

Genetic differentiation of the northern Far East cyprinids, *Phoxinus* and *Rhynchocypris*

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SUMMARY: Although minnows of the genera *Phoxinus* and *Rhynchocypris* (Cyprinidae) are abundantly distributed in the northern Far East region, disagreements on their taxonomy have persisted. Gene products of 18 allozyme or protein coding loci of 21 populations of minnows from the northern Far East were investigated in order to resolve their classification and genetic relationships. Six monophyletic clusters were identified, being consistent with *Phoxinus phoxinus*, *Rhynchocypris oxycephalus*, *R. percnurus*, *R. czekanowskii*, *R. kumgangensis* and *R. semotilus*, plus one paraphyletic group referred to as *R. lagowskii*.

KEYWORDS: allozyme, electrophoresis, taxonomy, Cyprinidae, phoxinin minnows

INTRODUCTION

In the northern Far East region, many phoxinin minnows have been reported and variously classified. Nakamura¹⁾ used the genus *Moroco* for three Japanese species, *M. jouyi*, *M. steindachneri* and *M. percnurus sakhalinensis*. On the other hand, Nakabo²⁾ included them in the genus *Phoxinus*, regarding them as subspecies of continental taxa, viz *P. oxycephalus jouyi*, *P. lagowskii steindachneri* and *P. percnurus sakhalinensis*. Kim³⁾ recognised six Korean phoxinin species in two genera: *Phoxinus phoxinus* and *Rhynchocypris oxycephalus*, *R. steindachneri*, *R. percnurus*, *R. kumgangensis* and *R. semotilus*, the last two being endemic to the Korean Peninsula. Chereshev⁴⁾ reported four species from Far East Russia, including them in the genus *Phoxinus*, *P. phoxinus*, *P. lagowskii*, *P. percnurus* and *P. czekanowskii*. He treated *oxycephalus* as a subspecies of *P. lagowskii*, and recognized some subspecies in both *P. percnurus* and *P. czekanowskii*, the latter being endemic to Russia.⁴⁾ Previously, Howes' worldwide revision of phoxinin minnows⁵⁾ divided the Asian species into four genera, namely, *Tribolodon jouyi*, *Lagowskiella lagowskii*, *L. czekanowskii*, *Eupallasella percnurus*, *Phoxinus phoxinus* and *P. semotilus*. *Tribolodon* was apparently a misidentification, that genus being more correctly applied to the Far Eastern dace, such as *T. hakonensis*.

Summarizing these studies led us to tentatively recognize seven groups of phoxinin minnows in the northern Far East region: *oxycephalus*, *lagowskii*, *percnurus*, *czekanowskii*, *kumgangensis*, *semotilus* and *phoxinus* groups, their generic classifications being ambiguous. Their morphological distinctions included: *oxycephalus* - a deeper caudal peduncle than the other species; *lagowskii* - a dark longitudinal lateral band on the body and a black blotch on the caudal base; *percnurus* - a thick body and lower jaw a little more prominent than or reveal with the upper jaw; *czekanowskii* - lateral line canal absent on body, but several pored scales above the pectoral fin; *kumgangensis* - two dark lateral bands on body; *semotilus* - a large dark spot on dorsal fin base; *phoxinus* - several irregular lateral cross bands on body.

However, because visual characteristics are somewhat subjective, they are of limited value in assessing the relationships of specimens from different localities. Accordingly, we analyzed allozyme allelic compositions of phoxinin minnows collected from the northern Far East region in an attempt to stabilize their classification.

MATERIALS AND METHODS

Twenty-one samples of tentatively-classified *oxycephalus*, *lagowskii*, *percnurus*, *czekanowskii*, *kumgangensis*,

Table 1 Sampling localities, date of collection and sample size

Abbrev.	Localities	Date	Sample size
<i>Rhynchocypris oxycephalus</i>			
1 Honshu	Koya River, Japan	June 1999	20
2 Korea	Somjin R., Korea	May 1996	20
3 Vladivostok	Sukhodol R., Russia	June 2000	22
4 Amur	Amur R., Russia	August 1997	20
<i>R. lagowskii</i> group			
5 Honshu	Mogami R., Japan	October 1997	20
6 Korea	Nakpung R., Korea	May 1995	20
7 Vladivostok	Razdolnaya R., Russia	June 2000	19
8 Amur	Amur R., Russia	August 1997	14
<i>R. percnurus</i>			
9 Hokkaido	Tombetsu R., Japan	June 1993	20
10 Amur	Amur R., Russia	August 1997	20
11 Sakhalin	Tym R., Russia	August 2000	6
12 Lena	Lena R., Russia	August 1998	20
13 Anadyr	Anadyr R., Russia	August 1998	20
<i>R. czekanowskii</i>			
14 Amur	Amur R., Russia	August 1998	22
15 Tumnin	Tumnin R., Russia	August 1998	4
16 Sakhalin	Tym R., Russia	August 2000	9
<i>R. kumgangensis</i>			
17 Korea	Pukhan R., Korea	May 1996	20
<i>R. semotilus</i>			
18 Korea	Songchon R., Korea	July 1999	16
<i>Phoxinus phoxinus</i>			
19 Korea	Samchokoship R., Korea	May 1996	20
20 Amur	Amur R., Russia	August 1997	20
21 Anadyr	Anadyr R., Russia	August 1998	20

semotilus and *phoxinus* groups were collected from the northern Far East region, Japan, Korea and Russia (Table 1, Fig. 1). The gene products of 18 allozyme or protein coding loci were investigated by starch gel electrophoresis (12 % gel): aspartate aminotransferase (E. C. 2.6.1.1, *AAT-1**, *AAT-2**), alcohol dehydrogenase (E. C. 1.1.1.1, *ADH**), glycerol-3-phosphate dehydrogenase (E. C. 1.1.1.8, *G3PDH**), glucose-6-phosphate isomerase (E.C. 5.3.1.9, *GPI-1**, *GPI-2**), isocitrate dehydrogenase (E. C. 1.1.1.42, *IDHP**), L-lactate dehydrogenase (E.C. 1.1.1.27, *LDH-1**, *LDH-2**, *LDH-3**), malate

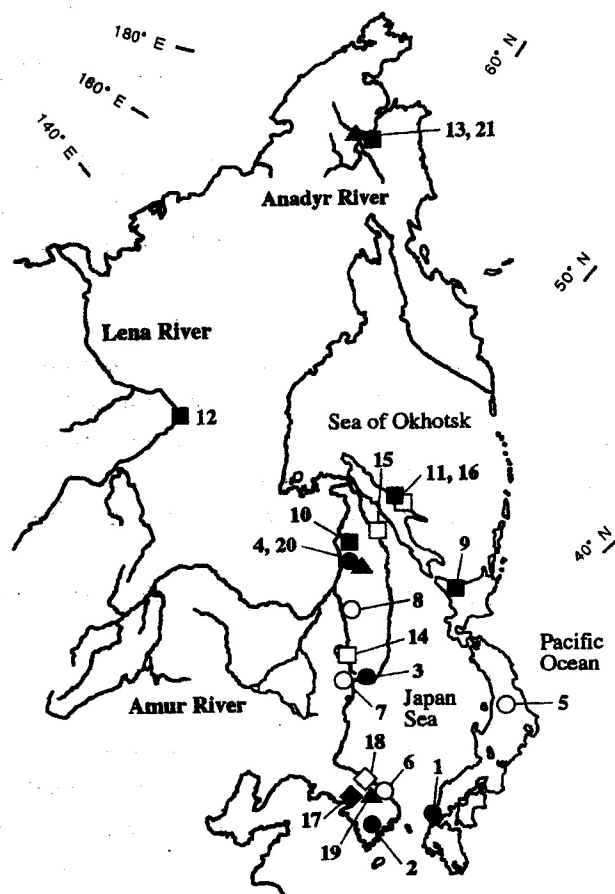


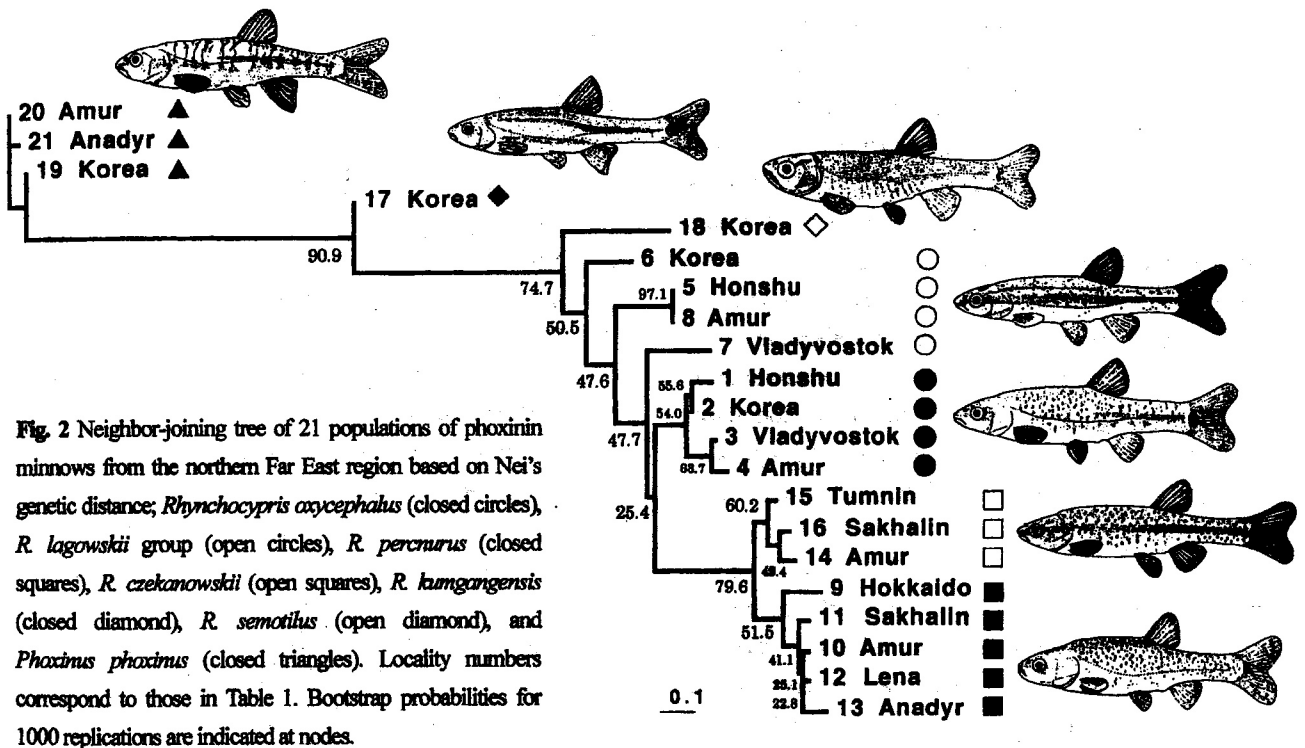
Fig. 1 Sampling localities of phoxinin minnows from the northern Far East region; *Rhynchocypris oxycephalus* (closed circles), *R. lagowskii* group (open circles), *R. percnurus* (closed squares), *R. czekanowskii* (open squares), *R. kumgangensis* (closed diamond), *R. semotilus* (open diamond), and *Phoxinus phoxinus* (closed triangles). Locality numbers correspond to those in Table 1.

dehydrogenase (E. C. 1.1.1.37, *MDH-1**, *MDH-2**, *MDH-3**), phosphoglucosmutase (E. C. 5.4.2.2, *PGM**), L-idoitol dehydrogenase (E. C. 1.1.1.14, *IDDH**), superoxide dismutase (E. C. 1.15.1.1, *SOD**) and general protein (*PROT-1**, *PROT-2**).

A neighbor-joining (NJ) tree⁶ based on Nei's genetic distance (*D*)⁷ between pairs of samples was constructed so as to determine their genetic relationships. Bootstrap probabilities (*p* in %) of nodes were calculated from 1000 replications.

Table 2 Number of dichotomous loci in range (above diagonal) and mean genetic distance with standard deviation (below diagonal) between pairs of seven species of *Rhynchocypris* and *Phoxinus*, and intra-specific genetic distance (on diagonal)

	<i>Rhynchocypris</i>						<i>Phoxinus</i>
	<i>oxycephalus</i>	<i>lagowskii</i> group	<i>percnurus</i>	<i>czekanowskii</i>	<i>kumgangensis</i>	<i>semotilus</i>	<i>phoxinus</i>
<i>R. o.</i>	0.12±0.06	4~7	6~9	6~8	11~12	9~11	15
<i>R. l.</i> group	0.43±0.09	0.31±0.16	8~10	6~10	9~12	8~11	15
<i>R. p.</i>	0.61±0.09	0.75±0.06	0.12±0.07	1~6	10~12	10~11	16
<i>R. c.</i>	0.54±0.08	0.64±0.11	0.27±0.08	0.09±0.04	11~12	11~10	15
<i>R. k.</i>	1.05±0.11	0.88±0.19	1.11±0.03	1.08±0.09	-	7	11
<i>R. s.</i>	0.84±0.07	0.71±0.11	0.90±0.02	0.90±0.06	0.48	-	16
<i>P. p.</i>	1.99±0.10	1.81±0.17	2.46±0.18	1.93±0.12	0.85±0.02	2.16±0.01	0.02±0.01



RESULTS

The *phoxinus* group was the most genetically distant from the others ($D = 0.85 \sim 2.46$, 11 ~ 16 loci being dichotomous), with *kumgangensis* and *semotilus* being the next distant from the remaining four groups ($D = 0.71 \sim 1.11$, 8 ~ 12 loci being dichotomous) (Table 2). The latter, including the *oxycephalus*, *lagowskii*, *percnurus* and *czekanowskii* groups, were relatively close to one another ($D = 0.27 \sim 0.75$, 1 ~ 10 loci being dichotomous). The

lagowskii group exhibited a greater intra-specific distance ($D = 0.31$) than the others ($D = 0.02 \sim 0.12$).

In the NJ tree (Fig. 1), the *phoxinus* group was distantly related to the others, which were clustered at a high bootstrap probability ($p = 90.9\%$). In the latter, *kumgangensis* and *semotilus* were connected as the outermost branches. The *oxycephalus*, *czekanowskii* and *percnurus* groups formed three monophyletic clusters of probabilities higher than 50% ($p = 54.0$, 60.2 and 51.5%, respectively), the latter two groups being jointed at a high

probability level ($p = 79.6\%$). On the other hand, the *lagowskii* group was divided into three paraphyletic lines.

DISCUSSION

The allozyme data indicated that two major divisions existed in the northern Far East phoxinin minnows, the genetic distance between them ($D = 0.85 \sim 2.46$) corresponding to the inter-generic level applied to American cyprinids, $D = 0.11 \sim 1.35$ (mean 0.73).⁸⁾ Therefore, the northern Far East phoxinin minnows should be classified into two genera, the *phoxinus* group in the present study being included in the genus *Phoxinus* and the others in the genus *Rhynchocypris*.⁵⁾

Because of their monophyletic nature, *oxycephalus*, *percnurus* and *czekanowskii* apparently represent three valid species of the genus *Rhynchocypris*, with *lungangensis* and *semotilus* also being likely valid species, being distantly related to the former. The genetic distances among them ($D = 0.27 \sim 1.11$) were a little higher than the inter-specific level determined for American cyprinids, $D = 0.04 \sim 0.97$ (mean 0.20).⁸⁾ On the other hand, the *lagowskii* group appeared to be genetically heterogeneous and paraphyletic, suggesting a possible subdivision of this group. In fact, the genetic distance among the *lagowskii* group ($D = 0.31$) corresponded to the species' level determined for cyprinids.⁸⁾

Consequently, the northern Far East phoxinin minnows should now be seen to represent two genera, including six species and one unresolved group; *Phoxinus phoxinus* (Linnaeus), *Rhynchocypris oxycephalus* (Sauvage and Dabry), *R. percnurus* (Pallas), *R. czekanowskii* (Dybowski), *R. lungangensis* (Kim) and *R. semotilus* (Jordan and Starks), and the *R. lagowskii* (Dybowski) group.

Although there remains a possibility of subspecies differentiation, such as between Japanese and Russian populations of *R. percnurus* or between Japan-Korean and Russian populations of *R. oxycephalus*, any such move, as well as classification of the *lagowskii* group, must follow

further research on a greater number of genetic markers from more populations representing a wider range.

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REFERENCES

1. Nakamura M. Cyprinid fishes of Japan. Research Institute of Natural Resources, Tokyo, 1969.
2. Nakabo T (ed). Fishes of Japan with pictorial keys to the species, 2nd ed. Tokai University Press, Tokyo, 2000.
3. Kim IS. Illustrated encyclopedia of fauna & flora of Korea Vol. 37. Freshwater fishes. Ministry of Education, Korea, Seoul, 1997. (in Korean).
4. Chereshevnev IA. Biogeography of freshwater fish fauna in the Russian Far East. Dalnauka, Vladivostok, 1998. (in Russian).
5. Howes GJ. A revised synonymy of the minnow genus *Phoxinus* Rafinesque, 1820 (Teleostei: Cyprinidae) with comments on its relationships and distribution. *Bull. British Mus. Nat. Hist. (Zool.)* 1985; 48: 57-74.
6. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 1987; 4: 406-425.
7. Nei M. Genetic distance between populations. *Am. Nat.* 1972; 106: 283-292.
8. Buth DG, Dowling TE, Gold JR. Molecular and cytological investigations. In: Winfield IJ, Nelson JS (eds), *Cyprinid fishes: systematics, biology and exploitation*. Chapman & Hall, London, 1991, pp. 83-126.