduction, histology and behavior have led to conflicting classifications (Kobayasi, 1976; Kim, 1980; Sawada, 1982; Kim and Lee, 1986; Lee and Lee, 1989; Kim and Park, 1997).

Kim (1997) and Nalbant (1993, 1994) recognized the following 8 genera in Korea: *Orthrias*, *Lefua*, *Misgurnus*, *Iksookimia*, *Koreocobitis*, *Cobitis*, *Niwaella* and *Choia*. The hypothesis of relationships presented by Kim and Nalbant (1993) was based on the condition of the first pectoral fin ray in males and the colour pattern of the body sides. All species of the two genera *Cobitis* and *Iksookimia* have the peculiar lamina circularis at the base of their pectoral fin in the male

as a secondary sexual characteristic, which is also an important characteristic to identify the cobitid species (Kim and Park, 1997). All loaches of *Iksookimia* are endemic to Korea, which were erected from the genus *Cobitis* by Nalbant (1993).

Recently, geographical variations of mitochondrial DNA were investigated through the genetic differences between the western and eastern *Cobitis koreensis* groups (Yang and Kim, 1995). In addition, extensive variations in mitochondrial DNA were studied among geographic populations of *Nemacheilus toni* (Park and Lee, 1991).

At present, no study has provided a widely accepted formulation of the evolutionary relationships within the family Cobitidae using genetic characteristic. In this paper we describe for the first time sequences of the mitochondrial cytochrome *b* gene of 8 cobitids and use them to evaluate the monophyly of the *Cobitis* and the paraphyly of the *Orthrias* and *Lefua*. We specifically address the phylogenetic relationships among the species of Cobitidae using one species of Cyprinidae (*Carassius auratus*) as a outgroup.

Materials and Methods

1. Sample

Details of the samples and species used in this study are presented in Table 1. The specimens were collected in the field and identified to species level using the method by Kim (1997). Tissue samples (fin) were dissected, avoiding cross-contamination, and stored at -80°C .

2. DNA extraction, amplification, and sequencing

Tissues were digested with proteinase K in the presence of extraction buffer (10~14 h at 55°C). DNA was purified with standard phenol/chloroform/isoamyl alcohol (25 : 24 : 1) and chloroform/isoamyl alcohol (24 : 1) extractions. Ethanol-precipitated DNA pellets were rinsed (70% ethanol), air dried and resuspended in TE buffer (Maniatis *et al.*, 1982).

PCR amplifications were performed following Kocher *et al.* (1989) using 50 ng of genomic DNA and 1 unit of Taq DNA polymerase (Ex-Taq Takara) per 50 μl of reaction volume. Thermal cycling was: initial hot start 4 min at 95°C ; followed

by 30 cycles of 30s at 94°C , 30s at 58°C , and 1 min 30s at 72°C , and an additional 7 min interval at 72°C . The 1,140 bps of the cytochrome *b* were amplified using primer cbup 1 and cblo 2 (Table 2). PCR products were prepared for cloning by removing the primers using the GENE CLEAN kit (BIO 101). The fragment was inserted into pGME-T vector (Promega) and used to transform JM 109 cell line. Automated DNA sequencing was performed using ABI PRISM 377 DNA sequencers, according to manufacture protocols. Sequencing primers were custom made (Table 2).

3. Phylogenetic analysis

Sequencing base composition, patterns of substitution for pairwise comparisons along the mitochondrial DNA cytochrome *b* sequences were aligned using the Clustal $\times 1.81$ (Thompson *et al.*, 1997). All phylogenetic analyses were executed using PAUP* test vision 4.0b4a (written by D. L. Swofford, 2000).

Different approaches to phylogenetic reconstruction were used to analyze the data set. They included maximum parsimony (MP), maximum likelihood (ML) and neighbor joining (NJ) trees. MP analyses were performed by the bootstrap

Table 1. List of Cobitidae species for analysis in the present study

Species	Family	Accession number	Locality/area
<i>Orthrias toni</i>	Cobitidae	–	Samchok/Korea
<i>Lefua costata</i>	Cobitidae	–	Samchok/Korea
<i>Misgurnus anguillicaudatus</i>	Cobitidae	AF051868	China
<i>Misgurnus anguillicaudatus</i>	Cobitidae	–	Yongdok/Korea
<i>Iksookimia koreensis</i>	Cobitidae	–	Gosan/Korea
<i>Iksookimia pumila</i>	Cobitidae	–	Buan/Korea
<i>Cobitis sinensis</i>	Cobitidae	–	Hyongsan R./Korea
<i>Cobitis melanoleuca</i>	Cobitidae	–	Samchok/Korea
<i>Niwaella multifasciata</i>	Cobitidae	–	Nam R./Korea
<i>Carassius auratus</i>	Cyprinidae	AB006953	Japan

–, present work

Table 2. List of primers used in this study, Usage (PCR amplification–P, Cycle sequence–C)

Primer name	Gene	Sequence	Usage
cbup 1	Cyt b	GGATTACAAGACCGATGCTTT	P
cblo 2	Cyt b	TGACTTGAAGAACCACCGTTG	P
cytup 3	Cyt b	ACTTATCCGCAACATTCATGC	C
cytup 4	Cyt b	CCCACATCTGCCGAGATGTA	C
cytup 5	Cyt b	TGACTAATTCGAAGCATGCAC	C
cytup 6	Cyt b	CTGATATCTCCACCGCCTTCT	C
cytup 7	Cyt b	CCCATAGGATTAACCTCAGACG	C
cytup 8	Cyt b	GACGCAGACAAAGTATCATTTCA	C
cytup 9	Cyt b	CTCGGACGCAGATAAAATTTTC	C
cytup 10	Cyt b	TTTGCATTCCACTTCTCTTC	C

method with heuristic searches with following search parameters: ignore uninformative characters, retain minimal trees, collapse zero length branches and branch swapping algorithm (tree-bisection-reconnection: TBR). ML analyses were performed using quartet puzzling with the parameter model of evolution of Hasegawa-Kishino-Yano (HKY85; Hasegawa *et al.*, 1985). HKY model has been requested transitions/transversions ratio = 2 ($\kappa = 4.00347$) and used empirical base frequencies. All sites were assumed to have evolved at the same rate.

A search for the NJ (Saito and Nei, 1987) was performed using the Kimura two-parameter distance (Kimura, 1980), which allows for unequal rates of transitions and transversions. The NJ tree was estimated to 1,000 bootstrap replicates to assess branch support.

Result

1. Nucleotide variation

The cytochrome *b* genes were sequenced in all 8 sampled specimens by a 650 bp segment of the 5' end of the gene. Completed cytochrome *b* sequences from 1 non-cobitid species, *Carassius auratus* (from Japan) and *Misgurnus anguillicaudatus* (from China) were downloaded from Genbank. *Carassius auratus* was designated as out-group taxa in all analyses. The nucleotide composition of the cytochrome *b* gene was homogeneous among congeners. The nucleotide composition of the cytochrome *b* sequences followed the pattern described for numerous fish (Cantatore *et al.*, 1994): a global deficit of guanosine (G: 15.9%) and approximately equal frequencies of the other three nucleotides (A: 27.8%, C: 25.9%, T: 30.3%). Base composition was quite constant among sequences: G+C content ranged from 39.4% to 42.6% (31.6% to 47.1% for variable sites only).

2. Phylogenetic analysis

Phylogenetic signal was detected in the aligned cytochrome *b* data set. In maximum parsimony analysis with heuristic search, over the 650 bp segment for 10 cobitid species, 415 nucleotide positions were constant, 84 variable characters were uninformative and 151 were informative for parsimony analysis. Maximum parsimony analysis recovered a single most parsimonious tree,

with a length of 453 steps and a consistency index (excluding uninformative characters) of 0.684 (Fig. 1, a). Cobitidae was monophyletic with respect to the other noncobitidae species in the analysis, and this clade is supported with high bootstrap (100%). *Misgurnus* is recovered as the sister taxon of a monophyletic *Cobitis-Iksookimia-Niwaella* clade. The monophyly of the *Cobitis-Iksookimia-Niwaella* clade is supported with high bootstrap (81%) and decay (5) values (Fig. 1, a). *Orthrias* and *Lefua* are recovered as monophyletic. This clade receives high support in bootstrap (98%) and has a branch length of 33 steps (Fig. 1, a). But, it was found that this clade was paraphyletic from other Cobitidae.

The two species, *Cobitis sinensis* and *Niwaella multifasciata* were strongly monophyletic and continued to be placed outside the rest of cobitids. This clade was supported high bootstrap (88%) and decay (5) value (Fig. 1, a). *Cobitis melanoleuca* clade was recovered as the sister taxon of a monophyletic clade consisting of *Iksookimia koreensis* and *I. pumila* and this *Iksookimia* clade supported with high bootstrap (99%) and decay (9) values, and the length of the branch leading to this clade is 17 steps. Also, *Misgurnus anguillicaudatus* from China and *M. anguillicaudatus* from Yongdok were paraphyletic, but this clade was weak.

Maximum likelihood tree analyzed by quartet puzzling (Fig. 1, b). Number of puzzling step was 1,000. This tree was similar to the maximum parsimony tree with heuristic search but some difference resided in the placement between *Misgurnus anguillicaudatus* from China and *M. anguillicaudatus* from Youngdok, Korea. Their monophyly was supported high puzzle value (97%).

The neighbor joining (NJ) tree was constructed using Kimura's (Kimura, 1980) two-parameter distance (Table 3). This tree was very similar to the maximum likelihood tree with quartet puzzling (Fig. 1, c).

The hypothesis of Kim (1997) and Nalbant (1994) was not significantly different from the topology using maximum parsimony, maximum likelihood and neighbor joining criteria. Some surprising results from this analysis were that a paraphyletic *C. melanoleuca* from *C. sinensis* clade is strongly supported in bootstrap analysis (89%) and *Orthrias-Lefua* clade is early diverged from other cobitids and this clade high bootstrap value (98%).

Table 3. Pairwise distance matrix of Kimura 2-parameter

	1	2	3	4	5	6	7	8	9	10
1. <i>N. multifasciata</i>	–									
2. <i>C. sinensis</i>	0.05478	–								
3. <i>I. koreensis</i>	0.10860	0.09736	–							
4. <i>I. pumila</i>	0.10671	0.09551	0.01089	–						
5. <i>C. melanoleuca</i>	0.11572	0.11191	0.07416	0.07952	–					
6. <i>M. anguillicaudatus</i> (from Yongdok)	0.11191	0.11955	0.12185	0.12379	0.13106	–				
7. <i>M. anguillicaudatus</i> (from China)	0.11175	0.12325	0.12948	0.12751	0.13087	0.09907	–			
8. <i>C. auratus</i>	0.20437	0.18396	0.19021	0.18812	0.20924	0.18828	0.18960	–		
9. <i>L. costata</i>	0.20734	0.19876	0.20956	0.20742	0.21811	0.21811	0.21209	0.20904	–	
10. <i>O. toni</i>	0.28013	0.26914	0.26195	0.25957	0.28352	0.26702	0.28202	0.27186	0.22776	–

tion ranging from rapid to conservative (Irwin *et al.*, 1991). Due to the variable rate of evolution among the codon positions, cytochrome *b* appears to be useful for testing phylogenetic hypotheses for a wide range of divergences within Actinopterygians (Lydeard and Roe, 1997).

Phylogenetic relationships between Korean loaches (Cypriniformes: Cobitidae) were investigated by comparing cytochrome *b* gene sequences from 8 species, which were newly sequenced. As a result, four groups of Korean cobitids were found. Specimen composition in these groups was almost unchanged in all the reconstructed trees. The first identified clade included specimens from the Nakdong river basin, namely *Cobitis sinensis* and *Niwaella multifasciata*. The second clade included *Iksookimia koreensis* from the Mankyong river and *I. pumila* from the Buan Baecheon river. The third clade included *Misgurnus anguillicaudatus* from China and Youngdok, Korea. The final clade included *Orthrias toni* and *Lefua costata*.

Most phylogenies agree to a certain extent with previous phylogenetic hypotheses based on morphological studies. Disagreement between the previous hypothesis about *Cobitis* suggests that *C. melanoleuca* was early diverged from *C. sinensis*. Also, new interesting relationships were found: *Orthrias-Lefua* appeared to be paraphyletic in Cobitidae observed and *Iksookimia* clade was a sister to *Cobitis melanoleuca*. It was agreed that *Orthrias* and *Lefua* were classified by the other family Balitoridae (Nelson, 1994). Interfamilial sequence divergences between Cobitidae and Cyprinidae ranged from 0.184 to 0.272. This value coincided with sequence divergence value between *Orthrias-Lefua* and *Cobitis-Iksookimia*. We might conclude from these statement

that *Orthrias-Lefua* had been reclassified as the family Balitoridae. The intergeneric sequence divergences of Cobitidae ranged from 0.097 to 0.282 by pairwise distance of Kimura 2-parameter (Table 3). Intra-generic sequence divergence between *C. sinensis* and *C. melanoleuca* was 0.112. The value was higher than genus *Sebastes* (Teleostei, Scorpaenidae) which was low levels (<10%) of intrageneric sequence divergence (Rocha-Olivares *et al.*, 1999). Also Cobitidae was monophyletic with respect to the other noncobitidae species in the analysis. But intragenus *Cobitis* appeared to be paraphyletic.

The phylogenetic location of some *Iksookimia* species was pervaded to *Cobitis*. The problem of intergeneric cyprinid hybrids was addressed (Briolay *et al.*, 1998). The present study also was addressed the question of cobitid intergeneric hybrids. Intergeneric hybrids by interbreeding seems to be the consequence of a high genetic flexibility in cobitid fishes. The placement of *C. sinensis* and *C. melanoleuca* confused the recovery of a monophyletic *Cobitis*. The phylogenetic placement of these two species within Cobitidae cannot be resolved with the cytochrome *b* data set. The aberrant placement of *Cobitis sinensis* was critically examined by including a complete cytochrome *b* sequence of *C. lutheri*, *C. striata* and *N. brevifasciata* (unpublished data).

Presently, a 2% mtDNA divergence rate pr MY has been assumed for bony fishes, however, divergence rates as low as 0.5% have been suggested (Cantatore *et al.*, 1994). Given the relatively slow rate of nucleotide substitution in fish mitochondrial DNA relative to "conventional" rate estimates for vertebrates (Martin *et al.*, 1992; Bermingham *et al.*, 1997), these levels of specific sequence differentiation are remarkable

and suggest that many of these populations have been isolated for millions of years. Long term isolation may explain the unique color patterns of cobitid fishes. Specific sequence divergences between *I. koreensis* and *I. pumila* detected 1.1%. Thus, we concluded that geographic isolation leads to different values of sequence divergence, and on the other hand, genes which are under strong environmental selection (e. g. for pigmentation) would still reflect the special adaptations of the different stocks on their habitat. This leads to a great variability of species. However, for a final conclusion among the possible explanations, further studies on the molecular phylogeny of loaches are required.

Overall, we observed considerable genetic divergence among species, and the data seems to confirm the growing necessity for taxonomic revision of Cobitidae. Estimates of genetic distinction and relationships afforded by analysis of mitochondrial DNA sequences provide an excellent source of information that, when combined with analysis of morphological characters, should yield a predictive classification. Mitochondrial DNA evolves rapidly and is thus particularly useful for resolving relationships among recently evolved groups. However, it has the disadvantage that all of the mitochondrial genes are inherited as a single linkage group so that only one independent gene tree can be inferred, regardless of the number of sequenced genes. Introns of nuclear genes are attractive candidates for independent sources of rapidly evolving DNA. As a consequence, compared with cytochrome *b*, introns were expected to enter more distinct character states over the course of evolution and had fewer multiple substitutions and lower levels of homoplasy (Prychitko and Moore, 2000).

The phylogenetic results we reported from molecular data were mostly congruent with previous morphology based studies, although some new points were made. This suggests that both approaches are reliable for the study of fish phylogeny. Therefore, in this study, the cytochrome *b* sequence data of mtDNA was also useful in elucidating the phylogenetic relationships among Cobitidae which show great ecological adaptability. These analyses have provided further evidence for the utility of cytochrome *b* sequence in addressing phylogenetic relationships among Cobitidae.

References

- Bermingham, E., S. MacCafferty and A.P. Martin. 1997. The Isthmus of Panama, molecular clocks, and the historical biogeography of neotropical freshwater fishes. In: T.D. Kocher and C. Stepien (eds.), Molecular systematics of fishes. Academic Press, New York, pp. 22 ~ 35.
- Briolay, J., N. Galtier, M. Brito and Y. Bouvet. 1998. Molecular phylogeny of Cyprinidae inferred from cytochrome *b* DNA sequence. *Mol. Phy. Evol.*, 9 : 100 ~ 108.
- Cantatore, P., M. Roberti, G. Pesole, A. Ludovico, F. Milella, M.N. Gadaleta and C. Saccone. 1994. Evolutionary analysis of cytochrome *b* sequences in some Perciformes: Evidence for a slower rate of evolution than in mammals. *J. Mol. Evol.* 39 : 589 ~ 597.
- Irwin, D.M., T.D. Kocher and A.C. Wilson. 1991. Evolution of cytochrome *b* gene of mammals. *J. Mol. Evol.* 32 : 128 ~ 144.
- Hasegawa, M., H. Kishino and T. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* 21 : 160 ~ 174.
- Kim, I.S. 1980. Systematic studies on the fishes of the family Cobitidae (Order Cypriniformes) in Korea. I. Three unrecorded species and subspecies of the genus *Cobitis* from Korea. *Kor. J. Zool.* 23 : 239 ~ 249.
- Kim, I.S. 1997. Illustrated encyclopedia of fauna & flora of Korea. Vol. 37. Freshwater Fishes. The Ministry of Education, Korea. (in Korean)
- Kim, I.S. and J.H. Lee. 1986. A chromosomal study on the genus *Cobitis* (Pisces: Cobitidae) in the southern part of Korea. *Bull. Korean Fish. Soc.*, 19 : 257 ~ 264. (in Korean)
- Kim, I.S. and J.Y. Park. 1997. *Iksookimia yongdoensis*, a new cobitid fish (Pisces: Cobitidae) from Korea with a key to the species of *Iksookimia*. *Ichthyol. Res.*, 44(3) : 249 ~ 256.
- Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. *J. Mol. Evol.*, 16 : 111 ~ 120.
- Kobayasi, H. 1976. Comparative study of karyotypes in the small and large race of spinous loaches (*Cobitis biwae*), *Zoological Magazine*, 85 : 84 ~ 87.
- Kocher, T.D., W.K. Thomas, A. Meyer, S.V. Edward, S. Paabo, F.X. Villablanca, and A.C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA.* 86 : 6196 ~ 6200.
- Lee, H.Y. and H.S. Lee. 1989. Cytogenetics of the cobitid fish (Pisces: Cobitidae) in Korea. I. Intra-population polymorphism in the distribution of

- constitutive keteochromation of spinous loach, *Cobitis taenia*. Korean J. Genetic., 11 : 97~104.
- Lydeard, C. and K.J. Roe. 1997. The phylogenetic utility of the mitochondrial cytochrome *b* gene for inferring relationships among actinopterygian fishes. In: T.D. Kocher and C. Stepien (eds.), Molecular systematics of fishes. Academic Press, New York, pp. 285~303.
- Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. In: "Molecular Cloning: a Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp. 15~32.
- Martin, A.P., G.J. Naylor, and S.R. Palumbi. 1992. The rate of mitochondrial DNA evolution in sharks are slow compared with mammals. Nature, 357 : 153~155.
- Nalbant, T. 1993. Some problems in the systematics of the genus *Cobitis* and its relatives (Pisces, Ostariophysi, Cobitidae). Rev. Roum. Biol. (Biol. Anim.), 38 : 101~110.
- Nalbant, T. 1994. Studies on loaches (Pisces: Ostariophysi: Cobitidae). I. An evaluation of the valid genera of Cobitidae. Trav. Mus. Hist. Nat. "Grigore Antipa", Vol. XXXIV : 375~380.
- Nelson, J.S. 1994. Order Cypriniformes. In: Nelson, J.S. (ed), Fishes of the world. Third edition. John Wiley & Sons, Ins., New York, pp. 139~140.
- Park, C.S. and H.Y. Lee. 1991. Systematic study on the fishes of family Cobitidae (Pisces: Cypriniformes): Extensive variation in mitochondrial DNA among geographic populations of *Nemacheilus toni*. Korean J. Ichthyol., 3 : 140~147.
- Prychitko, T.M. and W.S. Moore. 2000. Comparative evolution of the mitochondrial cytochrome *b* gene and nuclear fibrinogen intron 7 in woodpeckers. Mol. Biol. Evol., 17 : 1101~1111.
- Rocha-Olivares, A., C.A. Kimbrell, B.J. Eitner, and R.D. Vetter. 1999. Evolution of a mitochondrial cytochrome *b* gene sequence in the species-rich genus *Sebastes* (Teleostei, Scorpaenidae) and its utility in testing the monophyly of the subgenus *Sebastomus*. Mol. Phyl. Evo., 11 : 426~440.
- Saito, N. and M. Nei. 1987. The neighbor-joining method : a new method for reconstructing phylogenetic trees. Mol. Biol. Evol., 4 : 106~125.
- Sawada, Y. 1982. Phylogeny and zoogeography of the superfamily Cobitidae (Cyprinoidae, Cypriniformes). Mem. Fac. Fish. Hokaido Univ., 28 : 65~223.
- Swofford, D.L. 2000. PAUP* Ver. 4.0b4a, Sinauer, Sunderland, MA.
- Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin, and D.G. Higgins. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res., 24 : 4876~4882.
- Yang S.Y. and J.H. Kim. 1995. Systematic study on the fishes of the family Cobitidae (Pisces, Cypriniformes) 6. A study on mitochondrial DNA RFLP in Korean cobitid fish, *Cobitis koreensis*. Institute for Basic Science. Inha Uni., 16 : 169~174. (in Korean)

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Mitochondrial Cytochrome *b* gene의 분석에 의한 한국산 미꾸리과 어류 (Cobitidae)의 계통

김소영 · 김익수 · 장광엽 · 장미희

전북대학교 생물학부

한국산 미꾸리과 어류의 계통유전학적 관계를 고찰하고자 8종의 mitochondrial cytochrome *b*의 유전자 서열을 비교한 결과 대부분 이전의 형태학적 연구의 결과와 일치하였다. 그러나 종개속 *Orthrias*과 쌀미꾸리속 *Lefua*의 분류학적 위치는 미꾸리과 Cobitidae와 paraphyletic group으로 나타났으며 이 두 속의 sequence divergence는 0.184~0.272으로 나타나 미꾸리과와 잉어과 사이의 divergence와 유사하였다. 한편 참종개속 *Iksookimia* 2종과 북방종개 *Cobitis melanoleuca*는 각각 다르게 분화한 결과를 보여 주었으며 또한 중국산 미꾸리와 한국산 영덕 미꾸리의 sequence divergence는 0.099로 종간의 divergence를 보여주어 주목되었다. 미꾸리과 어류 가운데 참종개속의 일부 어류는 분류학적 위치로 보아 이들의 기원이 미꾸리과의 속간 잠종기원으로 생각된다.