Diversity and conservation of the northern Far East Asian freshwater fishes, with a case study on freshwater sculpins

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SUMMARY: The freshwater fishes in the northern Far East were investigated to elucidate the species richness, ecological complexity and genetic diversity. The fishes in Hokkaido Island, Sakhalin Island, Primor'e and the Amur River basin were composed of 27 families, 83 genera and 130 species which possessed high ecological and genetic diversity. Concerning the freshwater sculpins, for example, one *Trachidermus*, one *Mesocottus* and 11 *Cottus* species are distributed in the regions. The mitochondrial DNA analyses made clear that the amphidromous, lacustrine and fluvial species have originated from the ancestral stocks of catadromous species such as *T. fasciatus* and *Cottus kazika*, showing the creation of species diversity. Mainly because of rapid changes of freshwater environments, however, nearly 10-40 % fish species to the total number of species distributed in the regions were found to be endangered, indicating the necessity of effective conservation actions.

KEY WORDS: species diversity, life-history diversity, genetic variability, phylogenetic relationships, speciation, endangered fish

INTRODUCTION

The freshwaters are composed of rivers, streams, lakes, swamps, waterways in rice field and so on, in which fish species having various modes of life-histories live: those are fluvial, anadromous, catadromous, amphidromous, estuarine, lacustrine and so forth. The diversity of these freshwater fishes are characterized by hierarchical structures from genes to ecosystems through populations and communities (Fig. 1).¹⁾

In the last decade, however, the environments of freshwaters have been rapidly changed mainly by great increase of human activities which had resulted in global warming, water pollution, reclamation of streams and lakes, artificial reservoirs and so on.^{2,3)} As a result, freshwater fishes had heavy damages on number of species and population sizes.

For example, an estimation of habitat losses in cold-water adapted fish by globally climatic warming was carried out using two charrs, *Salvelinus malma* and *S. leucomaenis*, and demonstrated that the habitat losses of charrs will advance according to the increase of average annual temperature.⁴⁾

Therefore, it is reasonable to state that we should face the research subjects of freshwater fish diversity and their conservation in both global and local scales. The main subjects for understanding the diversity of freshwater fishes in the northern Far East are as follows: the first is to elucidate the species richness which includes abundance of species numbers and population sizes in each species.; the second is to make clear the ecological diversity in which complexity of life-history patterns and reproductive styles, population dynamics and interspecific relationships in fishes are included; and the third is to elucidate the variability of genetic population structure and phylogeography in each fish species, and phylogenetic relationships among the species.

DIVERSITY AND CONSERVATION OF THE NORTHERN FAR EAST ASIAN FRESHWATER FISHES

In order to estimate the diversity of freshwater fishes in northern Far East, our research group have had several expeditions in the regions for recent 10 years. In this paper, I describe briefly about some findings of species, ecological

Fig. 1. Habitat diversity and hierarchical structure of biodiversity in the freshwater fishes.
S: spawning, G: growth.

and genetic diversities of fishes.

The expeditions were carried out mainly in Hokkaido Island, Japan and Sakhalin Island, Primor'e and the Amur River basin in Russia. Concerning the species richness, the native freshwater fishes are composed of 27 families, 83 genera and about 130 species in all the four regions. In each region, Hokkaido is inhabited by 36 genera 59 species, Sakhalin by 39 genera 39 genera 57 species, Primor'e by 33 genera 45 species, and Amur River basin by 70 genera 90 species, demonstrating the most species richness in Amur River basin (Table 1).⁵⁾

From the view point of life-history types, these fishes are classified into pure freshwater fish, secondary freshwater fish, diadromous fish and peripheral fish. Furthermore, diadromous fishes are subdivided into anadromous, catadromous and amphidromous fish.^{6,7)} Comparing to the poorness of pure freshwater fish species in Hokkaido, Sakhalin and Primor'e, remarkably abundant species are found in Amur River basin. The species richness of these fishes in Amur River basin is supposed to have occurred mainly due to the connection of this river with a branch of Yellow River basin about 100 thousands years ago.^{8,9)} In addition, one more peculiarity is found in the abundance of

amphidromous fish species in Hokkaido Island which are composed of *Plecoglossus altivelis*, 3 *Cottus* species and 12 gobioid species.⁷⁾ This may be due to the past northward dispersal of these fishes from Honshu Island.

About the diversity of reproductive styles, only the feature in Hokkaido Island is presented because of almost no data in Russia. Hokkaido's freshwater fishes are limited to have 7 reproductive styles. As nonguarders, there are 4 styles, and the other 3 styles are found in guarders; For example, osmerid species are nonguarders and have the style as rock and gravel spawners with pelagic larvae. On the other hand, gobioid species are guarders and have the styles as hole nesters.⁷⁾

Concerning the genetic diversity, we have obtained some new findings of peculiar genetic structure and genetic divergence in several taxa of the norther Far Eastern freshwater fishes. Of the findings, following two examples are shown: One is for threespine sticklebacks and the other is for Japanese fluvial sculpins.

In threespine sticklebacks, it has been believed that they comprise only one species, *Gasterosteus aculeatus* around Japan. Our recent studies on the fishes, however, demonstrated clearly that two high-genetically divergent

Table 1. Abundance of fish species distributed in freshwaters of the northern Far East

Family	Japan	Far East Russi		
	Hokkaido Island	Sakhalin Island	Primor'e	Amur River basin
Petromyzontidae	4 (2)*	2 (1)	2 (1)	2 (1)
Acipenseridae	1 (1)	1 (1)	0 (0)	2 (2)
Clupeidae	1 (1)	0 (0)	1 (1)	1 (1)
Salmonidae	9 (3)	8 (3)	8 (4)	8 (4)
Coregonidae	0 (0)	0 (0)	0 (0)	1 (1)
Thymallidae	0 (0)	0 (0)	1 (1)	0 (0)
Osmeridae	7 (4)	3 (2)	2 (2)	1 (1)
Salangidae	1 (1)	1 (1)	0 (0)	1 (1)
Esocidae	0 (0)	1 (1)	0 (0)	1 (1)
Dalidae	0 (0)	0 (0)	0 (0)	1 (1)
Anguillidae	1 (1)	0 (0)	0 (0)	0 (0)
Cyprinidae	3 (5)	18 (13)	13 (9)	44 (33)
Cobitidae	3 (3)	3 (3)	3 (3)	3 (3)
Siluridae	0 (0)	1 (1)	0 (0)	2 (2)
Bagridae	0 (0)	1 (1)	0 (0)	7 (5)
Adrianichthyidae	0 (0)	0 (0)	0 (0)	0 (0)
Hemirhamphidae	1 (1)	1 (1)	1 (1)	0 (0)
Gadidae	0 (0)	0 (0)	0 (0)	1 (1)
Lotidae	0 (0)	1 (1)	0 (0)	1 (1)
Gastrosteidae	5 (2)	5 (2)	2 (2)	2 (2)
Syngnathidae	0 (0)	0 (0)	1 (1)	0 (0)
Mugilidae	2 (2)	1 (1)	1 (1)	2 (1)
Percichthyidae	0 (0)	0 (0)	0 (0)	1 (1)
Percidae	0 (0)	0 (0)	0 (0)	1 (1)
Gobiidae	16 (8)	6 (3)	7 (5)	5 (4)
Cottidae	4 (1)	2 (2)	2 (1)	2 (2)
Pleuronectidae	1 (1)	2 (2)	1 (1)	1 (1)
27 families	59 (36)	57 (39)	45 (33)	90 (70)
Total	27 families 83 genera 130 species			

* Figures in parentheses indicate the number of genera.

forms (Nei's $D=0.43$) exist around Japan.¹⁰⁾ One form was conveniently named as Pacific Ocean form, and the other as Japan Sea form. The two forms were found to be reproductively isolated from each other in several sympatric populations. Therefore, the two forms should be classified as different species. The other example is of a cold-water adapted sculpin, *Cottus nozawae*. This species is endemic to Japan, distributed in Hokkaido Island and Tohoku District, the northernmost part of Honshu Island. The mitochondrial DNA analysis showed a great genetic divergence between two regional populations from Hokkaido and Tohoku District.¹¹⁾ In the southern margin of its range, moreover, haplotype displacements were observed between tributary populations within a single river-system, probably because of their habitat fragmentation in this region and their strong sedentarity.¹²⁾

In the last two decades, however, the species diversity has gradually decreased mainly because of human-induced environmental changes. Of about 250 freshwater and blackish water fish species distributed in Japan, 3 species or subspecies were already extinct, and 88 species or subspecies are now endangered (the Environmental Agency, Japan Government).¹³⁾ A comparison of the relative

abundance of extinct and endangered species among several regions or countries shows that the percentage to the total number of species is considerably high in whole Japan, whereas that in Hokkaido Island is not so high (Table 2). The official conservation actions should be performed for the endangered freshwater species in Japan.

Table 2. Comparison of abundance of endangered freshwater fish species among several regions

Region	Total number of species distributed	Number of endangered species (%)
Japan	250	91 (36)
Hokkaido Island	59	9 (15)
Far East Russia	130	unknown
Europe	122	74 (61)
Sweden	16	4 (27)
North America	292	82 (28)
Australia	65	22 (34)

A CASE STUDY ON THE ORIGIN OF SPECIES DIVERSITY IN FRESHWATER SCULPINS

In the Far Eastern Asia, the freshwater sculpins are composed of one *Trachidermus*, one *Mesocottus* and 11 *Cottus* species, which have various types of life-histories such as catadromous, amphidromous, lacustrine and fluvial types.

In Japan, for example, the freshwater sculpins are thought to comprise one *Trachidermus* and 7 *Cottus* species.¹⁴⁾ Of the species, *Trachidermus fasciatus* and *Cottus kazika* are catadromous, *C. amblystomopsis*, *C. pollux* SE, *C. pollux* ME and *C. hangiongensis* are amphidromous, *C. reinii*, which are endemic to Lake Biwa, is lacustrine, and *C. nozawae* and *C. pollux* LE are fluvial. Recent allozyme and mtDNA studies showed that Japanese freshwater sculpin species comprise two major clades and the lacustrine *C. reinii* is a genetic variant within the amphidromous *C. pollux* SE, the latter suggesting that they should be a single species (Fig. 2). Therefore, the valid *Cottus* species in Japan is thought to be 7 species, and among them 5 species (*kazika*, *pollux* ME, *pollux* LE, *reinii* and *nozawae*) are endemic to Japan.

In order to elucidate the origin, dispersal and speciation events of these sculpins, the phylogenetic relationships among the freshwater sculpins in the Far Eastern Asia were inferred from nucleotide sequences of mitochondrial 12S rRNA gene and control region (1,895 base-pairs).

Fig. 2. Maximum likelihood tree of Japanese *Cottus* and *Trachidermus* species, deduced from 12S rRNA sequence data (622 base pairs). ** significantly positive, $p < 0.01$.

The sculpin samples used in the present study were collected mainly from Japan, Russian Far East and Southern Korea. In the continental areas, *Mesocottus haitei* was collected from the Amur River, Russia, *C. cognatus*, which is well-known to be also distributed in Alaska of North America, was collected from Anadyr of Russia, *C. kessleri* from Lake Baikal, *C. poecilopus* from the Amur River basin, Korea and Poland, *C. amblystomopsis* from the Tummin River, Russia, *C. czerskii* from the Sedanka River near Vladivostok, *C. hangiongensis* from Korea, and *T. fasciatus* from Korea. In addition, *C. gobio* from St. Petersburg, Russia and *C. aleuticus* from Alaska of North America were also used as material species in this study. These continental samples were used together with the samples collected from Japan for mtDNA sequencing analysis. In this analysis, two marine sculpins, *Leptocottus armatus* from North America and *Myoxocephalus stelleri*, from Hokkaido, Japan were used as the outgroups.

In the first step, we examined the phylogenetic relationships among the sculpin species collected from the Far Eastern Asia, Europe and North America. Figure 2 shows the Neighbor Joining tree (NJ tree) constructed by the sequence data. The NJ tree had almost similar topology with the most parsimonious tree (MP tree) and the Maximum likelihood tree (ML tree), but only MP and ML trees supported the monophyly of *Trachidermus* and *Cottus* clade. The MP tree indicated that there are following two greatly divergent genetic groups in the Far Eastern *Trachidermus* and *Cottus* species: Group I consists of *T. fasciatus* and *C. kazika* with catadromous life-histories, and Group II consists of the remaining *Cottus* species with amphidromous, lacustrine and fluvial life-histories. This result strongly suggests that the Far Eastern *Cottus* species

are not monophyletic but paraphyletic, because *C. kazika* is more closely related to *T. fasciatus* than the other *Cottus* species examined.

In Group II, several monophyletic species pairs were found. Interestingly, such sister relationships supported by high bootstrap probabilities (above 90 %) are seen between amphidromous *C. amblystomopsis* and fluvial *C. nozawae*, and between amphidromous *C. pollux* ME and fluvial *C. pollux* LE, suggesting the parallel evolution might have occurred from an ancestral amphidromous species to a fluvial one.¹⁵⁾ In contrast, the samples of *C. poecilopus* from Russia and Korea were found to be somewhat distantly related from each other and to be paraphyletic or polyphyletic, strongly suggesting that they should be classified as distinct taxonomic entities (Yokoyama and Goto, unpublished data).

In the second step, we attempted to estimate the evolutionary process of life-history diversity in the Far Eastern freshwater sculpins, by mapping the data of a reproductive character, marine spawning or freshwater spawning, on the phylogenetic tree. From this analysis, it is supposed that the amphidromous, lacustrine and fluvial life-styles with freshwater spawning habits have been derived from the catadromous life-style with marine spawning habits (Yokoyama and Goto, unpublished data). Unfortunately, however, the evolutionary process of the amphidromous, lacustrine and fluvial life-styles remains to be solved in the present study.

In summary, the origin and dispersal of Far Eastern *Trachidermus* and *Cottus* species are supposed to be as follows: the oldest freshwater sculpins such as *T. fasciatus* and *C. kazika* having the primitive morphological characters and marine spawning habit have originated in the southern part of Far East, and then one of the ancestral stocks have migrated westward to Europe through the Siberian Continent and the other eastward to the North America Continent, following the successive speciations. Of the freshwater sculpins in Far East having already-mentioned diversity and evolutionary histories, following 3 species or local populations are endangered: *Trachidermus fasciatus*, *Cottus reinii* and the Tohoku populations of *Cottus nozawae* (Table 3).¹³⁾ Except for *Cottus poecilopus* in Russia, the ranges of all the species are restricted to narrow areas. Therefore, the official conservation plans and actions should be made immediately for these endangered and restrictedly distributed freshwater sculpins.

Table 3. Endemism and the ranks of endangered state of *Trachidermus* and *Cottus* species in the northern Far East

Species	Endemic to:	Rank in RED LIST *
<i>Trachidermus fasciatus</i>	Kyushu Island, Korea, south-eastern China	Vulnerable
<i>Cottus kazika</i>	Honshu, Shikoku and Kyushu Islands	
<i>Cottus reinii</i> (pollux SE)	Honshu and Shikoku islands	Vulnerable
<i>Cottus pollux</i> ME	Hokkaido, Honshu, Shikoku and Kyushu islands	
<i>Cottus pollux</i> LE	Honshu, Shikoku and Kyushu Islands	
<i>Cottus hangiongensis</i>	Hokkaido and Honshu islands, Korea	
<i>Cottus amblystomopsis</i>	Hokkaido and Sakhalin islands, Primor'e	
<i>Cottus nozawae</i>	Hokkaido Island, Tohoku District	Endangered local population
<i>Cottus cognatus</i>	Anadyr	
<i>Cottus czerskii</i>	Primor'e	
<i>Cottus poecilopus</i>	Primor'e, Amur River basin	
<i>Cottus poecilopus</i> (Yellow-fin sculpin)	Korea	

* Based on Environment Agency, Japan Government (1999).

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