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Oryzias sakaizumii, a new ricefish from northern Japan (Teleostei: Adrianichthyidae)

Toshinobu Asai*,*, Hiroshi Senou** and Kazumi Hosoya***

Oryzias sakaizumii, new species, is described from Japanese freshwaters along the northern coast of the Sea of Japan. It is distinguished from its Japanese congener, *O. latipes*, by a slightly notched membrane between dorsal-fin rays 5 and 6 in males (greatly notched in *O. latipes*); dense network of melanophores on the body surface (diffuse melanophores in *O. latipes*); distinctive irregular black spots on posterior portion of body lateral (absent in *O. latipes*); and several silvery scales arranged in patches on the posterior portion of the body (few in *O. latipes*).

Introduction

Ricefishes, adrianichthyid fishes of the atherinomorph order Beloniformes, comprise 32 mostly small species, including the new species described herein (Herder & Chapuis, 2010; Magtoon, 2010; Parenti & Hadiaty, 2010). The family Adrianichthyidae has been classified in three subfamilies with four genera – *Adrianichthys*, *Oryzias*, *Xenopoecilus*, and *Horaichthys* – since 1981 (Rosen & Parenti, 1981; Nelson, 2006). In the latest revision of Adrianichthyidae, Parenti (2008) classified ricefishes in two genera based on a phylogenetic analysis: *Adrianichthys* with four species and *Oryzias* with 24 species. Ricefishes broadly range throughout mainly fresh and brackish wetlands of Central Eurasia; Central, East and Southeast Asia; and the Indian Subcontinent. They are found south along the Indo-Australian Archipelago across Wallace's line to the Indonesian islands of Timor and Sulawesi (Kottelat, 1990a–b; Takehana et al., 2005).

Oryzias latipes was originally described as *Poecilia latipes* by Temminck & Schlegel (1846), from Siebold's collection now at the RMNH, the Netherlands. Subsequently, this species was classified in the genus *Haplochilus* by Günter (1866), an incorrect spelling of *Aplocheilus*, hence *Aplocheilus latipes* (Jordan & Snyder 1901). The new genus *Oryzias* of Jordan & Snyder (1906) has since been generally accepted for this well-known Japanese species.

More recently, allozyme studies have shown that Japanese wild populations of *O. latipes* or medaka, could be divided into two genetic groups (Sakaizumi et al., 1983; Sakaizumi, 1984). Ge-

* Laboratory of Aquatic Ecology, Major in Environmental Management, Postgraduate School of Agriculture, Kinki University, 3327-204 Nakamachi, Nara 631-8505, Japan. E-mail: four-seasons@zpost.plala.or.jp
** Kanagawa Prefectural Museum of Natural History, 499 Iryuda, Odawara, Kanagawa 250-0031, Japan. E-mail: senou@nh.kanagawa-museum.jp
*** Corresponding author

netic differentiation between these populations was also confirmed by mitochondrial DNA analysis. At present, the wild populations of *O. latipes* complex which is distributed in East Asia may be divided into four distinct genetic populations: the Northern population from the northern coast of the Sea of Japan, the Southern population from the Pacific coast of eastern, western, and southern Japan, the East Korean population from eastern and southern Korea, and the China–West Korean population from China and western Korea by sophisticated molecular genetic investigations (Takehana et al., 2003, 2004a–b). Divergence time of the two Japanese populations was estimated to be about 4 to 5 million years ago using a molecular clock hypothesis (Takehana et al., 2003). In contrast, the estimated age of divergence based on a Bayesian model dates as far back as about 18 million years ago (Setiamarga et al., 2009). This genetic distance is generally considered equivalent to that of the genus level (Frankham et al., 2002). Both pieces of evidence indicate that the two regional medaka populations in Japan were genetically divergent for a long enough time to support a higher taxonomic classification. However, these populations have not been fully investigated taxonomically.

By giving an in-depth examination to the Northern population of the *O. latipes* complex, it is distinguished from all other congeners including *O. latipes* (Temminck & Schlegel, 1846). Thus, the purpose of this paper is to describe the Northern population as a new species, *Oryzias sakaizumii*.

Material and methods

Specimens were preserved in 70 % ethanol after fixation in 10% formalin. Character descriptions, counts, and measurements follow Hubbs & Lagler (2004) unless otherwise stated: straight-line distance from pectoral-fin base to pelvic-fin base, and from anterior origin of dorsal-fin base to caudal-fin base. Measurements are of straight-line, point-to-point, distances recorded with digital vernier calipers; they are expressed as a range of percentage of standard length (SL) or head length (HL), with the value for the holotype in parentheses after the range. Counts were made mainly from radiographs by soft X-rays and from cleared and counterstained specimens prepared using the protocol of Kawamura & Hosoya (1991). Samples of spermatozoa removed from some specimens were cryopreserved with liquid nitrogen because of the possibility for regeneration of the species within the near future.

The materials examined herein were deposited in the following institutions: BMNH, Natural History Museum, London; CAS, California Academy of Sciences, San Francisco; FMNH, Field Museum of Natural History, Zoology Department, Chicago; KPM-NI, Kanagawa Prefectural Museum of Natural History, Kanagawa, Japan; KUN-P, Kinki University, Nara, Japan; NSMT-P, National Museum of Nature and Science, Tokyo; RMNH, Naturalis – Nationaal Natuurhistorisch Museum, Leiden; and USNM, National Museum of Natural History, Smithsonian Institution, Washington.

Oryzias sakaizumii, new species

(Figs. 1–2)

Oryzias latipes (not Temminck & Schlegel, 1846): Senou, 2002: 547 (in part); Parenti, 2008: 553 (in part).

Holotype. KUN-P 42428, 28.9 mm SL, male (Figs. 1a, 4a, 5a); Japan: Fukui, Tsuruga, irrigation canal of the wetland Nakaikemi (Fig. 7); 35°39'25.7" N 136°05'20.1" E, altitude 48 m; T. Asai, 30 Oct 2010.

Paratypes. KPM-NI 12669, 27.3 mm SL, male; KPM-NI 12670, 27.5 mm SL, female; Japan: Akita, Nikaho; H. Karube, 17 Oct 2003. – KPM-NI 13340, 25.3 mm SL, male; KPM-NI 13343, 27.2 mm SL, female; Japan: Yamagata, Shinjo; H. Senou, 24 May 2004. – KPM-NI 17074, 28.5 mm SL, male; KPM-NI 17075, 29.8 mm SL, female; Japan: Niigata, Sado; H. Karube, 26 May 2006. – KPM-NI 14041, 27.0 mm SL, male; KPM-NI 14045, 28.8 mm SL, female; Japan: Fukushima, Aizuwakamatsu; H. Aiki, 21 Nov 2004. – NSMT-P 105973, 27.0 mm SL, male; NSMT-P 105974, 34.3 mm SL, female; Japan: Niigata, Nagaoka, storage reservoir; T. Asai & S. Asai, 13 Jul 2010. – NSMT-P 105971, 28.6 mm SL, male; NSMT-P 105972, 33.0 mm SL, female; Japan: Ishikawa, Notojima, irrigation canal; T. Asai, 10 Jul 2010. – KPM-NI 7495, 26.4 mm SL, male; KPM-NI 7496, 26.4 mm SL, female; Japan: Toyama, Himi, pond; 12 Nov 2000. – BMNH 2011.4.8.1–2, 2, 29.0 mm SL, male; 29.0 mm SL, female; CAS 231819, 2, 27.5 mm SL, male; 28.1 mm SL, female; FMNH 119687, 2,

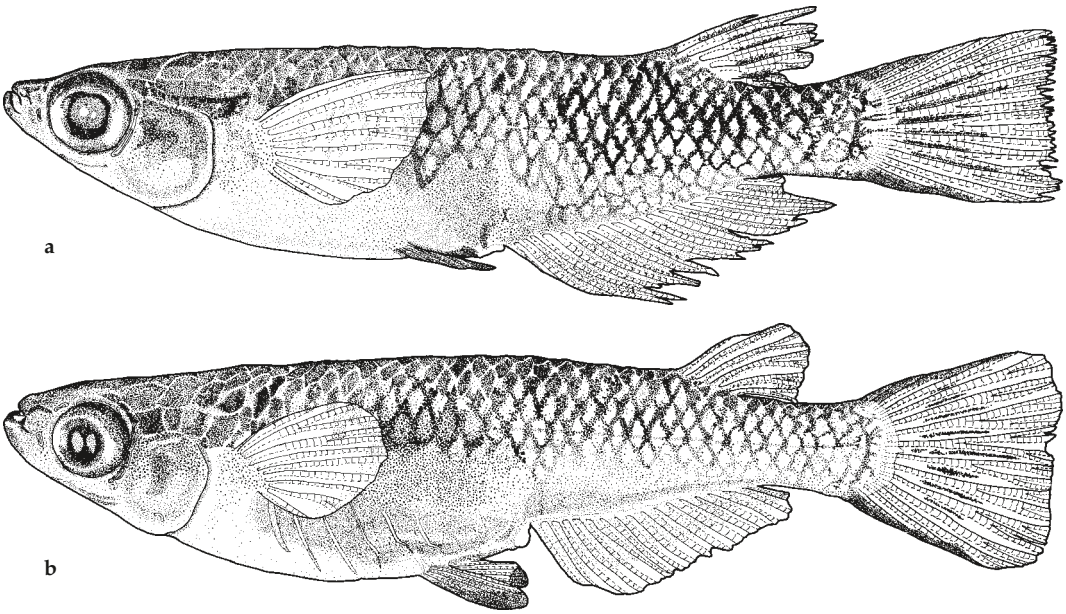

Fig. 1. *Oryzias sakaizumii*; Japan: Fukui Prefecture: Tsuruga: Nakaikemi wetland; **a,** KUN-P 42428, holotype, male, 28.9 mm SL; **b,** KUN-P 42431, paratype, female, 29.9 mm SL.

28.3 mm SL, male; 29.1 mm SL, female; KPM-NI 27949, 27.4 mm SL, male; KPM-NI 27953, 25.1 mm SL, female; RMNH.PISC. 37658, 26.8 mm SL, male; RMNH.PISC. 37659, 27.4 mm SL, female; USNM 400884, 2, 26.4 mm SL, male; 26.6 mm SL, female; KUN-P 42429, 27.0 mm SL, female; KUN-P 42430, 29.8 mm SL, male; KUN-P 42431, 17, 24.2–29.9 mm SL, 10 males, 7 females; KUN-P 42602, 10, 25.6–28.8 mm SL, all males, spermatozoa cryopreserved with liquid nitrogen; KUN-P 42605, 4, 25.5–28.8 mm SL, 2 males, 2 females; collected with holotype. – KUN-P 42604, 2, 28.8 mm SL, male; 30.1 mm SL, female; Japan: Hyogo, Toyooka, Maruyama River side, irrigation canal; T. Asai, T. Terawaki & F. Matsuo, 5 Oct 2010.

Non-type specimens. KUN-P 42140, 6, 29.1–32.6 mm SL, 3 males, 3 females (cleared and counterstained specimens); same locality as holotype; T. Asai, 8 Aug 2008. – KUN-P 42432, 12, 22.3–26.9 mm SL, 5 males, 7 females; collected with holotype.

Diagnosis. *Oryzias sakaizumii* is distinguished from all other congeners including *O. latipes* that are distributed throughout east Asia by the following combination of characters: distribution of melanophores, dense network of melanophores along each scale margin on the body surface in both sexes; depth of notch in membrane between dorsal-fin rays 5 and 6 less than half the length of rays in males; distinctive irregular black spots on the posterior portion of body lateral; and several silvery scales in patches on the posterior portion of the body.

Description. Counts and proportional measurements of holotype and selected paratypes are summarized in Table 1. Dorsal-fin rays 6 (6). Anal-fin rays 16–19 (18). Pelvic-fin rays 6 (6). Pectoral-fin rays 10–11 (10). Principal caudal-fin rays i,4–5/4–5,i (i,4/5,i). Procurrent fin rays, dorsal 4–6 (5), ventral 4–6 (4). Total vertebrae 29–31 (30), abdominal 11–13 (11) + caudal 17–19 (19).

Small, with maximum size of specimens examined 34.3 mm SL. Head small to moderate; head length 24.0–28.1 (26.6), depressed dorsoventrally; occiput depth 72.2–92.4 (79.4); and head width 63.1–84.2 (65.2). Body compressed laterally, short, slender to somewhat deep-bodied; body depth 22.6–28.5 (24.6). No pronounced abdominal concavity between pelvic fins and anterior part of anal fin in female. Mouth superior, up-turned, broad in a straight line and squared, slightly rounded dorsally; jaws subequal or lower jaw projecting slightly beyond upper jaw, each jaw with minute conical teeth; lips

Fig. 2. *Oryzias sakaizumii*; Japan: Fukui Prefecture: Tsuruga: Nakaikemi wetland: **a,** KPM-NI 27949, paratype, fresh male, 27.4 mm SL; **b,** KPM-NI 27953, paratype, fresh female, 25.1 mm SL.

slightly fleshy. Dorsal body profile relatively straight from head to mid-dorsum and gently curving from mid-dorsum to dorsal-fin origin; ventral body formed moderately into an arch from head to anal-fin origin.

Dorsal surface of head slightly concave just anterior to orbit, interorbital width 48.6–61.2 (53.5). Snout short, length 23.1–29.7 (23.4). Eye large, diameter 33.8–43.8 (37.5). Orbits confluent with dorsal profile of head or project slightly. Gill slit opening to superior margin of pectoral-fin base, not obscured by gill membrane; gill raker rudimentary; isthmus located inferior to posterior margin of eye. Dorsal fin placed far posteriorly.

Scales relatively large and deciduous, cycloid of varied shapes. No scales on rostral zone. Cephalic lateral-line system absent on dorsal, lateral, jugular, and jaw surfaces of head; sensory organs barely present, not developed as tube, and only U-shaped canaliform; nostril opens upper jaw posterosuperior, and lachrymal in front of eye opens on outer surface.

Pelvic fin abdominal. Anal fin base long, nearly one-quarter of SL. Membrane between dorsal-fin rays 5 and 6 notched in male. All fins soft-rayed. Filamentous dorsal- and anal-fin rays in male; dorsal-fin rays elongated; anal fin extending posterior, forming a nearly "parallelogram-shaped" fin slightly tapering caudad in males, nearly isosceles triangular-shaped fin tapering caudad in females; membrane between each anal-fin ray indented along outer margin in males (Figs. 1–2), entire and slightly concave in females. Pectoral-fin rays expanded medially, and posterior half of anal-fin rays with segmented papillary process. Medial-most pelvic-fin ray connected along one-half its length to body via a membrane. Pelvic-fin rays of some mature females elongated, nearly reaching anal-fin origin. Caudal fin truncate, medial part branched.

Males with a short, tubular urogenital papilla; females with an enlarged, slightly bilobed urogenital papilla.

Fig. 3. *Oryzias latipes*; Japan: Ehime Prefecture: Kitauwa: Kitanadakuninaga: **a,** KPM-NI 14468, fresh male; **b,** KPM-NI 14473, fresh female.

Coloration in life. Body translucent, light brown, and with minute melanophores as described below in preservative. As seen through abdominal wall, peritoneum silvery, subrectangular in females, smaller and subtriangular in males; both sexes with a silvery operculum and base of pectoral fin. Silvery scales in relatively large numbers of patches posteriorly on flank. Generally, all fins and belly lightly colored pale yellow. Caudal fin with yellowish dorsal and ventral submarginal bands. Dorsal edge of pectoral fin, posterior margin of dorsal-fin, and anterior and posterior edges of anal-fin with yellow bands. Pelvic fin overall yellowish. Pelvic and anal fins with widely scattered black chromatophores. Caudal-fin membrane between each fin ray with a few hairline longitudinal stripes. A straight black line tapering caudad and running along occiput surface to caudal-fin origin, excluding base of dorsal fin. Bisymmetrical twin crescent arches on occiput surface in silvery-blue. Eye dorsal view, silvery-blue; ventral-view, silvery.

Coloration in preservative. Specimens pale yellowish-gray dorsally, pearl-white ventrally, just after fixation. Belly burnt-orange just after fixation, gradually losing color. Charcoal to blackish peritoneum viewed through abdominal wall as subrectangular in females or smaller and subtriangular in males. A dark row of melanophores diverging anteriorly and joining basal line over posterior end of eye from dorsal surface of head to caudal-fin origin, excluding base of dorsal fin. A faint midlateral black hairline extending from posterior end of head lateral to base of caudal fin, and a straight black hairline extending from posterior end of anal-fin base to caudal-fin origin. A faint to discrete black chromatophore line extending from just dorsal to anal-fin origin, along body just dorsal to anal-fin base, and to posterior end of anal fin. Anal fin hyaline. Membrane of caudal fin with a hairline longitudinal stripe of melanophores just dorsal to first fin ray above midline and just ventral to first fin ray below midline. Dorsal- and anal-fin membranes between neighboring fin rays

with a black hairline. Pelvic-fin minutely speckled with black chromatophores in males. Black chromatophores clustering into a lunate fleck on posterior end of caudal peduncle. Urogenital papilla clear. Both sexes with dense network of melanophores on both flanks. Posterior portion of flank with distinctive irregular black spots.

Sexual dimorphism. Filamentous rays of dorsal fin elongated and membrane between dorsal-fin rays 5 and 6 notched in males, whereas not elongated and notched, forming an asymmetric fan-shaped fin in females (Figs. 1–2). Whole rays of anal fin extended, forming a nearly "parallelogram-shaped" fin slightly tapering caudad in males, nearly isoceles triangular-shaped fin tapering caudad in females. Medial pectoral-fin rays and posterior half of anal-fin rays with small papillary processes (contact organs), segmented in males [see Wiley & Collette (1970) for a review]. No contact organs in females. Membrane between each anal-fin ray indented along outer margin in

Table 1. Morphometric and meristic data for holotype and selected paratypes of *Oryzias sakaizumii* from Nakaikemi wetland, Tsuruga, Fukui Prefecture, Japan.

	holotype	paratypes					
	male	males (n = 19)			females (n = 16)		
		range	mean	SD	range	mean	SD
Total length (mm)	35.3	28.7–36.7	33.0	2.2	30.1–40.5	34.3	2.7
Standard length (mm)	28.9	24.2–29.8	27.2	1.5	24.4–34.3	28.5	2.5
Percentage of standard length							
Body depth	24.6	23.0–28.5	25.7	1.5	22.6–27.1	25.1	1.2
Body width	13.3	11.6–18.3	15.8	1.8	12.2–18.7	16.7	1.6
Head length	26.6	24.7–28.1	26.2	0.8	24.0–27.0	25.7	1.0
Depth of caudal peduncle	10.6	9.2–11.0	10.2	0.5	9.0–10.7	9.8	0.5
Length of caudal peduncle	16.7	15.1–18.1	16.8	0.7	14.9–17.9	16.3	0.9
Height of dorsal fin	17.6	16.7–20.2	18.9	1.1	12.9–14.9	13.9	0.6
Length of dorsal-fin base	7.6	7.2–10.2	8.6	0.8	6.4–9.9	7.5	0.8
Height of anal fin	19.0	16.1–19.7	17.8	0.9	11.5–17.2	13.8	1.4
Length of anal-fin base	26.4	24.3–28.8	26.6	1.2	23.5–27.1	24.6	0.9
Pectoral fin length	21.3	17.5–21.3	19.2	1.1	17.6–21.1	19.3	1.1
Length of pectoral-fin base	9.8	6.9–9.8	8.5	0.7	6.4–9.9	8.0	0.8
Pelvic fin length	11.1	8.2–12.6	10.7	1.0	10.4–14.4	12.4	0.9
Pre-dorsal length	75.8	75.0–78.5	77.3	0.9	76.2–79.3	77.7	0.8
Pre-anal length	61.1	58.4–62.9	60.6	1.2	59.8–62.9	61.7	0.8
Pre-pelvic length	49.5	47.5–51.3	49.4	1.2	47.2–52.7	50.7	1.7
Length from pectoral base to ventral base	20.5	19.2–24.0	21.3	1.4	20.4–25.8	22.8	1.4
Length from dorsal-fin base to caudal-fin base	17.7	16.5–20.6	17.9	0.9	16.2–19.7	17.6	0.9
Percentage of head length							
Head width	65.2	66.3–77.4	70.9	3.5	63.1–84.2	74.0	5.8
Occiput depth	79.4	73.2–91.7	82.2	4.4	72.2–92.4	83.0	4.9
Snout length	23.4	23.1–29.7	26.8	1.9	24.6–29.4	27.0	1.4
Interorbital width	53.5	51.6–61.2	55.3	2.6	48.6–60.4	54.8	2.9
Eye diameter	37.5	33.8–43.8	39.1	2.5	34.2–41.2	37.1	1.7
Counts							
Dorsal-fin rays	6	6	6	–	6	6	–
Anal-fin rays	18	16–18	17.2	0.6	17–19	17.8	0.8
Pectoral-fin rays	10	10–11	10.4	0.5	10–11	10.8	0.4
Pelvic-fin rays	6	6	6	–	6	6	–
Principal caudal-fin rays	i,4/5,i	i,4–5/5,i	i,4.1/4.9,i	0.2/0.2	i,4/4–5,i	i,4/4.9,i	–/0.3
Procurrent caudal-fin rays	5/4	4–5/4–6	4.8/5.0	0.4/0.5	4–6/4–6	4.6/4.9	0.6/0.7
Total caudal-fin rays	20	20–22	20.8	0.5	18–23	20.4	1.3
Abdominal vertebrae	11	11–12	11.8	0.4	11–13	11.8	0.6
Caudal vertebrae	19	17–19	18.1	0.6	17–19	18.1	0.4
Total vertebrae	30	29–31	29.9	0.5	29–31	29.8	0.5

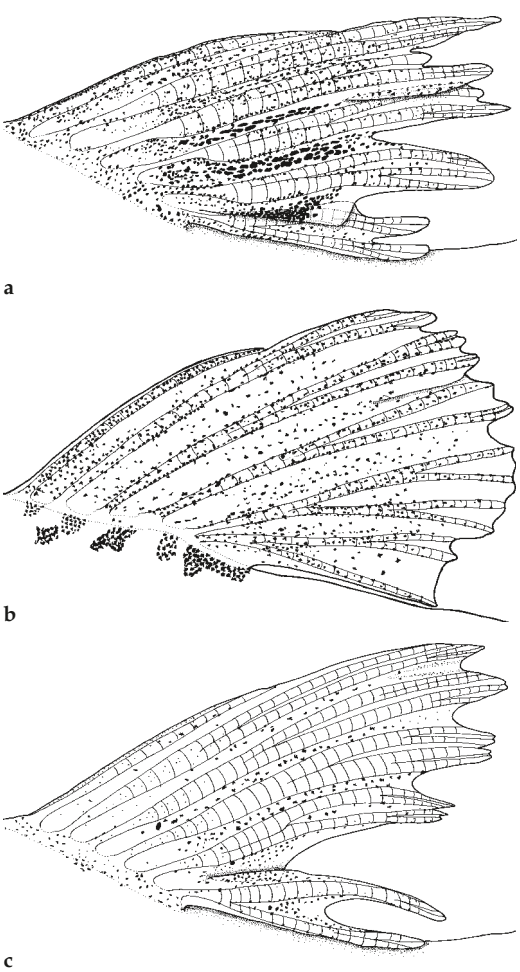

Fig. 4. Comparison of dorsal fin shape in the Japanese species of *Oryzias*: **a,** *Oryzias sakaizumii*, KUN-P 42428, holotype, male, 28.9 mm SL; **b,** *O. sakaizumii*, KUN-P 42431, paratype, female, 29.9 mm SL; **c,** *O. latipes*, KUN-P 42601, male, 33.3 mm SL.

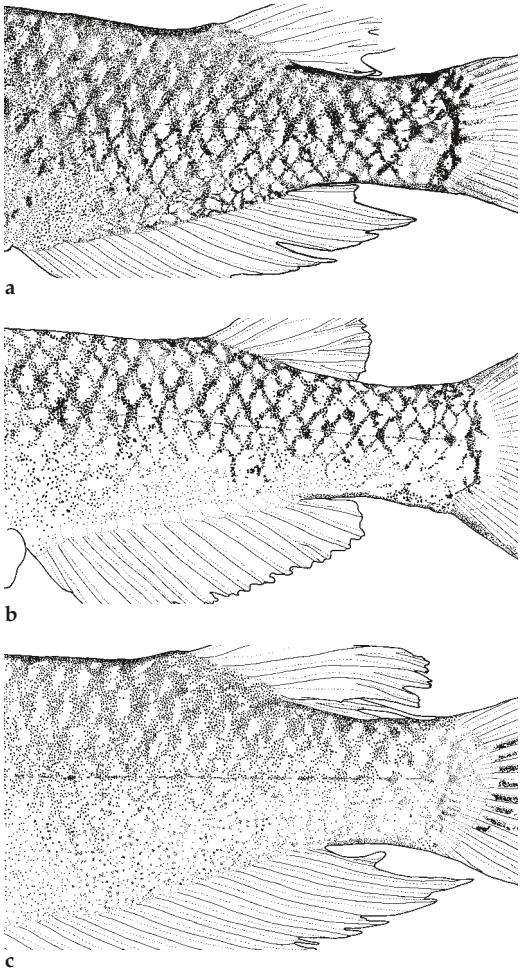

Fig. 5. Melanophore pattern in the Japanese species of *Oryzias*: **a,** *Oryzias sakaizumii*, KUN-P 42428, holotype, male, 28.9 mm SL; **b,** *O. sakaizumii*, NSMT-P 105974, paratype, female, 34.3 mm SL; **c,** *O. latipes*, KUN-P 42169, male, 26.2 mm SL.

males, entire and slightly concave in females (Fig. 1). Male with a short, tubular urogenital papilla; females with enlarged, slightly bilobed urogenital papilla. Pelvic-fin rays elongated, nearly reaching to anal fin origin in females.

During the breeding season, xanthophores appear in both sexes over the whole body, and the melanophores of males remain scattered but increase in density on the body lateral and the dorsal, anal, and caudal fins. Melanophores are especially prominent in pelvic fins, all jet-black. Contact organs become well-developed during this period.

Distribution and habitat. *Oryzias sakaizumii* is found in freshwaters along the Sea of Japan coast in the northern half of Honshu, from the Pacific coast of Momoishi, Aomori Pref. to Hyogo Pref. around Tango, Tajima district that distributional western limit is Hamasaka (Sakaizumi et al., 1983; Sakaizumi, 1984; Takehana et al., 2003) (Fig. 6). It inhabits ponds, marshes, and streams, such as irrigation canals amid paddy fields in flat alluvial lowlands (Sakaizumi, 1986). This species is well adapted to aquatic life on temporary floodplain wetlands, since it mainly inhabits regions around paddy fields that were originally back

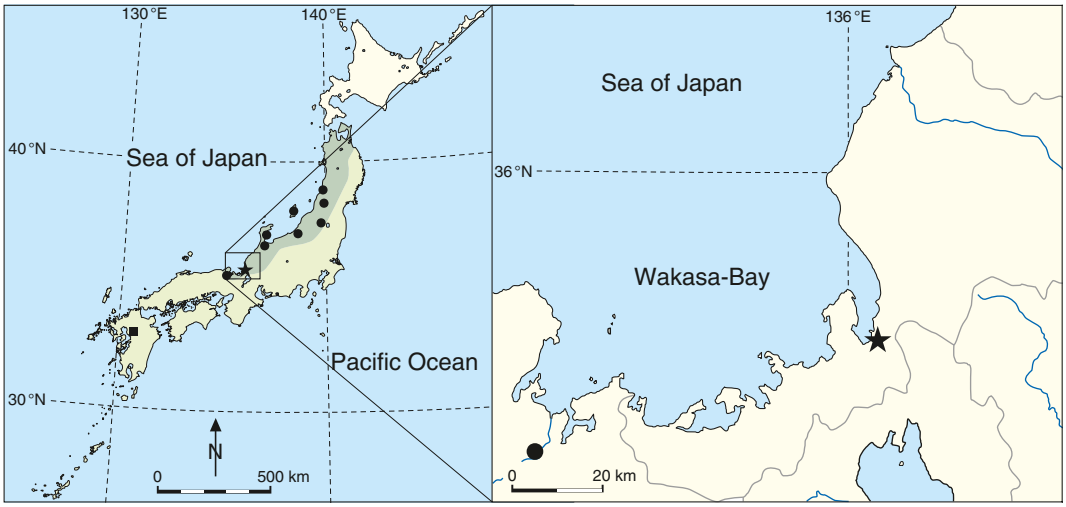

Fig. 6. Distribution and collection sites of *Oryzias sakaizumii*. ★, type locality; ●, localities of paratypes; ■, presumed type locality of *O. latipes*; ▒, distribution range of *O. sakaizumii*; □, distribution range of *O. latipes*.

marshes prior to agricultural development. The wetland Nakaikemi is conserved under the control of Tsuruga City, Fukui Prefecture to preserve the natural habitat for the new species, *O. sakaizumii*, and therefore this location is the most suitable as type locality.

Ecology. As does its closely related congener, *O. latipes*, a female of *O. sakaizumii* spawns about 20 spherical eggs every day over a few months from early May to early September. Spawned eggs with attachment filaments are suspended for a while on the abdominal region of the female between the pelvic fins and anterior part of anal fin. Thereafter, most of the eggs carried by females are rubbed off on waterweed appropriate for rearing. The remaining eggs may fall to the ground. Hatching occurs within a few weeks. This species is omnivorous, and in some cases, may eat eggs. It forms schools beneath the water's surface and actively searches for food in sunny, warm afternoons. In contrast, both during winter and on rainy, cloudy, and cold days, it keeps under submerged sediment such as leaf litter. This species has a life span of only 1 year in the wild, whereas it lives for a few years in captivity (pers. obs.).

Conservation. The wild populations of *Oryzias sakaizumii* have been decreasing rapidly due to habitat degradation (pers. obs.). Three main factors are responsible: consolidation in paddy fields such as land remodeling and concrete lining on three faces of irrigation canals; pesticide application to rice crops; and alien invasive species. For example, the western mosquitofish *(Gambusia affinis)* was introduced into the habitat of *O. sakaizumii* and subsequently expanded its distributional area in Hyogo Prefecture. In addition, the largemouth bass *(Micropterus salmoides)* invaded the habitat of *O. sakaizumii* in a part of Niigata Prefecture. *Oryzias sakaizumii* has been categorized as threatened through ranks such as Threatened II (Fukui Prefecture), Near Threatened (Niigata Prefecture), and an important rare wild species (Aomori Prefecture). In recent years, this species has been observed to have a patchy distribution, restricted to a limited number of localities, and it is captured with much difficulty in field surveys.

Etymology. The specific epithet, *sakaizumii*, honors Dr. Mitsuru Sakaizumi, a molecular geneticist at Niigata University, who pioneered in and contributed to the field of molecular phylogenetic study of Japanese ricefishes. Proposed English name: Northern medaka.

Remarks. *Oryzias sakaizumii* is similar to the most closely related species, *O. latipes*, in general body shape, but differs in having the depth of the notch in the membrane between the dorsal-fin rays 5

Fig. 7. Type locality of *Oryzias sakaizumii*, Nakaikemi wetland, Tsuruga, Fukui Prefecture, Japan, photographed 4 June 2011. **a,** paddy field; **b,** swamp located behind paddy field in **a**.

and 6 less than half the length of the rays in males (vs. more than half the length of the rays in *O. latipes*; Figs. 2–3); a dense network of melanophores along each scale margin on body side (vs. diffuse melanophore pigmentation scattered over the mid-portion of each scale); a distinctive irregular black spots present on the posterior portion of body (vs. absent); and 10–23 silvery scales in patches on the posterior portion of body (vs. 0–9).

From our observation of the lectotype and paralectotypes of *O. latipes* in RMNH, we conclude that they are identical with the Southern popula-

tion in the body shape and lack the diagnostic characters of *O. sakaizumii* (the slightly notched dorsal-fin membrane, the dense network of melanophores, the distinctive irregular black spots, and the numerous silvery scales), confirming that the Northern population is a new species, *O. sakaizumii*.

Parapatric populations of the Northern and Southern populations coexist within the drainage basin of River Shinano, Nagano Prefecture, and River Yura, Kyoto Prefecture, which are the distributional boundary zones between *O. latipes* and *O. sakaizumii* (Kume & Hosoya, 2010). In the Yura River drainage, the two species have also been reported to exhibit different schooling behaviors (Uono et al., 2011). Therefore, a mechanism for reproductive isolation could be present as a result of this differing behavior between populations. The Northern population and the Southern population should be treated as distinct biological species (sensu Mayr & Ashlock, 1991).

Genetic hybrid populations exist within the drainage of River Maruyama, Hyogo Prefecture and around Kumihama, Amino, Kyoto Prefecture, which are distributional boundary zones between the two species (Sakaizumi, 1984). These populations are related herein to *O. sakaizumii*, as they are in the same clade by mtDNA analysis (Takehana et al., 2003), ribosomal DNA (Sakaizumi, 1987), and because they share morphological characters.

As for relationships with congeners, the continental forms of medaka have been divided into two phyletic groups: the East Korean form and the China–West Korean form, which are distributed throughout East Asia (Sakaizumi & Jeon, 1987; Takehana et al., 2004a–b). However, these forms have not been fully investigated taxonomically. The China–West Korean form is considered to be *O. sinensis* (sensu Parenti, 2008). Taxonomically, the East Korean form, which has been regarded as *"O. latipes"* in Korea, is considered to be in the same clade as *O. sakaizumii*, since it shares characters such as the slightly notched membrane between dorsal-fin rays 5 and 6, and the dense network of melanophore [see Kim & Park (2002) for illustration]. Furthermore, it seems that the Korean *"O. latipes"* could be a subspecies of *O. sakaizumii* or another unnamed species; they differ by characters such as the presence of minute black spots on lower part of caudal fin, and the caudal peduncle without black chromatophores clustering into a lunate fleck.

Comparative materials. *Oryzias latipes*: – RMNH 2713a, lectotype, 35.7 mm SL, female; Japan. RMNH 2713b, paralectotype, 32.7 mm SL, female; Japan. RMNH 2713c, paralectotype, 30.2 mm SL, female; Japan. – KUN-P 42139, 14, 24.7–32.4 mm SL, 5 males, 9 females (2 males, 2 females cleared and counterstained); Japan: Kyoto, Mutobe. – KUN-P 42141, 18, 22.4–30.4 mm SL, 8 males, 10 females (3 males, 4 females cleared and counterstained); Japan: Nara. – KUN-P 42601, 1, male, 33.3 mm SL; Japan: Ehime, Iyo. – NSMT-P 58863, 13, 24.4–31.3 mm SL, 8 males, 5 females (2 males, 1 female cleared and counterstained); Japan: Saga, Bizen-Yamaguti. – KUN-P 42131, 10, 21.7–35.9 mm SL, 6 males, 4 females (2 males, 2 females cleared and counterstained); Japan: Okinawa, Kunigami. KUN-P 42135, 10, 25.3–35.9 mm SL, 5 males, 5 females; Japan: Okinawa, Kunigami.

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Literature cited

Frankham, R., J. D. Ballou & D. A. Briscoe. 2002. Introduction to conservation genetics. Cambridge University Press, Cambridge, xxi + 617 pp.

Günther, A. C. L. G. 1866. Catalogue of the fishes in the British Museum. Vol. 6. Catalogue of the Physostomi, containing the families Salmonidae, Percopsidae, Galaxidae, Mormyridae, Gymnarchidae, Esocidae, Umbridae, Scombresocidae, Cyprinodontidae, in the collection of the British Museum. British Museum, London, xv+368 pp.

Herder, F & S. Chapuis. 2010. *Oryzias hadiatyae*, a new species of ricefishes (Atherinomorpha: Beloniformes: Adrianichthydae) endemic to Lake Masapi, Central Sulawesi, Indonesia. Raffles Bulletin of Zoology, 58: 269–280.

Hubbs, C. L. & K. F. Lagler. 2004. Fishes of the Great lakes region. Revised edition. University of Michigan Press, Bloomfield Hills, xxxii + 276 pp.

Jordan, D. S. & J. O. Snyder. 1901. A preliminary check list of the fishes of Japan. Annotationes Zoologicae Japonenses, 3: 31–159.

Jordan, D. S. & J. O. Snyder. 1906. A review of the Poecilidae or killifishes of Japan. Proceedings of United States National Museum, 31: 287–290.

Kawamura, K. & K. Hosoya. 1991. A modified double staining technique for making a transparent fish-skeletal specimen. Bulletin of National Research Institute of Aquaculture, 20: 11–18. [In Japanese].

Kim, I. S. & J. Y. Park. 2002. Freshwater fishes of Korea. Kyo-Hak Publishing, Seoul, 465 pp. [In Korean].

Kottelat, M. 1990a. Synopsis of the endangered Buntingi (Osteichthyes: Adrianichthyidae and Oryziidae) of Lake Poso, Central Sulawesi, Indonesia, with a new reproductive guild and descriptions of three new species. Ichthyological Exploration of Freshwaters, 1: 49–67.

— 1990b. The ricefishes (Oryziidae) of the Malili Lakes, Sulawesi, Indonesia, with description of a new species. Ichthyological Exploration of Freshwaters, 1: 151–166.

Kume, K. & K. Hosoya. 2010. Distribution of Southern and Northern populations of Medaka *(Oryzias latipes)* in the Yura River drainage of Kyoto, Japan. Biogeography, 12: 111–117.

Magtoon, W. 2010. *Oryzias songkhramensis*, a new species of ricefish (Beloniformes; Adrianichthyidae) from Northeast Thailand and Central Laos. Tropical Natural History, 10 (1): 107–129.

Mayr, E. & P. D. Ashlock. 1991. Principles of systematic zoology. McGraw-Hill, New York, xx + 475 pp.

Nelson, J. S. 2006. Fishes of the World, 4th edition. Wiley, Hoboken, xx + 601 pp.

Parenti, L. R. 2008. A phylogenetic analysis and taxonomic revision of ricefishes, *Oryzias* and relatives (Beloniformes, Adrianichthyidae). Zoological Journal of the Linnean Society, 154: 494–610.

Parenti, L. R. & R. K. Hadiaty. 2010. A new remarkably colorful, small ricefish of the genus *Oryzias* (Beloniformes, Adrianichthydae) from Sulawesi, Indonesia. Copeia, 2010: 268–273.

Rosen, D. E. & L. R. Parenti. 1981. Relationships of *Oryzias*, and the groups of Atherinomorph fishes. American Museum Novitates, 2719: 1–25.

Sakaizumi, M. 1984. Rigid isolation between the Northern population and the Southern population of the Medaka, *Oryzias latipes*. Zoological Science, 1: 795–800.

— 1986. Genetic diversity and evolution in wild populations of the medaka *Oryzias latipes* (Pisces: Oryziatidae). Pp. 161–179 in: K. Iwatsuki, P. H. Raven & W. J. Bock (eds.), Modern aspects of species. University of Tokyo Press, Tokyo.

Sakaizumi, M. 1987. Trends of modern biogeography, biogeography of killifish using genetic techniques. The Heredity, 41: 17–22. [In Japanese].

Sakaizumi, M., N. Egami & K. Moriwaki. 1983. Allozymic variation and regional differentiation in wild population of the fish *Oryzias latipes*. Copeia, 1983: 311–318.

Sakaizumi, M. & S. R. Jeon. 1987. Two divergent groups in the wild populations of medaka *Oryzias latipes* (Pisces: Oryziatidae) in Korea. Korean Journal of Limnology, 20: 13–20.

Senou, H. 2002. Adrianichthyidae medakas. P. 547 in: T. Nakabo (ed.), Fishes of Japan with pictorial keys to the species. English edition. Tokai University Press, Tokyo.

Setiamarga, D. H. E., M. Miya, Y. Yamanoue, Y. Azuma, J. G. Inoue, N. B. Ishiguro, K. Mabuchi & M. Nishida. 2009. Divergence time of the two regional medaka populations in Japan as a new time scale for comparative genomics of vertebrates. Biology Letters, 5: 812–816.

Takehana, Y., N. Nagai, M. Matsuda, K. Tsuchiya & M. Sakaizumi. 2003. Geographic variation and diversity of the cytochrome *b* gene in Japanese wild populations of medaka, *Oryzias latipes*. Zoological Science, 20: 1279–1291.

Takehana, Y., K. Naruse & M. Sakaizumi. 2005. Molecular phylogeny of the medaka fishes genus *Oryzias* (Beloniformes: Adrianichthyidae) based on nuclear and mitochondrial DNA sequences. Molecular Phylogenetics and Evolution, 36: 417–428.

Takehana, Y., S. R. Jeon & M. Sakaizumi. 2004a. Genetic structure of Korean wild population of the medaka *Oryzias latipes* inferred from allozymic variation. Zoological Science, 21: 977–988.

Takehana, Y., S. Uchiyama, M. Matsuda, S. R. Jeon & M. Sakaizumi. 2004b. Genetic variation and diversity of the cytocrome *b* gene in wild population of medaka *(Oryzias latipes)* from Korea and China. Zoological Science, 21: 483–491.

Temminck, C. J. & H. Schlegel. 1846. Pisces. Pp. 173–268 in: Fauna Japonica, sive descriptio animalium quae in itinere per Japoniam suscepto annis 1823–30 collegit, notis observationibus et adumbrationibus illustravit. Ph. Fr. de Siebold. Parts 10–14. Leiden.

Uono, T., T. Hamaguchi, K. Kume & K. Hosoya. 2011. Comparison of fish schooling behavior between the Southern and Northern populations of *Oryzias latipes* in the Yura River system, Kyoto Prefecture. Journal of Japan Society on Water Environment, 34: 109–114. [In Japanese].

Wiley, M. L. & B. B. Collette. 1970. Breeding tubercules and contact organs in fishes: their occurrence, structure, and significance. Bulletin of the American Museum of Natural History, 143: 145–216.

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