Molecular phylogeny of *Lethenteron* species from the Far East, with their taxonomic problems

**YUJI YAMAZAKI**
Department of Biology, Faculty of Science, Toyama University, Toyama 930-8555, Japan (yatsume@sci.toyama-u.ac.jp)

**SUMMARY:** Allozyme analyses were performed to disclose the phylogenetic relationships among *Lethenteron* species occurring in the Far East. As a result, several complete allele substitutions were found between all sympatric pairs of four *Lethenteron* taxa, strongly suggesting the existence of reproductive isolation among them. Therefore, these four taxa, *L. japonicum*, *L. kessleri*, and the northern and southern forms of *L. reissneri*, should be regarded as discrete species. *Lethenteron japonicum* appeared to be closely related to *L. kessleri* and the northern form of *L. reissneri*, whereas the southern form of *L. reissneri* was greatly divergent from the other three *Lethenteron* species, suggesting that the former three species are monophyletic. In the monophyletic group, the two nonparasitic (and fluvial) species, *L. kessleri* and the northern form of *L. reissneri*, are supposed to have speciated from the ancestral stocks of parasitic (and anadromous) *L. japonicum* in different geological times. The present study also indicates that some taxonomic problems are comprised in fluvial, nonparasitic lamprey species from the Far East. At present, some fluvial lamprey species and local populations are faced with endangered situation in Japan and Korea.

**KEY WORDS:** lamprey, allozyme, genetic divergence, speciation, conservation

**INTRODUCTION**

Because lampreys, usually designated as Agnatha with hagfishes, have been considered as a representative of primitive vertebrates, they have received special attention in taxonomy, phylogeny, ecology and other diverse fields. Many lamprey genera include several species, variously characterized by parasitic-anadromous or nonparasitic-fluvial life-cycle. In such species’ assemblages, the latter are generally called satellite species, the nonparasitic-fluvial forms being thought to have been most likely derived from congeneric parasitic-diadromous species.

Only three *Lethenteron* species, parasitic-anadromous *L. japonicum* and nonparasitic-fluvial *L. kessleri* and *L. reissneri*, have been formally described from the Far East, the latter two being treated as satellite species. Recently, allozyme analyses have disclosed two genetically divergent groups (northern and southern forms) within *L. reissneri* distributed in Japan and Korea. The phylogenetic relationships among *Lethenteron* taxa from the Far East including the two forms of *L. reissneri* were not clear. Additionally, although the identification between *L. kessleri* and *L. reissneri* was based on morphological characteristics, their taxonomy had been objected. Therefore, it is clear that some taxonomic and phylogenetic problems still need resolution in *Lethenteron* taxa from the Far East.

In this study, allozyme analyses were performed for specimens collected from Japan, Korea and the Far Eastern region of Russia, in order to clarify the genetic characteristics of each *Lethenteron* taxa occurring in the Far East and the phylogenetic relationships among these taxa.

**MATERIALS AND METHODS**

Samples of four *Lethenteron* taxa were collected from Japan, Korea and the Far Eastern region of Russia from 1993 to 1998. All identifications were based on morphological and genetic characteristics. As an outgroup, *Entosphenus tridentatus* population collected from the Snake River, tributary of the Columbia River was examined.

Allozyme analyses were performed based on Yamazaki & Goto. Allele frequencies were calculated directly from observed genotypes. Nei’s unbiased genetic distance (D) was calculated between all possible pairs of samples from allele frequencies. A dendrogram based on the D values was generated using the UPGMA method to estimate the genetic relationships among populations.
RESULTS

Diagnostic alleles among taxa

Twenty-seven gene loci encoding 15 enzymes and one general protein were assumed in the present study as follows: AAT-1*, 2*, ACP*, AH-1*, 2*, EST*, FH*, G3PDH*, G6PDH*, GPI-1*, 2*, IDDH*, IDHP-1*, 2*, 3*, 4*, LDH*, MDH-1*, 2*, 3*, 4*, MEP-1*, 2*, PGDH*, PGM*, PROT-1* and 2*. Four Lethenteron taxa could be distinguished from each other by having diagnostic alleles at 1 to 11 loci. The samples of *L. japonicum* were distinguished from *L. kessleri* samples by one locus, from the northern form of *L. reissneri* by 2 loci, and from the southern form of *L. reissneri* by 11 loci. The samples of *L. kessleri* were distinguished from the northern form of *L. reissneri* by 3 loci, and from the southern form of *L. reissneri* by 11 loci. The northern-form samples of *L. reissneri* were distinguished from the southern-form samples of *L. reissneri* by 11 loci. All possible pairs of taxa, except that between *L. kessleri* and the northern form of *L. reissneri*, were found in at least one river. No hybrids between the taxa were found in any sympatric samples.

Genetic variability and differentiation

Nei's unbiased genetic distances ($D$) between all possible pairs of samples are shown in Table 1.

<table>
<thead>
<tr>
<th>Number of population</th>
<th><em>L. japonicum</em></th>
<th><em>L. kessleri</em></th>
<th>Northern form</th>
<th>Southern form</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. japonicum</em></td>
<td>12</td>
<td>0.007</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. kessleri</em></td>
<td>8</td>
<td>0.055</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td>Northern form of <em>L. reissneri</em></td>
<td>20</td>
<td>0.268</td>
<td>0.292</td>
<td>0.117</td>
</tr>
<tr>
<td>Southern form of <em>L. reissneri</em></td>
<td>19</td>
<td>0.687</td>
<td>0.692</td>
<td>0.711</td>
</tr>
<tr>
<td><em>E. tridentatus</em></td>
<td>1</td>
<td>0.720</td>
<td>0.723</td>
<td>0.746</td>
</tr>
</tbody>
</table>

Fig. 1 Collection site of *Lethenteron* taxa from the Far East. Figures refer to sample sites listed in Figure 2.

Fig. 2 A UPGMA dendrogram using Nei's unbiased genetic distance for 60 samples of *Lethenteron*, based on 27 loci of biochemical genetic markers. Circle, triangle, solid and open squares indicate *L. japonicum*, *L. kessleri*, the northern and southern forms of *L. reissneri*, respectively. Star indicates the sample of *Entosphenus tridentatus* collected from the Columbia River, North America.

Of *L. reissneri* by 2 loci, and from the southern form of *L. reissneri* by 11 loci. The samples of *L. kessleri* were distinguished from the northern form of *L. reissneri* by 3 loci, and from the southern form of *L. reissneri* by 11 loci. The northern-form samples of *L. reissneri* were distinguished from the southern-form samples of *L. reissneri* by 11 loci. All possible pairs of taxa, except that between *L. kessleri* and the southern form of *L. reissneri*, were found in at least one river. No hybrids between the taxa were found in any sympatric samples.

Table 1 Average values of Nei's unbiased genetic distance between pairs of populations of four *Lethenteron* and one *Entosphenus* taxa.
three taxa was most distantly connected with that of the southern-form samples of *L. reissneri*, excluding the outgroup (*Entosphenus tridentatus*).

**DISCUSSION**

In the *Lethenteron* taxa from the Far East, several complete allele substitutions were found between all sympatric pairs of taxa, indicating the existence of reproductive isolation between them. Consequently, the present data strongly suggest that each of the four taxa should be recognized as fully discrete species.

Judging from the somewhat low genetic divergence, the following three *Lethenteron* species, *L. japonicum*, *L. kessleri*, and the northern form of *L. reissneri*, must be monophyletic, suggesting the latter two species are satellite species of the former species. On the other hand, the southern form of *L. reissneri* is greatly divergent from the other three *Lethenteron* species. These results were also supported by molecular phylogenetic analyses deduced from mitochondrial DNA CO I gene region sequence (Yamazaki et al., unpubl. data).

As the speciation hypothesis in lampreys, the nonparasitic, fluvial forms were thought to have been most likely derived from congeneric parasitic, diadromous species. According to the present study based on allozyme analyses, each of *L. kessleri* and the northern form of *L. reissneri* was thought to have been derived from *L. japonicum* or its ancestor. However, it may be difficult to determine whether or not the southern form of *L. reissneri* has evolved monophyletically from the other *Lethenteron* species (or its ancestor) presently distributed in the Far East, because of a large genetic divergence between the former and the latter.

The present study also indicates that some taxonomic problems are comprised in fluvial and nonparasitic lamprey species from the Far East. Allozyme analyses enabled us to distinguish between the two forms of *L. reissneri*. However, the specimen from the Far Eastern region of Russia including the type locality of *L. reissneri*, the Amur River basin, did not match either of them (Fig. 3). Therefore, specimens reported as *L. reissneri* from the Japanese Archipelago and the Korean Peninsula may, in fact, represent a different species from *L. reissneri* (sensu stricto) described from the Amur. There is clearly a need for further comparison of the specimens used in this study with the type specimens of *L. reissneri*.

In addition, specimens collected from the Primorski and Amur regions, where *L. reissneri* has been the only fluvial lamprey reported, have similar genetic characteristics to those from Hokkaido, Sakhalin, and the Yakutsk region (Yamazaki et al., unpubl. data), such having formerly been treated as *L. kessleri*. These indicate a need for further morphological and genetic studies with a special emphasis on specimens collected from the Anadyr River system, where both *L. kessleri* and *L. reissneri* have been reported.

Recently, the numbers and ranges of various animal species have decreased, probably as a consequence of drastic environmental changes by human activities. The three fluvial *Lethenteron* species are also regarded as vulnerable species, because of reduction of their numbers and ranges, as well as some other lampreys. These declines indicate a necessity for taking measures to conserve lamprey and fish species.

**ACKNOWLEDGMENTS**

I thank Dr. A. Goto, Hokkaido University, for his invaluable suggestions in the course of this study. I am also grateful to Mr. V. V. Tsiger, TINRO Center, Dr. S. R. Jeon, Sang-Myung University, Dr. H. Sakai, National Fisheries University, Dr. K. Takata, Shinshu University and Dr. M. Nishida, University of Tokyo, for providing valuable suggestions and field assistance.

**REFERENCES**