Title	First Japanese record of the sharpbelly Hemiculter leucisculus (Basilewsky, 1855) (Cypriniformes: Cyprinidae) from Okayama Prefecture, western Honshu
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First Japanese record of the sharpbelly *Hemiculter leucisculus* (Basilewsky, 1855) (Cypriniformes: Cyprinidae) from Okayama Prefecture, western Honshu

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Abstract. Seven specimens of the sharpbelly *Hemiculter leucisculus* (Basilewsky, 1855) were collected by angling from a stream connected to the Hyakken River emptying into Kojima Bay, Okayama city, Okayama Prefecture, western Honshu, Japan, on 19 July 2016. This collection represents a new Japanese record for the species as an alien fish. The specimens are identified based on their morphology and genetic analysis of CO1 of mitochondrial DNA. One of the sequences of CO1 from six specimens is accorded with a previously reported haplotype H2, and that from the remaining one specimen has a newly obtained haplotype (H5) close to H2. Although the species has not yet been confirmed for its establishment in the stream, some individuals were found on the sampling date to jump out of the water, and this behavior resembles one associated with the spawning of the species observed in China. It is necessary to monitor the species in the stream and adjacent waters.

Key words: *Hemiculter leucisculus*, alien fish, new country record, Japan

During a sampling of a domestic alien cyprinid, *Ischikauia steenackeri* (Sauvage, 1883), to examine its parasite fauna in a stream connected to the Hyakken River flowing into Kojima Bay, Okayama city, Okayama Prefecture, seven cyprinid specimens similar to *I. steenackeri* were collected but differed from the species. They were later identified as the sharpbelly *Hemiculter leucisculus* (Basilewsky, 1855) based on their morphology and molecular analysis. We report here this collection as the first record of the species from Japan.

The specimens were collected from the stream (34°37′19.1″N, 133°58′48.8″E) by angling and brought alive or on ice to the laboratory of Hiroshima University on 19 July 2016. Five dead fish were stored frozen until dissection. All fish were examined for their parasites, fixed in 10% formalin, and later preserved in 70% ethanol. Before fixation, their right pectral fins were removed from the fish and stored in 99% ethanol for molecular analysis. Voucher specimens are deposited in the fish collection of the National Museum of Nature and Science, Tsukuba city, Ibaraki Prefecture, Japan (NSMT-P131564).

Counts and measurements followed Hubbs & Lagler (2004). Gill rakers were counted from the left gills removed for the parasitological survey. DNA was extracted from the right pectral fins using the DNeasy blood and tissue kit (Qiagen) in accordance with the manufacturer's instructions. The DNA was amplified by polymerase chain reaction (PCR) using the primer pair FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and FsihR1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3') to amplify first subunit of cytochrome oxidase (CO1) (Ward *et al*. 2005). A total of 25 μL PCR reaction consisted of 1 μL of DNA template, 1× ExTaq Buf-

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fer (TaKaRa), 0.2 mM of each dNTP, 1 μM of each primer, and 2.5 units of TaKaRa Ex Taq DNA Polymerase (TaKaRa). PCR was carried out with the following protocol: 94°C for 60 sec followed by 35 cycles of 94°C for 30 sec, 54°C for 30 sec, and 72°C for 1 min, and 10 min of final hold at 72°C. PCR products were purified using NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel) and sequenced with a 3130X Genetic Analyzer (Applied Biosystems) with the same primers that were generated the PCR products and submitted to the DDBJ (DNA Data Bank of Japan) database (LC316767–316773). CO1 sequences of *H. leucisculus* were downloaded from GenBank (HQ536384, HQ536385, HQ536386, HQ536387, HQ536388, HQ536389, KF492988, KF492989, KF647872, KF956522), edited and aligned with newly obtained sequences (625bp) using MEGA6 (Tamura *et al*. 2013).

Hemiculter leucisculus (Basilewsky, 1855)
Berg, 1909
Japanese name: Kawa-iwashi
(Fig. 1)

Fig. 1. *Hemiculter leucisculus* (Basilewsky, 1855) (NSMT-P 131564, 98.3 mm in standard length) collected from Okayama Prefecture, western Honshu, Japan.

Table 1. Measurements of seven specimens of *Hemiculter leucisculus* collected from Okayama Prefecture, western Honshu, Japan.

Specimen	1	2	3	4	5	6	7
Standard length (mm)	98.3	86.6	113.6	101.7	107.7	102.7	98.0
Percent of standard length							
Head length	22.6	24.0	21.1	22.3	21.3	18.6	21.4
Body depth	23.8	22.3	22.3	19.8	22.0	22.8	22.3
Body width	10.2	10.3	12.1	9.0	11.5	10.7	10.1
Depth of caudal peduncle	9.2	9.9	9.3	8.2	9.7	9.8	9.1
Length of caudal peduncle	15.7	15.9	15.6	13.3	15.1	14.7	14.8
Preanal length	69.2	68.4	71.7	69.2	71.1	70.3	72.0
Prepelvic length	47.3	49.1	50.4	48.7	50.5	50.8	50.3
Height of dorsal fin	17.0	19.5	18.8	20.0	17.5	16.5	15.4
Length of dorsal fin base	9.2	10.6	10.0	9.7	10.2	10.0	9.8
Height of anal fin	11.4	10.4	11.3	10.3	11.8	9.5	11.8
Length of anal fin base	12.1	12.5	11.3	12.5	11.7	12.0	12.2
Pectoral fin length	20.2	20.4	21.1	20.0	19.5	21.8	19.2
Percent of head length							
Head width	42.5	37.5	54.9	43.9	52.2	52.7	44.8
Snout length	25.7	22.9	30.8	29.1	26.8	33.9	24.7
Orbit diameter	25.2	22.1	29.3	26.0	25.9	33.9	23.3
Interorbital width	28.3	24.6	33.1	30.0	32.9	39.3	28.9

Specimens. NSMT-P 131564, seven specimens, 86.6–113.6mm in standard length, a stream connected to the Hyakken River emptying into Kojima Bay, at Kuwano, Naka District, Okayama city, Okayama Prefecture, Japan, 19 July 2016.

Description. Morphometric data are shown in Table 1. Body elongate. Mouth oblique, without barbels. Caudal fin strongly forked. Gill rakers 17–20. Belly with keel from throat to anus. Lateral line bending down under pectoral fin, rising behind end of anal fin obliquely in three scales, extending along middle of caudal peduncle. In life, dark dorsally, silvery laterally, whitish ventrally.

Dorsal fin with 3 simple and 6–7 branched rays. Anal fin with 3 simple and 11–12 branched rays. Pectoral fin with 1 simple and 12–13 branched rays. Pelvic fin with 1 simple and 7–8 branched rays. Pored lateral-line scales 51–56. Scale rows between lateral-line scales and dorsal-fin origin 8–9; between lateral-line scales and anal-fin origin 2.

Remarks. This fish was originally described by Basilewsky (1855) as *Culter leucisculus* from Pekin, China. The Japanese name, "Kawa-iwashi" was proposed by Oshima (1923) for *Cultriculus kneri* (Warpachowski, 1888) collected in Taiwan, but the species has been regarded as *Hemiculter leucisculus* (Berg 1909; Wu 1964; Vasil'eva & Kozlova 1988). The specimens examined in this study approximately conform to the descriptions of *H. leucisculus* collected in China (Wu 1964) and Russia (Vasil'eva & Kozlova 1988). Also, the sequences of CO1 determined from our seven specimens are almost identical to the reported sequences of *H. leucisculus*: one of those from six specimens is accorded with a haplotype H2 determined from Azerbaijan (Mustafayev *et al*., 2015), while that from the remaining one specimen has a new haplotype H5 that is close to H2 (Table 2).

Hemiculter leucisculus is natively distributed in Korea, China, Vietnam, Far-East Russia, and Taiwan (Wu 1964) but has established as alien species in Uzbekistan, Afghanistan, Iran, Kazakhstan, Turkmenistan, Iraq, and Azerbaijan (Mustafayev *et al*. 2015; Wang *et al*. 2016). The present collection in Japan represents a new country record for *H. leucisculus* as an alien fish. As many cyprinids are imported as live fishing bait from China to Japan (Saito *et al*. 2011), the species is likely to be included in those imported fishes and actually found as an ornamental fish at pet shops in Japan. Under these situations, the Japanese population of the species may have originated from release of some imported individuals by hobbyists or their accidental escape from aquaria.

Hemiculter leucisculus is a typical r-selected species and has the high invasive potential (Wang *et al*. 2016). During our sampling, some individuals of the species were observed to jump out of the water: a similar behavior was previously reported in China to be associated with the spawning of the species (Wu *et al*. 1979). No information is available on the establishment of the species in Japan, but it is necessary to monitor its abundance and reproduction, es-

Table 2. Five haplotypes of CO1 from *Hemiculter leucisculus*. H1 to H4 were reported by Mustafayev *et al*. (2015), and H5 is a newly found haplotype.

	Variable site								
Haplotype	9	237	300	399	432	462	468	513	531
H1	G	G	A	G	G	G	A	C	T
H2	A	T		A					
H3	A	T	G	A	A		G	T	
H4	A	T	G	A					
H5	A	T		A		A			G

pecially in the stream sampled and adjacent waters.

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