A Pelagic Cyprinid of Lake Biwa Gnathopogon caerulescnens and a Brooklet-Dwelling Relative G.elongates formed a Hybrid Swarm in a Dammed Reservoir Lake Ono

Journal of National Fisheries University 60 (1) 43－50 (2011)

A Pelagic Cyprinid of Lake Biwa *Gnathopogon caerulescens* and a Brooklet-Dwelling Relative *G. elongatus* formed a Hybrid Swarm in a Dammed Reservoir Lake Ono

Harumi Sakai[1†], Norihisa Nakashima[2], Takashi Uno[2], Masami Yonehana[2], Shinsuke Kitagawa[2] and Masayuki Kuwahara[3]

Abstract : A planktivorous pelagic cyprinid of Lake Biwa *Gnatopogon caeruleslens* was transplanted to a dammed reservoir Lake Ono in Yamaguchi Prefecture in 1953, but nowadays fish seemingly intermediate between *G. caerulescens* and a brooklet-dwelling relative *G. elongatus* has propagated. Morphological and genetic comparisons clearly revealed the Lake Ono fish intermediacy between *G. caerulescens* and *G. elongatus* and strongly suggested that the Lake Ono population is a hybrid swarm between them. In order to transplant *G. caerulescens* to other waters from Lake Biwa hereafter, it should be introduced to sufficiently large and deep lakes capable of assuring its pelagic and planktivorous lifestyle, and contamination of *G. elongatus* should be eliminated at the same time.

Key words : Cyprinidae, Lake Biwa, transplantation, hybrid swarm, morphology, allozyme, mitochondrial DNA

Introduction

Lake Biwa is the oldest and largest lake in Japan, being more than four million years old with a surface area of 674km^2 and a maximum depth of 104m[1)]. Fifteen endemic fish species/subspecies inhabit in the lake, having evolved lifestyles adapted to the lake environment[2)]. One of them, a cyprinid fish *Gnathopogon caerulescens* (Sauvage) (Fig. 1B) lives on zooplankton in the pelagic region of Lake Biwa[3)], being thought to have evolved from a brooklet-dwelling relative *G. elongatus* (Temminck & Schlegel) (Fig. 1C), a benthic omnivorous feeder that inhabits western Japan[4)]. *Gnathopogon caerulescens* has been transplanted from Lake Biwa to many ponds and reservoirs for decades because of its high commercial value[5)] and has colonized in several freshwaters[3)].

Such an example is the case of Lake Ono, a reservoir in Yamaguchi Prefecture, with a surface area of 25km^2 and a maximum depth of 39m, appeared in 1950 by the construction of the Koto River Dam. For several years since the appearance of water body, more than ten species of freshwater fish had been introduced from Lake Biwa to Lake Ono for propagation purpose, and *G. caerulescens* was also transplanted in 1953[6)]. Nowadays there are many *Gnathopogon* fish in Lake Ono, but the fish look like intermediate between *G. caerulescens* and *G. elongatus* (Fig. 1A). Morphological and genetic intermediacy of the Lake Ono fish, therefore, was practically examined in comparison with *G. caerulescens* and *G. elongatus*.

Materials and Methods

Fish Collection

Gnathopogon fish from Lake Ono, *G. caerulescens* from

2011年7月15日受付. Received Jury 15, 2011.

[1]Department of Applied Aquabiology, National Fisheries University
[2]Graduate of Department of Applied Aquabiology, National Fisheries University (the then Department of Aquaculture, Shimonoseki University of Fisheries)
[3]Lake Biwa Museum, Shiga Prefecture
[†]Corresponding author : sakaih@fish-u.ac.jp

Lake Biwa and *G. elongatus* from the Ane River, an inlet of Lake Biwa, were frozen immediately after collection for allozyme analysis and morphological observation (Table 1, Fig. 2).

Fins of different samples from Lake Ono, *G. caerulescens* and *G. elongatus* from Lake Biwa, *G. elongatus* from seven rivers in Japan, and *G. strigutus* (Regan) from the Amur River, Russia, and *Pungtungia herzi* Herzenstein from the Kawatana River, Yamaguchi Prefecture, were clipped and fixed in ethanol for mitochondrial DNA analysis (Table 2, Fig. 2).

Allozyme Analysis

Small pieces of muscle and liver were cut off from the frozen samples for allozyme analysis, and the remaining bodies were fixed in a formalin solution for morphological observation.

To examine the genetic intermediacy of the Lake Ono fish, gene products of 17 allozyme or protein coding loci were investigated by standard horizontal starch gel electrophoresis and zymogram methods[7]: glycerol-3-phosphate dehydrogenase (E.C.1.1.1.8, *G3pdh**), glycerol-6-phosphate isomerase (E.C.5.3.1.9, *Gpi-1**, *Gpi-2**), isocitrate dehydrogenase (E.C.1.1.1.42, *Idhp-1**, *Idhp-2**, *Idhp-3**), L-lactate dehydrogenase (E.C.1.1.1.27, *Ldh-1**, *Ldh-2**), malate dehydrogenase (E.C.1.1.1.37, *Mdh-1**, *Mdh-2**, *Mdh-3**), phosphogluconate dehydrogenase (E.C.1.1.1.44, *Pgdh**), phosphoglucomutase (E.C.5.4.2.2, *Pgm**), superoxide dismutase (E.C.1.15.1.1, *Sod**), and general protein (*Prot-1**, *Prot-2**, *Prot-3**).

Morphological Observation

At first, jaw protrusion of *Gnathopogon* fish from Lake Ono was compared with those of *G. caerulescens* and *G. elongatus*. Then, nine relative lengths in % of standard

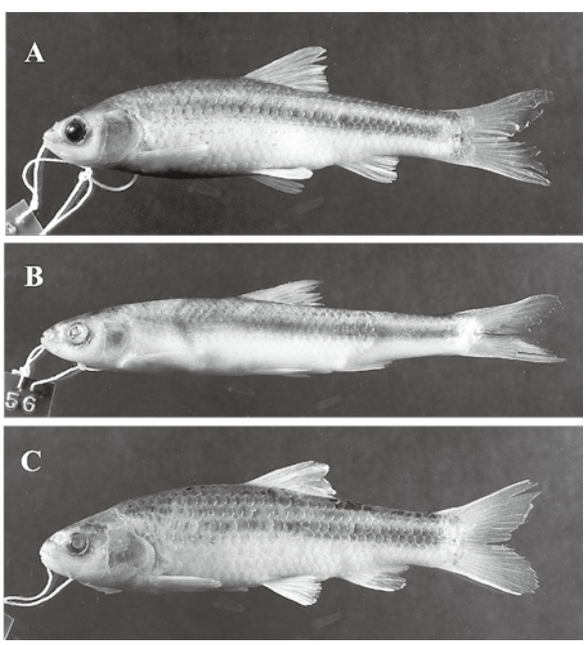

Fig. 1. *Gnathopogon* from Lake Ono, 66.5mm SL (standard length) (A), *G. caerulescens* from Lake Biwa, 80.2 mm SL (B) and *G. elongatus* from the Ane River, 68.8 mm SL (C).

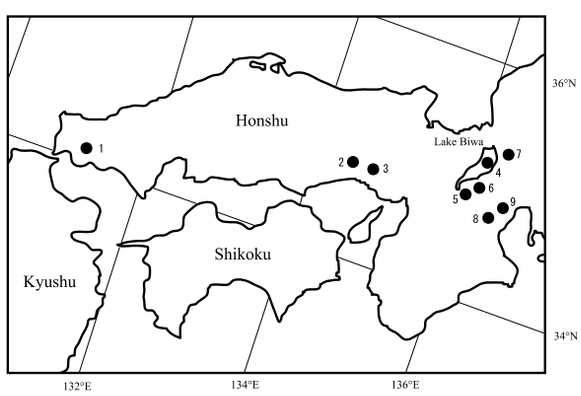

Fig. 2. Map of western Japan showing sampling locations : Lake Ono (1), Kizu River (2), Kako River (3), Lake Biwa (4), Yasu River (5), Kusatsu River (6), Ane River (7), Anraku River (8) and Suzuka River (9).

Table 1. Sample data for morphological observation and allozyme analysis

	Gnathopogon from Ono	*G. caerulescens*	*G. elongatus*
Locarity	Lake Ono, Yamaguchi Pref.	Lake Biwa, Shiga Pref.	Ane River, Shiga Pref.
Loc. N. in Fig. 2	1	4	7
Date	April 1988	May 1988	July 1988
No. ind.			
morphology	102	91	64
allozyme	90	100	78
Standard length (mm)	40.5 - 70.0	60.1 - 93.1	22.1 - 68.8

length, four relative lengths in % of head length, and six counts were compared (see Table 7 for characteristics measured). Counts and measurements followed Nakamura's method[3]. The number of vertebrae was counted with soft X-ray projection, the Weberian apparatus being counted to be four.

To present the intermediacy of Lake Ono fish, principal component analyses were carried out for relative lengths as well as for counts, both in log-standardized values.

Mitochondrial DNA Analysis

Mitochondrial 16S rRNA sequence was analyzed in order to infer genetic relationships of *G. caerulecens*, *G. elongatus*, *G. strigatus* and *Pugtungia herzi* as an out-group, and to know maternity of *Gnathopogon* from Lake Ono. Total DNA was isolated from a piece of fin by standard methods: proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation. PCR amplification was carried out to amplify partial mtDNA 16S rRNA gene using two primer pairs, L1854 (5'-AAACCTCGTACCTTTTGCAT-3')-H2582 (5'-ATTGCGCTACCTTTGCACGGT-3') and L2503 (5'-CACAAGCCTCGCCTGTTTACCA-3')-H3058 (5'-TCCGGTCTGAACTCAGATCACGTA-3')[8] with a thermal cycler (Gene Amp PCR System 2400, Perkin Elmer). Amplified and purified DNA was sequenced directly on an automated DNA sequencer (ABI PRISM 310, Applied Biosystems). The nucleotide sequences determined were deposited in DDBJ / EMBL / GenBank.

The DNA sequences were edited and aligned manually with the multiple-sequence editor DNASIS ver. 3.4 (Hitachi Co. Ltd.). Neighbor-joining dendrogram[9] based on Kimura's two-parameter model distance (K2P)[10] was constructed with 1000 bootstrap replications using the computer package PHYLIP v. 3.57c[11]. Weighting scheme of transitions and transversions (Ts/Tv) was set as 4. Including insertion/deletion, 1263 base pairs were used to infer genetic relationships of *Gnathopogon* and 736 base pairs of anterior half were analyzed to know maternity of *Gnathopogon* from Lake Ono.

Results

Genetic Intermediacy

Of 17 loci examined, five loci were variable (Table 3), exhibiting no significant departure from the Hardy-Weinberg expectation (chi-square test, $p>0.05$). Allelic frequency of *Gnathopogon* from Lake Ono was intermediate between *G. caerulescens* and *G. elongatus* in most of major alleles. Nearly perfect allelic displacement was exhibited in the locus *Prot-3** between *G. caerulescens* (**b*) and *G. elongatus* (**a*), and *Gnathopogon* from Lake Ono had both alleles (see also Fig. 3).

Table 4 indicates genetic contributions of *G. caerulescens* (44.1 % in mean) and *G. elongatus* (55.9 % in mean) to the allelic constitution of *Gnathopogon* from Lake Ono,

Table 2. Sample data for mitochondrial 16s rRNA sequence analysis and accession numbers deposited to DDBJ / EMBL / GenBank

	Locarity	Loc. N. (Fig. 2)	Date	No. ind.	Abbreviation	Acces. N.
Gnathopogon from Ono	Lake Ono, Yamaguchi Pref.	1	May 2001	17	Ono 1 - 17	AB622881 - AB622897
G. caerulescens	Lake Biwa, Shiga Pref.	4	September 1997	4	Biwa-c 1 - 4	AB622898 - AB622901
	Lake Biwa, Shiga Pref.	4	June 1999	2	Biwa-c 5, 6	AB622902, AB622903
	Lake Biwa, Shiga Pref.	4	January 2002	2	Biwa-c 7 - 8	AB622904, AB622905
G. elongatus	Kizu River, Hyogo Pref.	2	November 2001	2	Kizu 1, 2	AB622906, AB622907
	Kako River, Hyogo Pref.	3	April 2001	3	Kako 1 - 3	AB622908 - AB622910
	Lake Biwa, Shiga Pref.	4	January 2002	1	Biwa-e	AB622911
	Yasu River, Shiga Pref.	5	January 2001	5	Yasu 1 - 5	AB622912 - AB622916
	Kusatsu River, Shiga Pref.	6	June 1999	5	Kusatsu 1 - 5	AB622917 - AB622921
	Ane River, Shiga Pref.	7	January 2002	2	Ane 1, 2	AB622922, AB622923
	Anraku River, Mie Pref.	8	October 2001	2	Anraku 1, 2	AB622924, AB622925
	Suzaka River, Mie Pref.	9	October 2001	3	Suzuka 1 - 3	AB622926 - AB622928
G. strigatus	Amur River, Russia	-	September, 1998	1	-	AB622929
Pungtungia herzi	Kawatana River, Yamaguchi Pref.	-	July 2001	1	-	AB622930

assuming the genetic origin of the Lake Ono population to be a mixing of alleles of the former two species.

Genetic variability in ratio of polymorphic loci and heterozygosity was much higher in *Gnathopogon* from Lake Ono than in *G. caerulescens* and *G. elongatus* (Table 5).

Morphological Intermediacy

Generally the lower jaw is more protruded and barbels are longer in *G. caerulescens* than in *G. elongatus* (Fig. 4). All individuals of *G. caerulescens* had a protruded lower jaw (Table 6). Upper jaw was protruded in most of *G.*

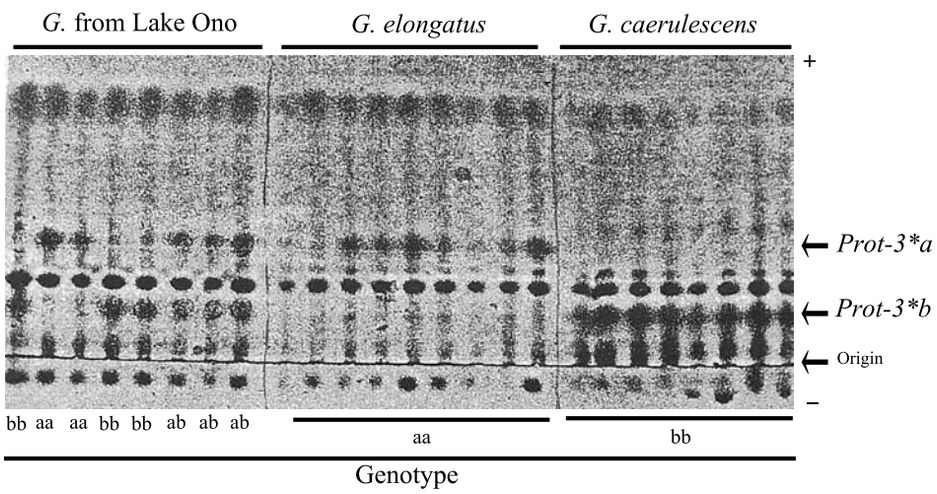

Fig. 3. Electrophoregram of general proteins for eight individuals of *Gnathopogon* from Lake Ono, nine individuals of *G. elongatus* from the Ane River, and eight individuals of *G. caerulescens* from Lake Biwa. *Gnathopogon caerulescens* and *G. elongatus* are fixed to alleles **b* and **a* in *Prot-3** respectively, and *Gnathopogon* from Lake Ono has both alleles.

Table 3. Allele frequency in variable loci of *Gnathopogon* from Lake Ono, *G. caerulescens*, and *G. elongatus*

Locus	Allele	*G.* from Lake Ono N = 90	*G. caerlulescence* N = 100	*G. elongatus* N = 78
*Gpi-1**	**a*	0.000	0.000	0.000
	**b*	0.867	0.930	0.904
	**c*	0.133	0.077	0.096
*Gpi-2**	**a*	0.039	0.005	0.006
	**b*	0.922**	0.950	0.872
	**c*	0.028	0.045	0.122
	**d*	0.011	0.000	0.000
*Pgdh**	**a*	0.000	0.048	0.009
	**b*	0.265**	0.606	0.089
	**c*	0.735**	0.346	0.830
	**d*	0.000	0.000	0.072
*Pgm**	**a*	0.767**	0.605	0.957
	**b*	0.156**	0.215	0.006
	**c*	0.056**	0.155	0.006
	**d*	0.022**	0.025	0.000
*Prot-3**	**a*	0.717**	0.000	0.994
	**b*	0.283**	1.000	0.006

**: intermediate between *G. caerulescens* and *G. elongatus*

Table 5. Genetic variability of *Gnathopogon* from Lake Ono, *G. caerulescens* and *G. elongatus* based on 17 protain coding loci

	G. from Lake Ono N = 90	*G. caerlulescence* N = 100	*G. elongatus* N = 78
Plymorphic loci	0.353	0.235	0.235
Heterozygosity (Ho)	0.100	0.073	0.051
Heterozygosity (He)	0.101	0.077	0.055

Table 4. Genetic contributions of *Gnathopogon caerulescens* and *G. elongatus* to the hybrid swarm of *Gnathopogon* from Lake Ono

		f	1-f
Locus	Allele	*G. caerlulescence*	*G. elongatus*
*Gpi-2**	**b*	0.641	0.359
*Pgd**	**b*	0.340	0.660
	**c*	0.196	0.804
*Pgm**	**a*	0.576	0.424
	**b*	0.718	0.282
	**c*	0.336	0.664
*Prot-3**	**a*	0.279	0.721
Average		0.441	0.559
SD		0.201	0.201

Formula: Ph = f×P1 + (1 - f)×P2
Ph: allele frequency in hybrid population, *Gnathopogon* from Lake Ono
P1: allele frequency in parental population 1, *G. caerulescens*
P2: allele frequency in parental population 2, *G. elongatus*
f, 1-f: mixing ratio for *G. caerulescens* and *G. elongatus*, respectively

Table 6. Number of individuals according to protruded jaws in *Gnathopogon* from Lake Ono, *G. caerulescens* and *G. elongatus*

	Protruded jaw		
	upper jaw	same	lower jaw
G. from Lake Ono	71	31	0
G. caerulescens	0	0	91
G. elongatus	51	12	1

elongatus and *Gnathopogon* from Lake Ono, but the ratio of upper jaw-protruded individuals of the Lake Ono population was lower.

Nearly all relative lengths and counts were significantly different among *Gnathopogon* from Lake Ono, *G. caerulescens* and *G. elongatus* (student's *T*-test, $p<0.05$), and all except for pelvic fin length, eye diameter and the number of predorsal scales in *Gnathopogon* from Lake Ono were intermediate between *G. caerulescens* and *G. elongatus* (Table 7).

Eigenvecters, eigenvalues and their cumulative contribution for principal component scores (PC 1, PC 2) in relative lengths and counts are indicated in Tables 8 and 9 respectively. PC scores of *Gnathopogon* from Lake Ono were scattered between *G. caerulescens* and G. *elongatus* both in relative lengths (Fig. 5) and counts (Fig. 6).

Genetic Relationships and Maternity

In the neighbor-joining dendrogram based on 16S rRNA sequence data (Fig. 7), *G. caerulescens* formed a single cluster, combining one individual of *G. elongatus* from the Yasu River (bootstrap probability 97%). The other haplotypes of *G. elongatus*, on the other hand, were divided into two distinct clusters, the eastern (the Lake Biwa basin and the Anraku and Suzuka Rivers) (99%) and western (the Lake Biwa basin and the Kizu and Kako Rivers) clusters (100%). Fish from the Lake Biwa basin, namely from the Kusatsu and Yasu Rivers, held haplotypes of both clusters. The western cluster of *G. elongatus* connected first with the *G. caerulescens* cluster

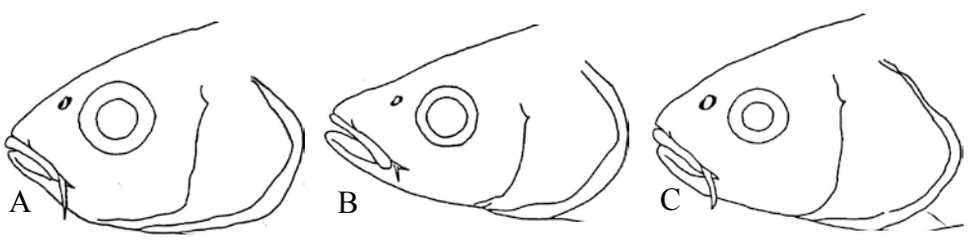

Fig. 4. Face profiles of *Gnathopogon* from Lake Ono (A), *G. caerulescens* from Lake Biwa (B) and *G. elongatus* from the Ane River (C).

Table 7. Mean relative lengths and counts with standard deviation in parentheses in *Gnathopogon* from Lake Ono, *G. caerlulescence*, and *G. elongatus*

	G. from Lake Ono N = 102	*G. caerlulescence* N = 91	*G. elongatus* N = 64	Significant difference *t*-test, $p<0.05$
Standard length in mm (SL)	40.5 - 70.0	60.1 - 93.1	22.1 - 68.8	
In % of SL				
Body depth	22.8 (1.3)*	19.4 (1.4)	24.6 (1.3)	*G* O-*Gc*-*Ge*
Head length (HL)	26.1 (1.3)*	25.4 (1.1)	30.3 (1.4)	*G* O-*Gc*-*Ge*
Predorsal length	48.7 (2.0)*	46.7 (1.5)	52.2 (1.6)	*G* O-*Gc*-*Ge*
Caudal peduncle length	22.5 (1.5)*	23.0 (1.4)	19.6 (1.6)	*G* O-*Gc*-*Ge*
Caudal depth	11.9 (0.8)*	9.3 (0.3)	12.2 (0.7)	*G* O-*Gc*-*Ge*
Pectoral fin length	17.1 (0.9)*	15.7 (0.9)	17.3 (1.6)	*G* O-*Gc, Gc*-*Ge*
Pelvic fin lenrth	15.2 (0.9)	13.7 (0.7)	15.2 (1.3)	*G* O-*Gc, Gc*-*Ge*
Dorsal fin length	21.1 (1.2)*	18.4 (1.0)	21.8 (1.5)	*G* O-*Gc*-*Ge*
Anal fin length	14.8 (1.0)*	11.8 (0.9)	16.3 (1.2)	*G* O-*Gc*-*Ge*
In % of HL				
Snout length	26.7 (2.2)*	26.6 (2.0)	27.3 (2.8)	insignificant
Barbel length	11.0 (2.2)*	6.2 (1.3)	13.3 (2.1)	*G* O-*Gc*-*Ge*
Eye diameter	23.0 (2.0)	19.6 (1.7)	20.3 (1.6)	*G* O-*Gc*-*Ge*
Interorbital width	31.4 (2.1)*	28.5 (2.3)	33.5 (3.2)	*G* O-*Gc*-*Ge*
Lateral line scales	37.1 (0.8)*	39.8 (1.3)	35.5 (1.3)	*G* O-*Gc*-*Ge*
Upper transverse scales	5.3 (0.4)*	6.1 (0.3)	5.1 (0.3)	*G* O-*Gc*-*Ge*
Lower transverse scales	4.2 (0.4)*	4.9 (0.4)	4 (0)	*G* O-*Gc*-*Ge*
Predorsal scales	12.5 (0.8)	14.7 (0.8)	12.8 (0.8)	*G* O-*Gc*-*Ge*
Vertebrae	37.6 (0.7)*	39.2 (1.6)	36.0 (0.9)	*G* O-*Gc*-*Ge*
Gill rakers	11.6 (1.5)*	16.8 (1.3)	8.4 (1.0)	*G* O-*Gc*-*Ge*

*: intermediate between *G. caerulescens* and *G. elongatus*

(100%), and then with the eastern cluster of *G. elongatus* (100%).

Out of 17 individuals of *Gnathopogon* from Lake Ono, 15 individuals were included in the western cluster of *G. elongatus*, especially in that of Lake Biwa group, and the other two were members of the *G. caerulescens* cluster (Fig. 8), indicating their maternities.

Discussion

The intermediacy of *Gnathopogon* from Lake Ono between *G. caerulescens* and *G. elongatus* was clearly evidenced by morphological characteristics. *Gnathopogon caerulescens* and *G. elongatus* were distinctly different in most characteristics, especially those of body shape metrics possibly relating to swimming behavior and those that may relate to feeding habits such as the number of gill rakers and barbel length. Such differences would be influenced by the character displacement phenomenon[12]. *Gnathopogon* from Lake Ono was actually intermediate between them in all such characteristics, also suggesting its ecological intermediacy.

The intermediacy was also evidenced by genetic markers. Allozyme allelic constitution was different between *G. caerulescens* and *G. elongatus*. Particularly alleles on *Prot-3** being nearly displaced, there must be little gene flow between them. The allelic constitution of *Gnathopogon* from Lake Ono, on the other hand, was

Table 8. Eigenvectors, eigenvalues and their cumulative contribution for PC 1 and PC 2 in relative lengths

	Eigenvectors	
	PC 1	PC 2
Body length	0.3148	-0.1104
Body depth	-0.2751	0.0870
Head length (HL)	-0.2463	0.2835
Predorsal length	-0.2670	0.1799
Caudal peduncle length	0.1924	-0.4440
Caudal depth	-0.2836	-0.0983
Pectoral fin length	-0.2160	-0.4453
Pelvic fin lenrth	-0.2373	-0.4441
Dorsal fin length	-0.2917	-0.2461
Anal fin length	-0.3011	-0.1369
Snout length	-0.2335	0.3148
Barbel length	-0.2933	0.0477
Eye diameter	-0.2661	-0.2267
Interorbital width	-0.2930	0.1819
Eigenvalue	8.92	1.43
Cumulative contribution (%)	63.72	73.94

Table 9. Eigenvectors, eigenvalues and their cumulative contribution for PC 1 and PC 2 in counts

	Eigenvectors	
	PC 1	PC 2
Lateral line scales	0.4276	-0.2348
Upper transverse scales	0.3942	0.1550
Lower transverse scales	0.4120	0.1349
Predorsal scales	0.3595	0.7935
Vertebrae	0.4146	-0.4529
Gill rakers	0.4368	-0.2605
Eigenvalue	4.24	0.56
Cumulative contribution (%)	70.68	79.97

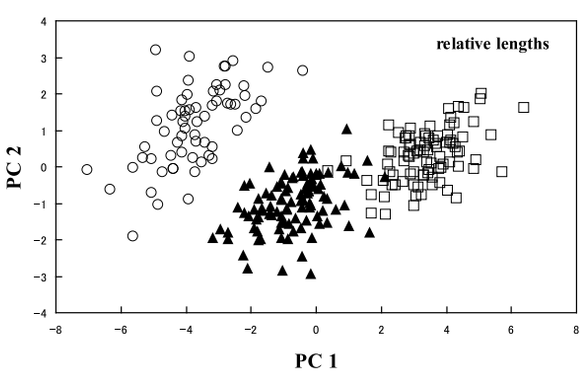

Fig. 5. Scattered diagram of principal component scores PC 1− PC 2 based on relative lengths of *Gnathopogon* from Lake Ono (closed triangles), *G. caerulescens* from Lake Biwa (open squares) and *G. elongatus* from the Ane River (open circles).

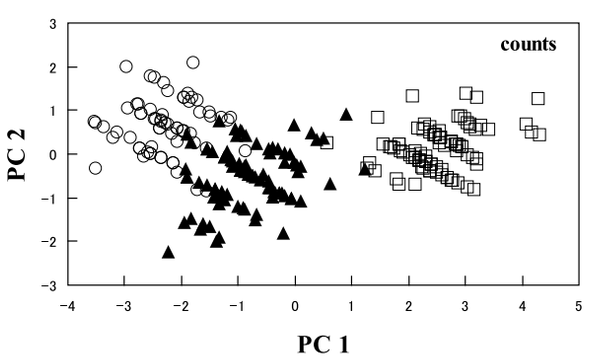

Fig. 6. Scattered diagram of principal component scores PC 1 − PC 2 based on counts of *Gnathopogon* from Lake Ono (closed triangles), *G. caerulescens* from Lake Biwa (open squares) and *G. elongatus* from the Ane River (open circles).

quite intermediate. Moreover, it was judged as a Mendelian population, being in the Hardy-Weinberg equilibrium.

The Lake Ono population, therefore, is inferred as a hybrid swarm[13] between *G. caerulescens* and *G. elongatus*. Such a total hybridization between distinct species in an exotic environment has sometimes occurred in many animals[13] including fish[14]. High genetic variability seen in the Lake Ono population must also support it because the variability in a hybrid population is generally expected to become higher than that of parental species[15]. Genetic contributions of *G. caerulescens* and *G. elongatus* to the hybrid swarm of Lake Ono were estimated at about 40% and 60% respectively.

According to phylogenetic analysis based on mitochondrial DNA sequence, haplotypes of *G. elongatus* were divided into two distinct groups, the eastern and western clusters. The western cluster was rather connected with the *G. caerulescens* cluster than the eastern cluster. It might be suggested that *G. caerulescens* has evolved from the western cluster group of *G. elongatu*s at Lake Biwa, adapting to the offshore environment. Haplotypes of the Lake Ono population were included in the western cluster of *G. elongatus* (15 individuals) as well as in the *G. caerulescens* cluster (2 individuals), but not in the eastern cluster of *G. elongatus*. Therefore, the hybrid swarm in Lake Ono is thought to have resulted from free crossings between *G. caerulescens* from Lake Biwa and *G. elongatus* from the western cluster group, the latter being the mother parent mostly.

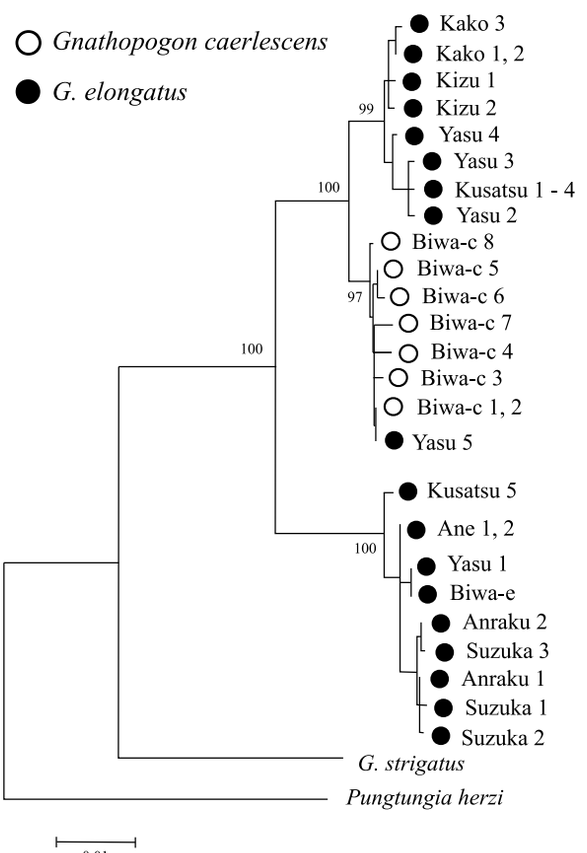

Fig. 7. Mitochondrial DNA phylogeny of *Gnathopogon* based on 16S rRNA region sequences by the neighbour-joining method with bootstrap probabilities in % of 1000 replications on major clusters. See Table 2 for haplotype abbreviations.

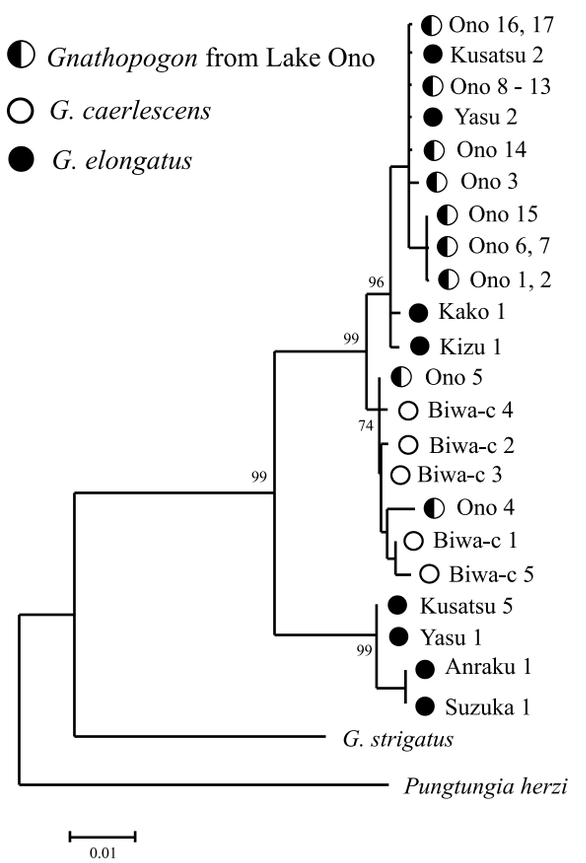

Fig. 8. Mitochondrial DNA phylogeny of *Gnathopogon* including those from Lake Ono based on sequences of the anterior half of 16S rRNA region by the neighbour-joining method with bootstrap probabilities in % of 1000 replications on major clusters. See Table 2 for haplotype abbreviations.

Because *G. elongatus* is thought to be non-native to Yamaguchi Prefecture[3,16], the Lake Ono environment is exotic not only to *G. caerulescens* but also to *G. elongatus*. It still remains ambiguous whether these two species had met in Lake Ono and hybridized at the new environment or the initially introduced stock to the lake had already been a hybrid population. Anyway, the hybrid swarm would have been able to propagate in a small lacustrine environment of Lake Ono that may present an intermediate environment between Lake Biwa suitable for *G. caerulescens* and brooklets suitable for *G. elongatus*.

Gnathopogon caerulescens has been introduced to other freshwaters from Lake Biwa and has colonized in several of them[3]. In many cases, however, the propagated fish does not look like *G. caerulescens* but is somewhat similar to *G. elongatus*[12]. The transplanted stock of *G. caerulescens* might have adapted to smaller lacustrine waters and changed their morphology[12], nevertheless some of the propagated populations must have been hybrid swarms like the Lake Ono population.

When *G. caerulescens* is transplanted to other waters from Lake Biwa hereafter, it should be done to so large and deep lakes as to be able to assure its pelagic and planktivorous lifestyle, and contamination of *G. elongatus* should be eliminated at the same time. Otherwise, fish similar to *G. elongatus* will come out through local adaptation or hybrid swarms like Lake Ono population will propagate through hybridization.

Acknowledgment

We are indebted to the staff of Ono Limnological Laboratory for help in collecting materials.

References

1) Research Group for Natural History of Lake Biwa : The Natural History of Lake Biwa. Yasaka Shobo Inc, Tokyo (1994) (in Japanese)

2) Yuma M, Hosoya K, Nagata Y : Distribution of the Freshwater Fishes of Japan: A Historical Overview. *Environ Biol Fish*, **52**, 97-124 (1998)

3) Nakamura M : Cyprinid Fishes of Japan. Res Inst Nat Res, Tokyo (1969) (in Japanese)

4) Kawanabe H : Fishes of Rivers and Lakes. Chuo-koronsha, Tokyo (1969) (in Japanese)

5) Matsuda S, Maehata M, Akiyama H: Fishes of Lake Biwa. Dai-ichi Hoki, Tokyo (1980) (in Japanese)

6) Fujioka Y : Fishes of the Koto River. *Bull Fac Ed Yamaguchi Univ*, **10**, 97-107 (1960) (in Japanese)

7) Hillis DM, Moritz C (eds) : Molecular Systematics. Sinauer, Sunderland (1990)

8) Watanabe K, Iguchi K, Hosoya K, Nishida M: Phylogenetic Relationships of the Japanese Minnows, *Pseudorasbora* (Cyprinidae), as inferred from Mitochondrial 16S rRNA Gene Sequences. *Ichthyol Res*, **47**, 43-50 (2000)

9) Saitou N, Nei M : The Neighbor-Joining Method: A New Method for Reconstructing Phylogenetic Trees. *Mol Biol Evol*, **4**, 406-425 (1987)

10) Kimura M : A Simple Method for Estimating Evolutionary Rates of Base Substitution through Comparative Studies of Nucleotide Sequences. *J Mol Evol*, 16, 111-120 (1980)

11) Felsenstein J : PHYLIP (Phylogeny Inference Package), Version 3.57c. University of Washington, Seattle (1995)

12) Hosoya K : Phylogenetic Relationships and Character Displacement in *Gnathopogon*. *In* : Mizuno N, Goto A (eds) Japanese Freshwater Fishes. Tokai University Press, Tokyo, 31-40 (1987) (In Japanese)

13) Mayr E : Animal Species and Evolution. Belknap Press of Harvard University Press, Cambridge, Massachusetts (1963)

14) Hubbs CL : Hybridization between Fish Species in Nature. *Syst Zool*, **4**, 1-20 (1955)

15) Campton DE : Natural Hybridization and Introgression in Fishes. *In* : Ryman N, Utter F (eds) Population Genetics and Fisheries Management. University of Washington Press, Seattle and London, 161-192 (1987)

16) Katayama M, Fujioka Y : Freshwater Fishes of Yamaguchi Prefecture. *Bull Fac Ed Yamaguchi Univ*, **21**, 73-97 (1972) (in Japanese)