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Longitudinal distribution patterns of three spined loach species (Cobitidae, *Cobitis*) in the Onga River system, northern Kyushu Island, Japan

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Abstract. Although the Onga River system is relatively small, it is home to three spined loach species, *Cobitis takatsuensis*, *C.* sp. 'yamato' complex, and *C. striata* (the Onga form of the middle race). The aim of the present study is to examine the relationship between the distribution pattern of the species and the physical parameters of the habitat. We mapped the distribution of these three species and measured six environmental factors at 86 points in this river system. Model selection was performed with a generalized linear model (GLM) using the AIC (Akaike's Information Criterion) to find the best model for the distribution pattern of each species. The dependent variable was the presence/absence of each species, and the independent variables were six environmental factors. The environmental factors that had a positive effect were turbidity for *C. takatsuensis*, turbidity and river gradient for *C.* sp. 'yamato' complex, and turbidity, river gradient and width of dry riverbed for *C. striata*. In addition, the coexisting fish species also differ among the three loaches. These results revealed that the three spined loach species select particularly different habitats and can therefore coexist in this small river system.

Key words: Cypriniformes, benthic fish, compartmentalization, habitat, niche

Introduction

Loaches of the genus *Cobitis* (family Cobitidae), are small benthic freshwater fishes and widely distributed through Europe and Asia (Kottelat & Freyhof 2007). They have a complicated geographical pattern in their genetic diversity (Kitagawa et al. 2003, 2005; Shimizu 2008), and are also known to show karyotype variations and to produce polyploid unisexual forms of hybrid origin (Saitoh et al. 2000, Bohlen & Ráb 2001, Vasil'ev & Vasil'eva 2008).

The spatial scale of habitat is generally expected to affect speciation rates (Mayr 1942, Rosenzweig 1995, Kisel & Barraclough 2010). For fishes inhabiting rivers, the spatial scale concerns the river length or river basin area. Some reports have indicated that the number of fish species is positively correlated with river size (Hirayama & Nakagoshi 2003, Nakajima et al. 2006, Kottelat & Freyhof 2007, Kim 2009). Following these hypotheses, spined loach species should tend to be represented with more species in large rivers.

Although the Onga River system in southern Japan is relatively small, it is home to three spined loach species, *Cobitis takatsuensis*, *C.* sp. 'yamato' complex, and *C. striata* (the Onga form of the middle race) (Saitoh et al. 2000, Nakajima et al. 2006, Kitagawa et al. 2009) (Fig. 1). In order to evaluate the speciation processes in *Cobitis*, it is important to know how these three species can coexist in this small river. However, there is no information available with regard to the distribution patterns of these three species.

The aim of the present study is to examine the relationship between the distribution patterns of three spined loach species and the physical parameters in the Onga River system.

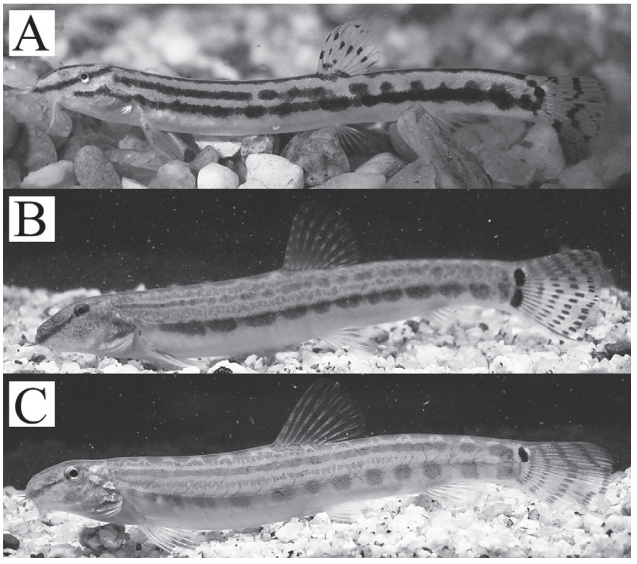

Fig. 1. *Lateral colour patterns of three cobitid fishes in the River Onga, Japan. A:* Cobitis takatsuensis*; B:* Cobitis *sp. 'yamato' complex; C:* Cobitis striata *(the Onga form of the middle race).*

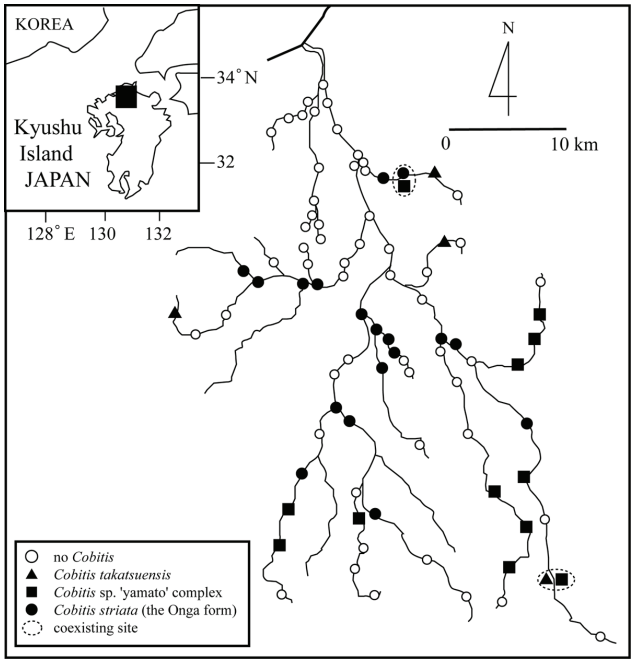

Fig. 2. *Map of the study area and distribution pattern of three* Cobitis *species in the Onga River system, northern Kyushu Island, Japan.*

Material and Methods

Study site and fish sampling

The Onga River system flows in the northern part of Kyushu Island, Japan. The main river has a length of 61 km, and its drainage area is 1030 km^2. The highest mountain in its drainage is Mt. Hikosan (alt. 1200 m). Fish sampling was conducted at 64 study sites in the Onga River system in the summer and autumn of 2007, with 22 study sites being added in 2009 to ensure that the entire area is covered (Fig. 2). The study sites ranged in altitude from 0 to 582 m. An approximately 100 m long stream segment, typically comprising pool, run, and riffle habitats, was sampled at each site. Fish specimens were collected with hand nets and cast nets. All captured fish were identified to the species level in the field, and then returned alive to the river. All specimens were identified according to Nakabo (2002) and Kitagawa et al. (2009).

Environmental measurements

We measured six physical environmental variables (altitude, river gradient, water depth, current velocity, width of dry riverbed (= flood plain), and turbidity) at 86 sites. Altitude and river gradient were measured on a digital map from the Geospatial Information Authority of Japan. Altitude is the centre point of each site, and river gradient was calculated for the longitudinal 1 km difference of elevation. Water depth was measured at each site by using a metal ruler, and the measured point was the deepest point of each site. Current velocity was determined at the fastest flowing point by using a portable current meter (model 3631, Yokogawa Electric Co., Tokyo, Japan). The measurement points of current velocities were set at 60 % water depth. Dry riverbed was determined by a laser measure (Trophy, Bushnell Co., Overland Park, USA). Turbidity was measured using a portable turbidity meter (HI93703B, Hanna Instruments Ltd., Bedfordshire, UK). The measurements of four environmental variables (water depth, current velocity, width of dry riverbed, and turbidity) were executed when normal water-level returned, which occurred at one month or more after fish sampling.

Data analysis

Model selection was performed with a GLM (generalized linear model) with a logit-link function and binomial distribution using the AIC (Akaike's Information Criterion) to find the best five models for the distribution pattern of each species (Burnham & Anderson 2002, Miyashita et al. 2008). The response variable was the presence or absence of each species, and the explanatory variables were the six environmental factors. Based on the AIC, we also computed Akaike weights (*wi*), which represent the probabilities that model *i* is the best model in the set of models. We also analysed the coexistence between each loach species and other fish species using the Chi-square test with the Yates' correction. All data analysis were performed using R ver. 2.12.0 (R Development Core Team 2010).

Table 1. *The fish species coexisting with each spined loach.*

	Chi-square	*p*
Cobitis takatsuensis		
Liobagrus reini	10.2	< 0.001
C. sp. 'yamato' complex		
Nipponocypris temminckii	12.4	< 0.001
Pseudogobio esocinus	9.1	< 0.01
Pungtungia herzi	22.8	< 0.001
Coreoperca kawamebari	18.0	< 0.001
Rhinogobius flumineus	9.8	< 0.01
C. striata (the Onga form)		
Carassius auratus	6.7	< 0.01
Acheilognathus rhombeus	12.3	< 0.001
Rhodeus atremius	15.3	< 0.001
Zacco platypus	6.6	< 0.01
Squalidus gracilis	16.0	< 0.001
Pseudobagrus nudiceps	9.8	< 0.01
Oryzias latipes	13.0	< 0.001

Results

The distribution patterns of the three species of spined loach are shown in Fig. 2. The appearance sites of three loaches almost perfectly segregated along longitudinal river line. *C. takatsuensis* was observed in a total of four sites. These sites were in the upstream area; however, the species was not collected in several sites of this area. *C.* sp. 'yamato' complex and *C. striata* were observed in a total of 13 and 18 sites, respectively. The sites where *C.* sp. 'yamato' complex was captured were in the upper-middle stream, whereas the sites where *C. striata* was captured were in the middle-lower stream. These two species of spined loach were relatively widely distributed in the Onga River system. A total of 40 fish species were found in this survey. There were fish species that significantly coexisted with each loach (Table 1). The coexisting fish species differ among the three loaches. The parameters of five models with the lowest AIC values are presented (Table 2). The environmental factor with a positive effect on the appearance rate has an estimate that was approximately twice as large as its standard error. The result shows that the positive environmental factor differs in each species. The environmental factors that had a positive effect were 'low turbidity' for *C. takatsuensis*, 'low turbidity' and 'low river gradient' for *C.* sp. 'yamato' complex, and 'low turbidity', 'low river gradient' and 'high width of dry riverbed' for *C. striata*. The average turbidity of *C. takatsuensis*, *C.* sp. 'yamato' complex and *C. striata* were 3.0 FTU (0.6 SD), 4.0 FTU (2.0 SD), and 6.0 FTU (2.5 SD), respectively. The average river gradient of *C.* sp. 'yamato' complex and *C. striata* were 17.0 m/km (15.0 SD) and 3.0 m/km (2.8 SD). The average width of dry riverbed of *C. striata* was 147.2 m (174.3 SD).

Discussion

The present distribution data indicate that the three species of spined loach are clearly segregated in the Onga River system. Each species has a different longitudinal distribution pattern. *C. takatsuensis* inhabited the upper part, *C.* sp. 'yamato' complex the middle part, and *C. striata* the lower part of the river (Fig. 2). The coexisting species also differ among the three species of spined loach (Table 1). For example, *Liobagrus reini*, *Nipponocypris temminckii*, and *Rhinogobius flumineus* are typical mountain stream fishes, on the other hand, *Carassius auratus*, *Zacco platypus* and *Oryzias latipes* are common species in middle-lower reaches of the rivers (Nakabo 2002, Nakajima et al. 2006). These results show that the habitat and preferred environment differ among of these three spined loaches.

C. takatsuensis inhabited the upper part of this river, however, the area of appearance was very limited. The only environmental factor that has a positive effect on *C. takatsuensis* was low turbidity. This result indicates that the distribution of this species is not simply determined by altitude or gradient. Only the upper part of the river is not always sufficient as a habitat for this species. Also the related species *C. shikokuensis*, recently separated from *C. takatsuensis* by Suzawa (2006), inhabits the gravel bottom of the mountain stream and needs clean water containing a high level of dissolved oxygen (Shimizu 2002). Although the preferred habitat of *C. takatsuensis* has been less distinct, these results show that *C. takatsuensis* inhabits a similar environment as *C. shikokuensis*. Both loaches are considered endangered species (Ministry of the Environment 2003), and this habitat specificity may be the reason for their reducing numbers.

Cobitis sp. 'yamato' complex is widespread in the upper-middle part of this river. The positive environmental factors were low turbidity and low gradient. The life history of this species remains incompletely understood. However, its distribution pattern in this river and the environmental factors influencing this distribution indicated that the upper-middle stream (the average gradient is about 17 m/km) with clean water might be the perfect environment for this species.

Table 2. *Information-theoretical statistics and coefficients selected for the top five models explaining the probability of appearance of the three spined loaches. Model selection was performed with a GLM with a logit-link function and binomial distribution.*

Model	AIC	ΔAIC	*wi*	Intercept	Turbidity	River Gradient	Width of Dry Riverbed	Water Depth	Current Velocity	Altitude
C. takatsuensis										
1	31867	0.00	0.064	–1.027 (0.968)	–0.567 (0.323)	-	-	-	-	-
2	32350	0.48	0.051	–1.484 (0.901)	-	-	-	–0.040 (0.029)	-	-
3	32569	0.70	0.045	–0.567 (1.090)	–0.418 (0.349)	-	-	–0.026 (0.028)	-	-
4	32581	0.71	0.045	–0.984 (1.292)	–0.838 (0.435)	–0.024 (0.018)	-	-	0.053 (0.029)	-
5	32735	0.87	0.041	–1.882 (1.296)	–0.500 (0.323)	-	-	-	0.023 (0.020)	-
Null	34544	2.68	0.017	–3.068 (0.500)	-	-	-	-	-	-
C. sp. yamato complex										
1	73111	0.00	0.085	0.157 (0.731)	–0.407 (0.158)	–0.018 (0.013)	-	-	-	-
2	73717	0.61	0.063	–0.826 (0.556)	–0.329 (0.146)	-	3.13E-04 (1.87E-04)	-	-	-
3	73774	0.66	0.061	–0.087 (0.749)	–0.421 (0.159)	–0.016 (0.013)	2.53E-04 (1.99E-04)	-	-	-
4	73824	0.71	0.059	–0.011 (0.782)	–0.380 (0.162)	–0.037 (0.024)	-	-	-	0.005 (0.004)
5	73926	0.81	0.057	–0.071 (0.757)	-0.426 (0.163)	–0.024 (0.014)	-	-	0.019 (0.017)	-
Null	77124	4.01	0.011	–1.889 (0.277)	-	-	-	-	-	-
C. striata (the Onga form of the middle race)										
1	82929	0.00	0.094	–0.457 (0.873)	–0.215 (0.119)	–0.133 (0.066)	2.61E-04 (1.30E-04)	0.006 (0.003)	-	-
2	83478	0.55	0.071	0.055 (0.793)	–0.195 (0.120)	–0.152 (0.066)	2.40E-04 (1.31E-04)	-	-	-
3	84129	1.20	0.051	–0.273 (0.821)	–0.186 (0.112)	–0.119 (0.060)	-	0.005 (0.003)	-	-
4	84174	1.24	0.050	0.163 (0.749)	–0.171 (0.113)	–0.138 (0.060)	-	-	-	-
5	84444	1.51	0.044	–1.222 (0.334)	-	–0.103 (0.054)	2.24E-04 (1.31E-04)	-	-	-
Null	94303	11.37	3.17E-04	–1.564 (0.236)	-	-	-	-	-	-

Values in parentheses represent SE.

C. striata is widespread in the middle-lower part of this river. The positive environmental factors were low turbidity, low gradient, and wide dry riverbed. The average appearance gradient was 3.0 m/km, which is lower than *C.* sp. 'yamato' complex (17 m/km). Although these two spined loaches are widely distributed in this river, the distribution area is clearly compartmentalised by gradient. The appearance of this species is also influenced by the width of dry riverbed. *C. striata* (the Setouchi form of the middle race), genetically related to the Onga form (Kitagawa et al. 2009), has been reported to scatter eggs on the submerged vegetation along the bank of the ditch (Saitoh 1990). A vegetation area generally exists in the dry riverbed (= flood plain) at the middle-lower reaches. Therefore, it is possible that the width of dry riverbed is correlated to a specific spawning site required by this species.

The present study reveals that habitat segregation is an important isolation mechanism among three species of spined loaches in the Onga River system. Although the three spined loaches inhabit one small river system, they almost never coexist in the same place. It is known that the longitudinal distribution of spined loach varies from species to species in one river system (Povž & Šumer 2000, Kottelat & Freyhof 2007, Nakajima et al. 2008, Kim 2009). We assume that the longitudinal distribution pattern of spined loach species may be decided by the compartmentalization of their spawning site. However, little is known about the spawning ecology of Japanese *Cobitis*, and it is necessary to accumulate such information.

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