Ichthyological Research
https://doi.org/10.1007/s10228-025-01032-z

FULL PAPER

The Biwa salmon, a new species of *Oncorhynchus* (Salmonidae) endemic to Lake Biwa, Japan

Yasuhiro Fujioka[1] · **Masayuki Kuwahara**[1,2] · **Ryoichi Tabata**[1] · **Yusuke Fuke**[3,4] · **Tetsuji Nakabo**[5]

Received: 30 March 2025 / Revised: 23 May 2025 / Accepted: 24 May 2025

Abstract

Belonging to the *Oncorhynchus masou* species complex, the Biwa salmon is described as the new species *Oncorhynchus biwaensis*, a Lake Biwa (Japan) endemic which has been treated as an unidentified species/subspecies since 1990. The new species can be easily distinguished from Masu salmon [*Oncorhynchus masou masou* (Brevoort 1856)] and Amago salmon (*Oncorhynchus masou ishikawae* Jordan and McGregor *in* Jordan and Hubbs 1925) among the species complex in having more pyloric caeca and fewer transverse scales above the lateral line. The identity of *Oncorhynchus rhodurus* Jordan and McGregor *in* Jordan and Hubbs 1925, often previously applied to the new species, is shown to differ from the latter. Similarly, *O. ishikawae*, also previously confused with the new species, is confirmed as Amago salmon. Types of the new species and examples of the other subspecies examined were confirmed as nonhybrids by DNA analysis.

Keywords Biwa salmon · Lake Biwa · MIG-seq · New species

Introduction

The *Oncorhynchus masou* complex, distributed in coastal marine and fresh waters of the northeastern Pacific, comprises Masu salmon, Amago salmon, Biwa salmon, and Taiwan salmon. The former three species, occurring around Japan, have been recently recognized as *Oncorhynchus masou masou* (Brevoort 1856), *Oncorhynchus masou ishikawae* Jordan and McGregor *in* Jordan and Hubbs 1925, and *Oncorhynchus* sp., respectively (Nakabo 2018; Fujioka et al. 2024), the two first-mentioned having both fluvial and sea-run forms, and the last-mentioned having fluvial (Fujioka and Fushiki 1988; Kuwahara and Iguchi 1994) and lacustrine forms.

However, the ecological forms of Amago salmon and Biwa salmon have been poorly understood until recently, both species having small distinctive red spots scattered on the body when young.

Oshima (1929, 1930, 1936) identified a "salmonid fish" with red spots on the body when young (fluvial form) distributed in western Japan, plus a lacustrine form in Lake Biwa, as *Oncorhynchus rhodurus* Jordan and McGregor *in* Jordan and Hubbs 1925, to which he applied the Japanese standard name "Biwamasu". Thereafter, the lacustrine form

---

This article was registered in the Official Register of Zoological Nomenclature (ZooBank) as 3EEA549D-42A2-4A0E-9313-A191FD2D1FD0.

This article was published as an Online First article on the online publication date shown on this page. The article should be cited by using the doi number.

✉ Yasuhiro Fujioka
yasuhirofujioka@outlook.jp

Masayuki Kuwahara
biwasalmon@nifty.com

Ryoichi Tabata
tabata-ryoichi@biwahaku.jp

Yusuke Fuke
yfa73986@gmail.com

Tetsuji Nakabo
tetsunabo@kcn.jp

[1] Lake Biwa Museum, 1091 Oroshimo, Kusatsu, Shiga 525-0001, Japan

[2] Authorized Non-Profit Organization Biwako Trust, 5-1-1 Hamaotsu, Otsu, Shiga 520-0047, Japan

[3] National Institute of Genetics, 1111 Yata, Mishima, Shizuoka 411-8540, Japan

[4] Faculty of Agriculture, Setsunan University, 45-1 Nagaotoge, Hirakata, Osaka 573-0101, Japan

[5] The Kyoto University Museum, Sakyo-ku, Yoshida, Kyoto 606-8501, Japan

only has been commonly recognized as "Biwamasu", and the fluvial form as "Amago".

Subsequently, a sea-run form identified as "Biwamasu" was found in the Kiso River, Aichi Prefecture (Oshima 1957), Kagami River, Yamaguchi Prefecture (Katayama and Fujioka 1966), and Nagara River, Aichi Prefecture (Kato 1968), being initially followed by Hikita (1962) and Nakamura (1963). However, Miyadi et al. (1963) recognized the lacustrine and sea-run forms, and the fluvial form as ecological variations among the species, applying the names *O. rhodurus* f. *rhodurus* for the formers, and *O. rhodurus* f. *macrostomus* for the latter.

Later, Kato (1973a, b, 1975) noted some morphological differences between the lacustrine form from the Ado River, Lake Biwa, sea-run form from Ise Bay/Nagara River, and fluvial form from the Nagara River. In addition, Kato (1978b) found both morphological and ecological differences between the lacustrine form from Lake Biwa and the fluvial form inhabiting some upper streams flowing into the lake, and suggested that the two forms should be treated as evolutionarily discrete populations. This suggestion laid the ground for subsequent determination of the taxonomic status of these forms, and appropriate conservation measures.

Similarly, Fujioka (1987, 1988a, 1990, 1991), Fujioka and Fushiki (1988, 1989a, b) and Fujioka et al. (1990a, b, 1991) reported several morphological, behavioral and physiological differences between the lacustrine (Lake Biwa) and fluvial forms during parr-smolt transformations.

During its taxonomic history, "Biwamasu" has been recognized as a subspecies included within the *O. masou* species complex, as *O. masou rhodurus* by Araga (1984), Kato (1985), Takasaki et al. (1994), Oohara and Okazaki (1996), and Murata et al. (1998), or *O. masou ishikawai* by Furukawa (1989).

However, Kimura (1990) treated "this salmonid fish" as an unidentified subspecies (*O. masou* subsp.), applying the English name "Biwa salmon" based on an examination of the holotypes of taxa related to the *O. masou* species complex. Thereafter, "Biwa salmon" has usually been recognized as *O. masou* subsp. (Hosoya 1993, 2002; Kuwahara and Iguchi 1994, 2007; Gwo et al. 2008; Hsu et al. 2010; Kuwahara et al. 2012, 2019; Kuwahara 2013; Tabata et al. 2016).

On the other hand, Kato (1986, 1987, 1988) recognized the three species of the *O. masou* complex as distinct species (*O. masou*, *O. ishikawae* and *O. rhodurus*) due to the morphological differences among them. Nakabo (2009) also recognized Biwa salmon as a distinct species, mainly due to the biological separation from Amago salmon shown by Kato (1978b) and Kuwahara and Iguchi (2007). Recently, "Biwa salmon" has been treated as a separate species (*Oncorhynchus* sp.), distinct from Masu salmon (*O. masou masou*) and Amago salmon (*O. masou ishikawae*) (Hosoya 2013; Kawase 2015; Amano et al. 2018; Nakabo 2018; Fujioka et al. 2024).

Because "Biwa salmon" have not been reviewed taxonomically since Kimura (1990), the species is here described as new, and included within the *O. masou* species complex. Recently, hybrid specimens between Biwa salmon and Amago salmon have been found in Lake Biwa and some inflowing rivers by Kuwahara et al. (2012, 2019). Because names proposed for hybrid specimens are excluded from the provisions of the International Code of Zoological Nomenclature (ICZN 1999: art. 1.3.3), the description herein of Biwa salmon as a new species of the family Salmonidae is based on specimens having no hybrid traces, confirmed by DNA analysis.

Because taxonomic reviews necessitate morphological comparisons of the types of nominal species, young individuals (excluding juveniles) of the other two *O. masou* subspecies were selected, since the types related to this species complex are also young individuals (Kimura 1990).

Materials and methods

All specimens examined were kept on ice when captured, and preserved at -20ºC in the laboratory until use. After thawing in cold water, the specimens were photographed and part of the right pelvic fin preserved in 100% ethanol pending molecular analysis. Approximately 10 scales were taken from the right side of the body between the dorsal fin base and lateral line before fixation in 10% formalin, washed in clear water, and mounted between two glass slides for observation. No specimens showing evidence of hybridization with other subspecies were used for the description of the new species. Examples of the two subspecies of *Oncorhynchus masou* were not collected from localities at which inter-subspecific hybridization had been recorded.

Morphology. The specimens examined were examined morphologically after fixation in 10% formalin for greater than a two month period. Specimens that could not be examined for both mitochondrial DNA (mtDNA) and nuclear DNA (described below) were excluded from the following morphological examination. Counts and measurements generally followed Nakabo (2002), and numbers of basal ridges and apical ridges on the body scales, Kato (1978a). Red spots on the body were counted on the left side before formalin fixation. The postorbital body length (from the end of the orbit to that of the hypural), and post orbital length (Nakabo 2002) are expressed relative to postorbital head length. Scale length and width followed Kato (1978a), expressed as the mean value of 10 scales. All measurements were made to 0.1 mm under a stereo microscope, using a caliper. Vertebrae and dorsal fin soft rays were counted from radiographs (CBW-80, SOFTEX Co., Ltd.). Scale shape and numbers were observed under 50 times magnification by Profile Projector V-16E, NIKON without staining, following the methods of Kato (1978a).

Molecular analysis. Total genomic DNA was isolated from a piece of fin preserved in 99% ethanol, using a DNeasy Blood & Tissue Kit or QIAamp DNA Micro Kit (QIAGEN, Hilden). Nucleotide sequence data for the mitochondrial cytochrome *b* (cyt*b*) gene (5' half of cyt*b*; 600 bp) were used to infer molecular identification. PCR amplification was conducted using the primer pair L15172 (5'- TGA GGA CAA ATA TCN TTY TGA GG -3'; Harada et al. 2002) and H15915 (5'- ACC TCC GAT CTY CGG ATT ACA AGA C -3'; Aoyama et al. 2000). The conditions for PCR amplification, purification of PCR products, and cycle sequencing reactions followed Tabata et al. (2016). Sequencing was performed using a DNA autosequencer (SeqStudio; Thermo fisher science, Waltham, MA), the resulting sequences were deposited in the DNA sequence database DDBJ/EMBL/GenBank (accession numbers: PV409879–PV409952). The cyt*b* nucleotide sequences were edited and aligned with ClustalW, and by eye using MEGA X (Kumar et al. 2018). Phylogenetic analyses were conducted using the maximum likelihood (ML) method. The unrooted ML tree was estimated using MEGA X under the TN model selected by Akaike's information criterion (AIC; base frequencies of A = 0.250, C = 0.320, G = 0.132, and T = 0.298). The relationships among mtDNA haplotypes were estimated as a statistical parsimony network using the TCS 1.2.1 software (Clement et al. 2000).

Assessment of the genetic characteristics of specimens based on genome-wide polymorphism utilized the multiplexed ISSR genotyping by sequencing method (MIG-seq; Suyama and Matsuki 2015). The MIG-seq library was prepared according to Watanabe et al. (2020) and Suyama et al. (2022). The index primer sets were those provided by Onuki and Fuke (2022). The library was outsourced to Novogene and 150 bp paired-end sequencing was performed on the NovaSeq 6000 (Illumina). Raw data were deposited in the NCBI Sequence Read Archive (SRA) (BioProject accession number: PRJNA1243010, BioSample accession numbers: SAMN47613482–47613562).

The raw data from NovaSeq 6000 were demultiplexed using the process_shortread program in Stacks 2.64 (Rochette et al. 2019), according to a 5 bp index. Quality control and trimming of the obtained reads were performed using fastp 0.23.4 (Chen et al. 2018). All reads were trimmed to 109 bases to remove tail and primer sequences: the first 35 bases (tail and primer sequences) from Read 1, and the first 17 bases (primer sequence) and the last 23 bases (for sequence length adjustment) from Read 2. Subsequently, reads containing low-quality bases (<Q30) and adaptor sequences were removed.

The filtered reads were mapped to the chromosome-level reference genome assembly of *O. m. masou* (GCF_036934945.1; Christensen et al. 2025) using strobealign 0.13.0 (Sahlin 2022), with the default settings. Subsequently, SNP calls were performed using the ref_map.pl pipeline in Stacks, outputting a VCF file with the first SNP per locus selected using --write-single-snp flag. The VCF files were filtered using vcftools 0.1.16 (Danecek et al. 2011) to remove loci with a depth of less than 3 (--min-meanDP = 3), and the populations program in Stacks under the following setting: 'remove variant sites shared by less than 75% of all individuals ($R = 0.75$), with heterozygosity greater than 75% (--max-obs-het = 0.75), and with fewer than three minor alleles (--min-mac = 3)'. Downstream analyses were conducted on files generated in plink format.

Principal component and clustering analyses were performed to assess the genetic segregation of each species. Principal component analysis was performed using PLINK v1.90b5 (Purcell et al. 2007). Clustering analysis was performed using ADMIXTURE 1.3.0 (Alexander et al. 2009) with 100 iterations at different seed values for the number of genetic clusters (K) ranging from 1 to 6. The results were summarized based on CV-error values. For each K, a re-analysis was performed based on the seed value showing the lowest CV-error value, so as to obtain the final output. The analyses and visualization of results were performed in R 4.3.1 (R Core Team 2023) using custom scripts.

Specimens examined. Deposited at LBM (Lake Biwa Museum, Kusatsu, Shiga, Japan), FAKU (The Kyoto University Museum, Sakyo-ku, Kyoto, Japan), NSMT (National Museum of Nature and Science, Tsukuba, Ibaraki, Japan), and OMNH (Osaka Museum of Natural History, Higashi-Sumiyosi-ku, Osaka, Japan).

Oncorhynchus biwaensis sp. nov.

(Standard Japanese name: Biwamasu; English name: Biwa salmon)

(Fig. 1; Table 1)

Salmo perryi (not of Brevoort 1856): Jordan and Snyder 1902: 579 (in part, Karasaki, Lake Biwa) [conspecific with "Masu salmon"]

Oncorhynchus masou (not of Brevoort 1856): Jordan et al. 1913: 42 (in part, Lake Biwa); Jordan and Thompson 1914: 211 (in part, Lake Biwa) [conspecific with "Masu salmon"]

Oncorhynchus macrostomus (not of Günther 1877): Jordan and McGregor *in* Jordan and Hubbs 1925: 135, pl. 4, fig. 2 (in part, Lake Biwa); Ohno and Ando 1931: 23, pl.

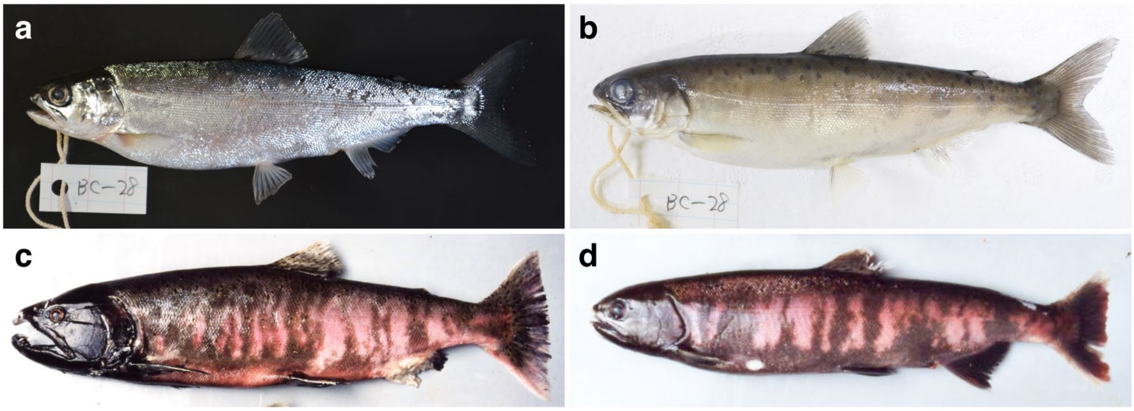

Fig. 1 *Oncorhynchus biwaensis* sp. nov. **a** Holotype, LBM 1210060173, 151.4 mm SL, from Lake Biwa, when fresh; **b** holotype, in sprits; **c** mature male, unregistered specimen, 49.5 cm SL; **d** mature female, unregistered specimen, 33.7 cm SL

Table 1 Counts of *Oncorhynchus biwaensis* sp. nov., *O. masou masou*, and *O. m. ishikawae*

	Oncorhynchus biwaensis sp. nov.			*O. m. masou*		*O. m. ishikawae*		*O. ishikawae*	*O. rhodurus*
	Holotype	Paratypes						Holotype*	Holotype*
	LBM 1210060173	*n* = 20		*n* = 20		*n* = 33		FMNH 58682	FMNH 59389
Standard length (mm)	151.4	112.2–241.0	Modes	92.2–190.2	Modes	99.6–165.7	Modes	151.2	446.0
Dorsal fin rays	14	14	14	15–16	15	14–16	15		
Anal fin rays	14	11–14	13	13–15	14	12–14	13		12
Pectoral fin rays	13	13–14	14	14–16	15	14–16	16		
Pelvic fin rays	9	9	9	9–10	10	9–11	10		
Gill-rakers on first arch	17	17–20	18	18–21	19	17–23	21	21	18
Lateral line scales	133	127–139	131	121–139	138	110–145	129	135	136
Scales above lateral line	22	19–22	21	28–35	29	28–36	32	34	36
Scales below lateral line	22	21–24	24	27–34	32	26–34	31	27	33
Number of basal ridges	21.1	18.0–44.5	–	11.6–20.3	–	6.4–17.3	–		
Number of apical ridges	17.9	14.9–36.2	–	5.3–11.7	–	6.1–12.7	–		
Branchiostegal rays	13	11–13	13	13–14	13	11–14	13	12–13	11–12
Number of pyloric caeca	60	43–65	56	32–56	46	28–49	36	51	42
Number of red spots	3	0–15	0	0	0	2–46	29		
Number of vertebrae	64	63–66	65	62–67	64	64–67	66	65	63

*After Jordan and Hubbs (1925) and Kimura (1990)

3, fig. 20 (in part, Lake Biwa) [conspecific with "Amago salmon"]

Oncorhynchus rhodurus (not of Jordan and McGregor *in* Jordan and Hubbs 1925): Oshima 1929: 133, photo in p. 137 (in part, Lake Biwa); Oshima 1936: 17 (in part, Lake Biwa); Okada and Nakamura 1948: 136 (in part, Lake Biwa); Aoyagi 1957: 32 (in part, Lake Biwa); Oshima 1957: 42, figs. in pp. 42 and 50 (in part, Lake Biwa); Hikita 1962: 44 (in part, Lake Biwa) [conspecific with "Amago salmon"]; Nakamura 1963: 97, fig. 18Aa, b (in part, Lake Biwa)

Oncorhynchus formosanus (not of Jordan and Oshima 1919): Oshima 1930: 1121, fig. 6 (in part, Lake Biwa) [conspecific with "Amago salmon"]

Oncorhynchus rhodurus var. *rhodurus* (not of Jordan and McGregor *in* Jordan and Hubbs 1925): Matsubara 1955: 206 (key; lakes in southern Japan) [conspecific with "Amago salmon"]

Oncorhynchus rhodurus f. *rhodurus* (not of Jordan and McGregor *in* Jordan and Hubbs 1925): Miyadi et al. 1963: 42, pl. 4, fig. 14 (Lake Biwa) [as intraspecific ecological variant]

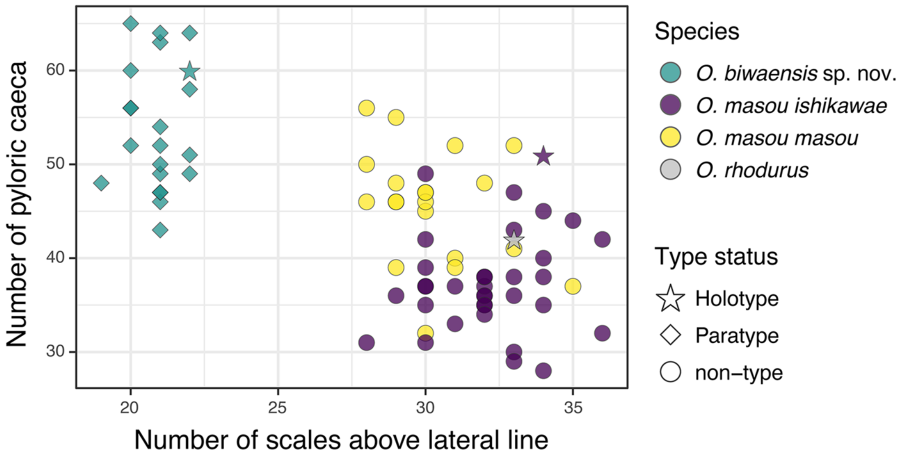

Fig. 2 Relationships between numbers of pyloric caeca and transverse scales above lateral line in *Oncorhynchus biwaensis* sp. nov., *O. masou ishikawae*, *O. m. masou* and holotype of *O. rhodurus*

Oncorhynchus rhodurus (not of Jordan and McGregor *in* Jordan and Hubbs 1925): Kato 1973a: 108, tables 1, 2 (Lake Biwa); Kato 1973b: 232 (Lake Biwa); Kato 1975: 192, fig. 2c (Lake Biwa); Kato 1978a: 53, fig. 4 (Lake Biwa); Kato 1978b: 199, fig. 5B–D (Lake Biwa); Kato 1981: 185, fig. 2C (Lake Biwa) [a discrete population of a distinct species]

Salmo (*Oncorhnchus*) *masou macrostomus* f. *ishikawai* (not of Jordan and McGregor *in* Jordan and Hubbs 1925): Miyadi et al. 1976: 86, pl. 6 (Lake Biwa) [as distinct intraspecific population]

Oncorhynchus masou rhodurus (not of Jordan and McGregor *in* Jordan and Hubbs 1925): Araga 1984: 39, pl. 43 (A–D) (Lake Biwa); Kato 1985: 47, figs. 2C, 3E (Ado River); Araga 1988: 39, pl. 43 (A–D) (Lake Biwa); Takasaki et al. 1994: 10153 (molecular analysis; Same River, Shiga Pref.); Oohara and Okazaki 1996: 189 (molecular analysis; Lake Biwa); Murata et al. 1998: 1864 (molecular analysis; Lake Biwa) [as distinct subspecies]

Oncorhynchus rhodurus (not of Jordan and McGregor *in* Jordan and Hubbs 1925): Kato 1986: 3 (Lake Biwa); Kato 1987: 112, figs. C, D (Lake Biwa); Kato 1988: 5, fig. 2B (Ado River) [as distinct species]

Salmo (*Oncorhnchus*) *masou ishikawai* (not of Jordan and McGregor *in* Jordan and Hubbs 1925): Furukawa 1989: 180, photos (Lake Biwa) [as distinct subspecies]

Oncorhynchus masou subsp.: Kimura 1990: 11 (Lake Biwa); Hosoya 1993: 262, figs. (Lake Biwa); Kuwahara and Iguchi 1994: 496 (Ishida River, Shiga Pref.); Hosoya 2002: 304, figs. (Lake Biwa); Kuwahara and Iguchi 2007: 15 (molecular analysis; Chinai, Ishida and Anegawa rivers); Gwo et al. 2008: 776 (molecular analysis; cultivated stock Biwa salmon, Inland Station of National Research Institute of Aquaculture); Hsu et al. 2010: e316 (cultivated stock Biwa salmon); Kuwahara et al. 2012: 195 (molecular analysis; Lake Biwa); Tega et al. 2012: 51 (molecular analysis; cultivated stock Biwa salmon, Samegai Trout Farm, Shiga Pref.); Tabata et al. 2016: 2601 (molecular analysis; Lake Biwa); Kuwahara et al. 2019: 67(molecular analysis; as distinct subspecies; several streams inflowing to Lake Biwa) [as distinct subspecies]

Oncorhynchus sp.: Nakabo 2009: 313 (Lake Biwa); Hosoya 2013: 367, figs. (Lake Biwa); Kawase 2015: 252, photos (Lake Biwa); Amano et al. 2018: 799 (ecological note; Lake Biwa); Fujioka et al. 2024: 138 (Lake Biwa) [as distinct species]

Holotype. LBM 1210060173 (mtDNA accession No.: PV409899; SRA accession No.: SAMN47613509), young female, 151.4 mm SL, 50 m depth, off Takashima, Shiga Pref., Lake Biwa, Japan, 35.1°N, 135.9°E, bottom trawl, coll. by Y. Kawata and Y. Fujioka, 25 Feb. 2022.

Paratypes. LBM 1210060151, 1210060161, 1210060162–1210060166, 1210060169–1210060172 (PV409881, 409889–409893, 409895–409898; SAMN47613487, 47613497–47613502, 47613505–47613508), 112.2–189.3 mm SL, 11 young males, and LBM 1210060146, 1210060149, 1210060168 (PV409879, 409880, 409894; SAMN47613482, 47613485, 47613504), 215.2–241.0 mm SL, 3 young females, data same as holotype. FAKU 211998 and 211999 (PV409882, 409883; SAMN47613490, 47613491), 188.1–185.7 mm SL, young males, data same as holotype. NSMT-P 151065 and 151066 (PV409884, 409885; SAMN47613492, 47613493), 151.4–172.7 mm SL, young males, data same as holotype. OMNH-P 53412 (PV409886, SAMN47613494), 154.6 mm SL, young female, OMNH-P 53413 (PV409887, SAMN47613496), 155.8 mm SL, young male, data same as holotype.

Diagnosis. A new species of the *O. masou* species complex with the following combination of characters: fewer dorsal, anal, pectoral and pelvic fin-rays [14 (mode 14), 11–14 (13), 13–14 (14) and 9 (9), respectively]; fewer scales above and below lateral line [19–22 (21) and 21–24 (24),

Table 2 Measurements of *Oncorhynchus biwaensis* sp. nov., *O. masou masou*, and *O. m. ishikawae*

	Oncorhynchus biwaensis sp. nov.			*O. m. masou*		*O. m. ishikawae*		*O. ishikawae*	*O. rhodurus*
	Holotype	Paratypes						Holotype*	Holotype*
	LBM 1210060173	*n* = 20	Average	*n* = 20	Average	*n* = 33	Average	FMNH 58682	FMNH 59389
Standard length (mm)	151.4	112.2–241.0	163.9	92.2–190.2	132.2	99.6–165.7	125.6	151.2	446.0
As % SL									
Total length	117.7	116.2–122.3	119.1	107.6–123.2	118.8	105.8–121.1	118.7	120.2	115.0
Fork length	108.8	108.3–110.8	109.6	89.4–113.5	109.8	90.1–113.0	110.4		
Body depth	24.6	20.9–26.7	23.3	23.5–30.5	28.0	21.4–25.7	23.9	24.8	30.7
Body width	12.1	11.2–13.6	12.5	12.4–17.8	15.2	13.2–16.0	14.7		
Head length	23.9	22.9–25.6	24.0	21.8–28.7	27.0	23.3–29.3	27.5	27.1	33.9
Snout length	6.0	5.5–7.0	6.2	5.1–8.0	7.3	6.5–8.2	7.2	6.4	
Eyed diameter	5.3	4.2–6.3	5.2	4.5–6.5	5.7	5.4–7.3	6.5	5.6	3.9
Interorbital width	7.3	6.7–8.0	7.4	7.3–10.2	8.7	7.0–10.0	8.6	7.3	11.0
Upper jaw length	12.3	11.8–13.1	12.5	12.3–16.6	15.4	13.6–17.5	15.6	15.0	24.9
Postorbital length	7.9	7.2–8.4	7.6	6.6–9.2	8.1	5.6–8.5	7.7		
Postorbital body length	90.6	89.2–92.3	90.8	78.7–89.3	87.2	79.4–90.9	88.1		
Predorsal length	46.3	44.4–49.3	46.7	42.9–50.7	48.3	44.0–51.3	49.0		
Postorbital head length	13.4	12.8–14.6	13.6	11.5–16.7	14.8	12.2–16.1	14.7		
Longest dorsal fin length	14.8	12.3–14.8	13.8	13.6–19.9	16.6	13.5–19.0	17.2		
Longest anal fin length	9.0	9.0–12.5	10.4	11.7–16.4	13.9	11.2–15.1	13.8		
Scale length	0.9	0.8–1.1	1.0	0.6–1.0	0.8	0.5–1.0	0.7		
Scale width	0.6	0.6–0.8	0.7	0.4–0.8	0.6	0.3–0.8	0.5		
Scale length/width	1.47	1.26–1.64	1.48	1.16–1.42	1.30	1.28–1.54	1.37		
Scale length of apical area/basal area	1.26	0.99–1.45	1.21	0.95–1.48	1.16	1.01–1.38	1.20		
Pectoral fin length	16.1	14.5–16.7	15.5	14.2–20.5	18.1	14.5–18.8	17.1		
Pelvic fin length	11.4	10.7–13.1	12.0	12.3–16.2	14.2	12.1–15.0	14.0		
Caudal fin length	23.3	20.4–26.5	23.4	20.4–25.1	23.1	18.2–24.7	22.3		
Caudal peduncle length	16.2	14.0–18.4	17.1	14.2–17.9	16.2	16.4–19.3	17.8		
Caudal peduncle depth	8.5	7.6–9.0	8.1	8.1–10.9	10.0	8.3–10.3	9.5	8.5	10.4

*These values calculated followed Jordan and Hubbs (1925) and Kimura (1990)

respectively)]; 43–65 (56) pyloric caeca (Fig. 2); 0–15 (0) red spots laterally on body less than ca. 20 cm SL; body particularly slender in immature juveniles, body depth 20.9–26.7% of SL (average 23.3%); snout short, moderately pointed, length 5.5–7.0% of SL (6.2%); eye small, diameter 4.2–6.3% of SL (5.2%); interorbit narrow, width 6.7–8.0% of SL (7.4%). Scales oblong, length/width ratio 1.26–1.64 (1.48); focus slightly forward of center, length of apical area/length of basal area 0.99–1.45 (1.21); ridges circular, not disappearing in apical area (Fig. 3a).

Description of young individuals and immature large males and females (holotype and paratypes). Counts and measurements shown in Tables 1 and 2. Body fusiform, but compressed laterally. Body fusiform, but compressed laterally. Head and snout rather short, interorbital rather narrow, upper jaw rather short. Predorsal length rather short, postdorsal length relatively short. Longest dorsal fin and longest anal fin relatively short. Pectoral and pelvic fin relatively short. Pectoral fin relatively short. Pelvic fit relatively short. Scale relatively oblong. Caudal peduncle relatively shallow.

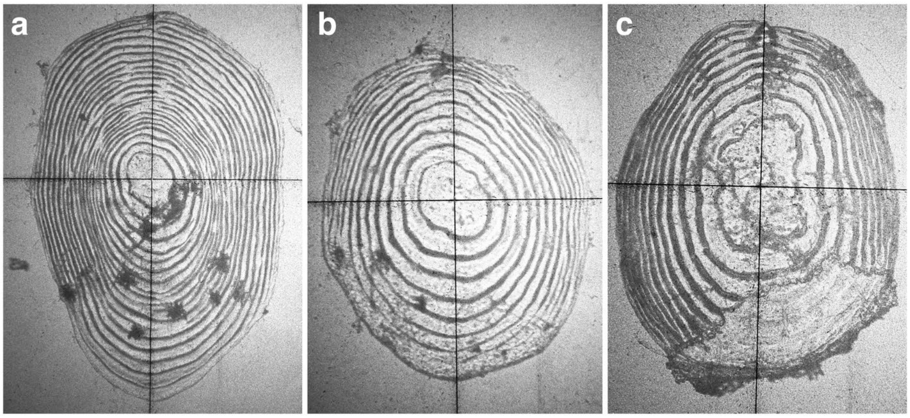

Fig. 3 Scales of species of *Oncorhynchus*: **a** *O. biwaensis* sp. nov., LBM 1210060173 (151.4 mm SL), holotype; **b** *O. masou masou*, LBM 1210060216 (105.7 mm SL); **c** *O. m. ishikawae*, LBM 1210060180 (120.2 mm SL)

Description of mature males and females. Body compressed well laterally. Head shape sexually dimorphic; jaws longer in males than in females, upper jaw convex, tip of upper jaw curved downward and tip of lower jaw curved upward in males.

Color of young individuals and immature large male and females when fresh (holotype and paratype) (Fig. 1a). Body silvery, with small dark spots scattered dorsally; parr marks indistinct. Pectoral fin faint transparent with faint dark uppermost margin. Pelvic fin whitish transparent with faint dark distal margin. Dorsal fin semi-transparent, with faint dark dorsal margin and without black tip. Anal fin whitish transparent with faint dark distal margin. Caudal fin semi-transparent with dark posterior margin, some dark spots on uppermost margin.

Color of young individuals and immature large males and females in spirits (Fig. 1b). Body dark brown, with small dark spots scattered dorsally, otherwise pale, with 7–11 parr marks. Pectoral fin semi-transparent, with faint dark dorsal margin. Pelvic fin semi-transparent, with faint dark anteriorly. Dorsal fin semi-transparent faint dark without black tip. Anal fin semi-transparent with faint dark at base. Caudal fin semi-transparent faint dark with darker posterior margin, some dark spots on uppermost margin.

Color of mature males (Fig. 1c) **and females** (Fig. 1d). Head, pectoral fin, pelvic fin and anal fin more brackish in males than in females. Body blackish brown with transverse cloud like pinkish or purplish markings on lateral side in both sexes.

Molecular characteristics. Partial mtDNA sequences could not be obtained from some individuals (Fig. 4d), which were therefore excluded from the type series (only specimens for which both partial mtDNA and nuclear genome-wide data were available are proposed as type specimens). All individuals identified as Biwa salmon on the basis of their external morphology and used in this study, were characterized by the mtDNA haplotypes detected in Biwa salmon by Tabata et al. (2016) (Fig. 5). The Holotype had the haplotype No.1. As a result of the nuclear genome-wide analysis, both the holotype and paratypes were recognized as belonging to the pure Biwa salmon cluster (Fig. 4a, d). While a very small amount of ancestry applicable to other species was detected in some individuals, these individuals also exhibited relatively high rates of missing data (Fig. 4d). Since missing data can lead to incorrect hybridization signals in clustering analysis (Yi and Latch 2022), we interpreted these signals as artifacts. Therefore, no individuals were suspected of resulting from interspecific hybridization.

Habitat. The type specimens were collected from the middle and bottom layers of Lake Biwa at depths between 40 m and 90 m.

Etymology. The specific name "*biwaensis*" referred to Lake Biwa, the original habitat of the new species.

Comparisons

Morphology. *Oncorhynchus biwaensis* sp. nov. have fewer scales above the lateral line [19–22 (mode 21)] than *O. masou masou* [28–35 (29)] and *O. m. ishikawae* [28–36 (32)], fewer scales below the lateral line [21–24 (24) vs 27–34 (32) and 26–34 (31), respectively], and more pyloric caeca [43–65 (56) vs 32–56 (46) and 28–49 (36), respectively] (Fig. 2; Table 1).

Proportionally (Table 2), Biwa salmon have a shorter head [22.9–25.6% of SL (average 24.0%)] than Masu salmon [21.8–28.7% of SL (27.0%)] and Amago salmon [23.3–29.3% of SL (27.5%)], shorter snout [5.5–7.0% of SL (6.2%) vs 5.1–8.0% of SL (7.3%) and 6.5–8.2% of SL (7.2%), respectively], narrower interorbital [width 6.7–8.0% of SL (7.4%) vs 7.3–10.2% of SL (8.7%) and 7.0–10.0% of SL (8.6%), respectively], shallower body [depth 20.9–26.7% of SL (23.3%) vs 23.5–30.5% of SL (28.0%) and 21.4–25.7% of SL (23.9 %), respectively], and a scale oblong length/width ratio 1.26–1.64 (1.48) compared with almost round

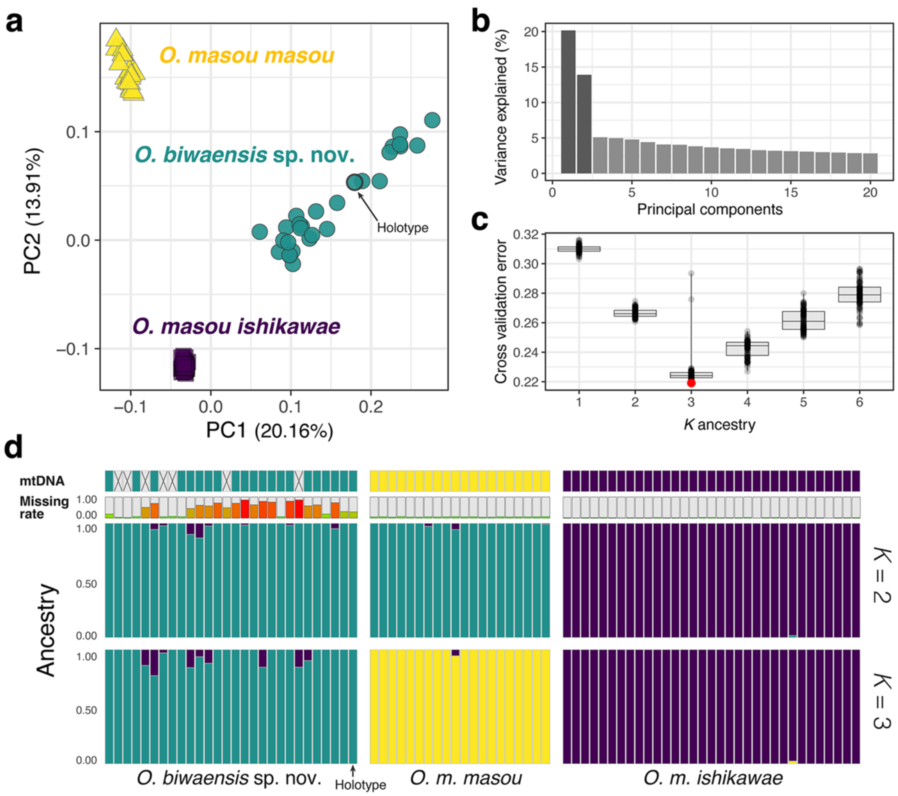

Fig. 4 Results of genetic analyses of species of *Oncorhynchus* based on genome-wide SNP data (1,654 SNPs) obtained by MIG-seq. **a** Principal component analysis; **b** bar plot of explained variance for each principal component (top two components shown in **a**); **c** comparison of CV-errors based on 100 replicates of ADMIXTURE analysis (output of run with smallest CV-error for each K value shown in **d**); **d** ancestry bar plots of ADMIXTURE analysis with $K = 2, 3$ (top two rows show results of mtDNA-based species delimitation and missing rate of SNP data)

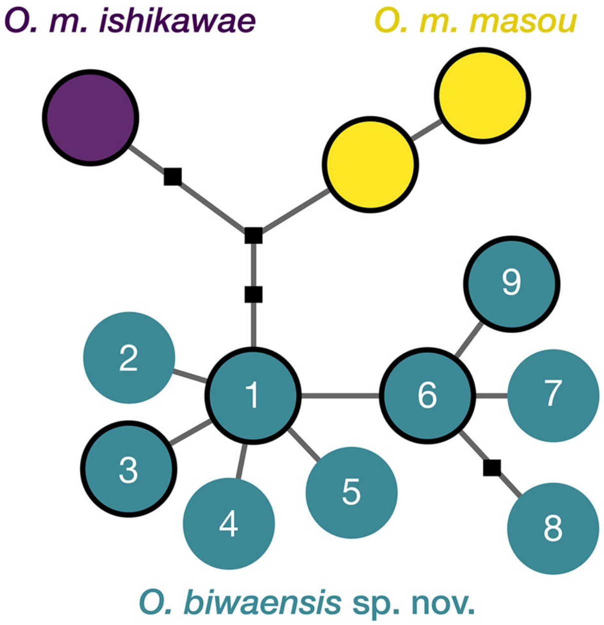

Fig. 5 Statistical parsimony network of mtDNA cytochrome *b* haplotypes from species of *Oncorhynchus* in this study and Tabata et al. (2016). *Circles* represent detected haplotypes and *squares* represent missing haplotypes. *Black-bordered circles* represent haplotypes detected in this study. Each branch indicates a single substitution. The numbers on the circles represent the haplotype names; Nos. 1 to 8 were identified in Tabata et al. (2016) and No. 9 is a newly identified haplotype in this study

scales [length/width ratio 1.16–1.42 (average 1.30) and 1.28–1.54 (1.37), respectively].

The scale focus, located slightly forward of center, apical area length/basal area length [0.99–1.45 (average 1.21)] and circular ridges present in the apical area in Biwa salmon (Fig. 3a) also contrast with scale characteristics in Masu and Amago salmon, the foci being located almost centrally in both latter species, the apical area length/basal area length being 0.95–1.48 (1.16) and 1.01–1.38 (1.20), respectively, in both, and circular ridges partly absent near the apical area in both (Fig. 3b, c).

Molecular characters. All individuals identified as Amago salmon and Masu salmon on the basis of their external morphology plus specific habitat had mtDNA haplotypes consistent with such identifications. Nuclear genome-wide analysis also revealed an absence of hybrid individuals. The mtDNA and nuclear genome analyses also supported the clear genetic differentiation between Biwa salmon, Amago salmon, and Masu salmon (Fig. 4).

Discussion

Species differentiation. The differences between Biwa salmon from Amago salmon and Masu salmon shown here did not contradict the previous morphology-based (Kato

1973a, 1978a, b, 1981, 1986, 1987) and molecular-based decisions regarding identity (Oohara and Okazaki 1996; Gwo et al. 2008; Tega et al. 2012; Tabata et al. 2016; Iwatsuki et al. 2019; Kuwahara et al. 2019; Yamamoto et al. 2020). The following discussion emphasizes previously recognized specific or subspecific differences.

Tega et al. (2012) found no genetic mixing due to hybridization between Biwa salmon (a wild population in Anegawa river, Shiga Prefecture, and cultured population at Shiga Prefectural Samegai Trout Farm) and Amago salmon (3 wild populations in Okayama Prefecture, and 2 cultured populations in Gifu Prefecture and at Shiga Prefectural Samegai Trout Farm), therefore stating that the two salmon species were clearly separated according to the DNA marker analysis.

Although Biwa salmon has been essentially reproductively isolated from Amago salmon in the Chinai, Ukawa, Otani, and Okawa Rivers in the Lake Biwa catchment, some hybrids have been found between the two, although characterized by low reproductive success (Kuwahara et al. 2019). However, Biwa salmon were found to have hybridized with Amago salmon originating from released fishes cultured at Shiga Prefectural Samegai Trout Farm (from parental stock transplanted from Gifu Prefecture) in upstream dams on the Aratani and Kawachidani Rivers (Lake Biwa catchment), resulting in a hybrid swarm (Kuwahara et al. 2019). Such hybridization implies different operating factors compared to the subspecific relationship between Masu salmon and Amago salmon resulting in hybrid swarms between them (see below) (Yamazaki et al. 2005; Kitanishi et al. 2017).

Amago salmon and Masu salmon, widely recognized as related subspecies because of their neighboring geographic distributions, resemble each other in both ecological and morphological aspects (Fujioka 2018), in addition to the presence of hybrid swarms of the two in the Jinzu River, Toyama Prefecture (Yamazaki et al. 2005), three rivers of the former river system in Gifu Prefecture, and the Kugami River, Tottori Prefecture (Kitanishi et al. 2017), due to the artificial introduction from their original habitat. As such, they have become good examples of subspecies categorization sensu "the Biological species concept" (see Mayr 1942).

Both Amago salmon and Masu salmon migrate upstream three or four months before the breeding season in spring (Nakamura 1963; Furukawa 1989; Mayama and Kimura 1989). However, majority of Biwa salmon migrate upstream just before the breeding season in autumn (Miyadi et al. 1963, 1976; Nakamura 1963; Kato 1978b; Furukawa 1989), and rarely in streams flowing into the lake in summer (Kuwahara and Iguchi 2007), resulting in the latter species being reproductively isolated from the others.

In Lake Biwa and its associated streams, Biwa salmon differ biologically from Amago salmon in age at maturity, growth rate and food choice (Kato 1973b, 1978b; Fujioka and Uenishi 2006). The former also differ physiologically from Amago salmon in lacking seawater adaptability (Fujioka 1988b; Fujioka and Fushiki 1989a; Nakajima et al. 2014), which further indicates a long period of reproductive isolation in the lake.

Regarding the speciation history among the *Oncorhynchus masou* complex, Tabata et al. (2016) demonstrated a Bayesian phylogenetic tree for the four taxa based on mtDNA 16S rRNA, CO1, ND5, cyt*b*, and CR regions. Accordingly, Biwa salmon was divided from the others, with Iwatsuki et al. (2020) similarly confirming the clear separation of Biwa salmon after examining 36 haplotypes of complete cyt*b* genes (1,141 bp). Yamamoto et al. (2020) also showed Biwa salmon to be well separated from the other taxa of the complex, based on both the cyt*b* and ND5 regions. Our results also support the clear separation of Biwa salmon from other species in genome-wide SNP data.

Accordingly, Biwasalmon is here described as a new distinct species, rather than a new subspecies of *O. masou*.

Scientific names. Biwa salmon has often been identified as a subspecies of *Oncorhynchus rhodurus*, or infraspecific form of *O. masou* (see synonym list), following Oshima's (1929) account. The species has also been referred to *Salmo perryi*, *Salmo* (*Oncorhynchus*) *masou macrostomus*, *Salmo* (*Oncorhynchus*) *masou ishikawai*, and *Oncorhynchus macrostomus* (see synonym list).

Kimura (1990) examined specimens related to the *O. masou* species complex, including holotypes of *Salmo macrostoma* (BMNH 1879.5.14), *Oncorhynchus ishikawae* (FMNH58682), *O. rhodurus* (FMNH 59389) and the specimen (FMNH 58687) identified as *O. macrostomus* by Jordan and McGregor *in* Jordan and Hubbs (1925), and identified the holotype of *S. macrostoma* as Masu salmon (originally described as *Salmo masou*) based on a specimen from Hakodate (Hokkaido) depicted in a color plate (pl. 9, fig. 2) (Brevoort 1856). However, no types of the latter are known. The holotype of *O. rhodurus* was not consistent with Biwa salmon due to having lower numbers of pyloric caeca, and higher numbers of scales above and below the lateral line (Fig. 2), instead being identified as a junior synonym of *O. masou* or *O. ishikawae*. The holotype of *O. ishikawae* was consistent with Amago salmon in the numbers of pyloric caeca, and scales above and below the lateral line, and FMNH 58687 was considered similarly (Fig. 2). Therefore, Kimura (1990) concluded that Masu salmon, Amago salmon, and Biwa salmon were *O. masou masou*, *O. m. ishikawae* and *O. m.* subsp., respectively, with Jordan and McGregor's "*O. macrostoma*" also being Biwa salmon. Biwa salmon has remained an unidentified subspecies or species since that account.

Kato (1978b) pointed out that Biwa salmon differed from Amago salmon in numbers of pyloric caeca and transverse row scales, and later (Kato 1986) provided a figure showing the relationship between numbers of pyloric caeca and scales above the lateral line, which clearly differentiated Biwa salmon, Masu salmon and Amago salmon. Figure 2, which also shows the relationship between numbers of pyloric caeca and scales above the lateral line, clearly separates Biwa salmon from Masu salmon and Amago salmon. In addition, plots for the holotypes of *O. rhodurus* and *O. m. ishikawae* (following Kimura 1990) indicate that both specimens fall into the clusters of *O. m. masou* and *O. m. ishikawae*. Therefore, Biwa salmon is conspecific with neither *O. rhodurus* nor *O. m. ishikawae*. In addition, the holotype of *O. ishikawae* had a black tipped dorsal fin (pl. IV-fig. 1 in Jordan and Hubbs 1925), indicating marine adaptability (Kubo 1974; Fujioka 1987), unlike "Biwa salmon" (see Fig. 1b).

The holotype of *O. ishikawae* (FMNH58682), was collected from Lake Biwa by Chiyomatsu Ishikawa and YojiroWakiya, during the visit of D. S. Jordan to Japan in 1922 (Jordan and Hubbs 1925). However, Oshima (1936) noted that Yojiro Wakiya had stated that the holotype was not collected from Lake Biwa but by himself from Miyako Bay (Miyagi Prefecture), facing the Pacific Ocean. Because of this, Oshima (1936) stated that the holotype of *O. ishikawae* should be identified as *Oncorhynchus masou* (Brevoort 1856). However, the specimen had a number of white spots (red when fresh) laterally on the body (Jordan and Hubbs 1925; Kimura 1990). Furthermore, no record of a specimen collected from Miyako Bay, Miyagi Prefecture by Y. Wakiya was included in Jordan and Hubbs (1925). Because no specimens of "Amago salmon" have been recorded from Miyako Bay, it is likely that the above assertion in Oshima (1936) was a memory lapse.

Comparative material examined. *Oncorhynchus masou ishikawae*, LBM 1210060174–1210060206 (mtDNA accession No.: PV409920–409952; SRA accession No.: SAMN47613530–47613562), 19 males and 14 females, 99.6–165.7 mm SL, Genta dani, Kozagawa River, Wakayama Pref., Japan, fishing, coll. by M. Kuwahara, Y. Takahashi and R. Uchiyama, 3–5 Aug. 2022. *Oncorhynchus masou masou*, LBM 1210060207–1210060226 (PV409900–409919; SAMN47613510–47613529), 18 males and 2 females, 92.2–190.2 mm SL, Kurumigahara River, Jinzu River system, Toyama Pref., Japan, electric fishing, coll. by Y. Tago, N. Nanjyo and K. Nomura, 7 Oct. 2022.

Acknowledgements We are grateful to Yasuhiko Tago, Nobuaki Nanjyo, Koji Nomura (Toyama Prefectural Agricultural, Forestry and Fisheries Research Center, Fisheries Research Institute), Yoshiaki Takahashi and Yoshikazu Nakata (Wakayama Pref.), Ayuto Matsuura (Kochi Pref.), Hiroshi Tsuchiya (Hokkaido Pref.), Ryu Uchiyama (Nature Photographer), Akira Yanagawa (Japan Water Agency), Hozi Iwatani (Fukui Prefectural Inland Water General Center), Ryuji Yasuda (SAKURAMASU RESTORATION), Takahisa Matsuura (Japan Fisheries Co-operative JF-Fukui), Ryoichi Oohashi and Osamu Oohasi (Gifu City), and Yoshimitsu Kawata (Ohmi Hachiman City) for aid in the collection of specimens, and to Seigo Kawase and Takehiro Deguchi (LBM), Mizuki Matsunuma (FAKU), Masanori Nakae (NSMT) and Shoko Matsui (OMNH) for registering the specimens examined. Graham S. Hardy for his critical reviewing of the manuscript. This study was supported by the 36th Takara Harmonist Fund research grant, and in part by JSPS KAKENHI Grant Number 21K14869 and 23K05899 for R. Tabata. Lastly, we deeply thank the late Katsuhiko Yoshiyasu (Osaka City) and late Jin Kondo (Nagoya City) for their encouragement of this study.

Declarations

Conflicts of interest There were no conflicts of interest relevant to this study.

Ethics approval All applicable institutional and national guidelines for the care and use of animals were followed. The survey and all experiments conducted complied with the current laws of Japan. All examined specimens were registered in the fish collections of LBM, FAKU, NSMT and OMNH.

References

Alexander DH, November J, Lange K (2009) Fast model-based estimation of ancestry in unrelated individuals. Genome Res 19:1655–1664

Amano Y, Kuwahara M, Takahashi T, Shirai K, Yamane K, Kawakami T, Yokouchi K, Amakawa H, Otake T (2018) Low-fidelity homing behaviour of Biwa salmon *Oncorhynchus* sp. landlocked in Lake Biwa as inferred from otolith elemental and Sr isotopic compositions. Fish Sci 84:799–813

Aoyagi H (1957) General notes on the freshwater fishes of the Japanese Archipelago. Taishukan Shoten, Tokyo

Aoyama S, Watanabe S, Ishikawa S, Nishida M, Tsukamoto K (2000) Are morphological characters distinctive enough to discriminate between two species of freshwater eels, *Anguilla celebesensis* and *A. interioris*? Ichthyol Res 47:157–161

Araga C (1984) *Oncorhynchus masou rhodurus* Jordan and McGregor. In: Masuda H, Amaoka K, Araga C, Uyeno T, Yoshino T (eds) The fishes of Japanese Archipelago. Tokai University Press, Tokyo, p 39

Araga C (1988) *Oncorhynchus masou rhodurus* Jordan and McGregor. In: Masuda H, Amaoka K, Araga C, Uyeno T, Yoshino T (eds) The fishes of Japanese Archipelago. 2nd ed. Tokai University Press, Tokyo, p 39

Brevoort JC (1856) Notes on some figures of Japanese fish taken from recent specimens by the artists of the U. S. Japan Expedition. In: Perry MC (ed) Narrative of the Expedition of an American Squadron to the China Seas and Japan, performed in the years 1852, 1853, and 1854 under the command of Commodore M. C. Perry, United States Navy, by order of the Government of the United States. Vol. 2. U.S. Senate Ex. Doc. No. 79. 33rd Congress. 2nd Session. Beverley Tucker, Washington, D.C., pp 253–288, pls 3–12

Chen S, Zhou Y, Chen Y, Gu J (2018) fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884–i890

Christensen KA, Flores A-M, Joshi J, Shibata K, Fujimoto T, Koop BF, Devlin RH (2025) Masu salmon species complex relationships and sex chromosomes revealed from analyses of the masu salmon (*Oncorhynchus masou masou*) genome assembly. G3 15:jkae278

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9:1657–1659

Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST, McVean G, Durbin R, 1000 Genomes Project Analysis Group (2011) The variant call format and VCFtools. Bioinformatics 27:2156–2158

Fujioka Y (1987) Parr-smolt transformation in biwa salmon *Oncorhynus rhodurus* reared in pond. Nippon Suisan Gakkaishi 53:253–260 [in Japanese with English summary]

Fujioka Y (1988a) Morphological changes associated with growth and parr-smolt transformation in biwa salmon *Oncorhynchus rhodurus* and amago salmon *Oncorhynchus rhodurus*. Nippon Suisan Gakkaishi 54:77–86 [in Japanese with English summary]

Fujioka Y (1988b) Seawater tolerance of eye period eggs and juveniles in biwa salmon *Oncorhynchus rhodurus* and amago salmon *Oncorhynchus rhodurus*. Bull Shiga Pref Fish Exp Stat 41:10–13 [in Japanese]

Fujioka Y (1990) Biwa salmon: its ecological and morphological characteristics. Tec Rep Hokkaido Salmon Hatch 159:25–38 [in Japanese with English summary]

Fujioka Y (1991) Morphological, physiological and ecological studies on biwa salmon. Bull Shiga Pref Samegai Trout Farm 3:1–112 [in Japanese]

Fujioka Y (2018) *Oncorhynchus masou* species group. In: Nakabo T (ed) The natural history of the fishes of Japan. Shogakukan, Tokyo, pp 139–141 [in Japanese]

Fujioka Y, Fushiki S (1988) Downstream migration and body silvering in underyearling biwa salmon *Oncorhynchus rhodurus*. Nippon Suisan Gakkaishi 54:1889–1897 [in Japanese with English summary]

Fujioka Y, Fushiki S (1989a) Seasonal changes in hypoosmoregulatory ability of biwa salmon *Oncorhynchus rhodurus* and amago salmon *O. rhodurus*. Nippon Suisan Gakkaishi 55:1885–1892

Fujioka Y, Fushiki S (1989b) Physiological studies on parr-smolt transformation in biwa salmon. Physiol Ecol Japan Spec 1:489–496

Fujioka Y, Fushiki S, Tagawa M, Ogasawara T, Hirano T (1990a) Seasonal changes in plasma thyroxine levels in biwa and amago salmon reared in the pond. Nippon Suisan Gakkaishi 56:249–254

Fujioka Y, Fushiki S, Tagawa M, Ogasawara T, Hirano T (1990b) Downstream migratory behavior and plasma thyroxine levels of biwa salmon *Oncorhynchus rhodurus*. Nippon Suisan Gakkaishi 56:1773–1779

Fujioka Y, Katoh T, Fushiki S, Nakagawa H (1991) Changes in fatty acid composition associated with body silvering in biwa salmon *Oncorhynchus rhodurus* and amago salmon *O. rhodurus*. Nippon Suisan Gakkaishi 57:2313–2320

Fujioka Y, Kawase S, Tabata R (2024) The fishes of Lake Biwa. Sunrise Publishing, Hikone [in Japanese]

Fujioka Y, Uenishi M (2006) Changes in habitat and food with growth of biwa salmon. Bull Shiga Pref Fish Exp Stat 51:51–63 [in Japanese with English summary]

Furukawa T (1989) Biwamasu *Salmo* (*Oncorhynchus*) *masou ishikawai*. In: Kawanabe H, Mizuno N (ed) Freshwater fishes of Japan. Yama-Kei, Tokyo, pp 180–181 [in Japanese]

Günther A (1877) Preliminary notes on a new fishes collected in Japan. Ann Mag Nat Hist (Ser 4) 20:433–446

Gwo JC, Hsu TH, Lin KH, Chou YC (2008) Genetic relationship among four subspecies of cherry salmon (*Oncorhynchus masou*) inferred using AFLP. Mol Phylogenet Evol 48:776–781

Harada S, Jeon SR, Kinoshita I, Tanaka M, Nishida M (2002) Phylogenetic relationships of four species of floating gobies (*Gymnogobius*) as inferred from partial mitochondrial cytochrome *b* gene sequences. Ichthyol Res 49:324–332

Hikita T (1962) Ecological and morphological studies of the genus *Oncorhynchus* (Salmonidae) with particular consideration on phylogeny. Sci Rep Hokkaido Salmon Hatchery 17:1–97 [in Japanese with English summary]

Hosoya K (1993) Salmonidae. In: Nakabo T (ed) Fishes of Japan with pictorial keys to the species. Tokai University Press, Tokyo, pp 256–266, 1266 [in Japanese]

Hosoya K (2002) Salmonidae. In: Nakabo T (ed) Fishes of Japan with pictorial keys to the species. English edition. Tokai University Press, Tokyo, pp 299–304, 1473–1474

Hosoya K (2013) Salmonidae. In: Nakabo T (ed) Fishes of Japan with pictorial keys to the species. 3rd edition. Tokai University Press, Hadano, pp 299–367, 1833–1835 [in Japanese]

Hsu TH, Wang ZY, Takata K, Onozato H, Hara T, Gwo JC (2010) Use of microsatellite DNA and amplified fragment length polymorphism for Cherry salmon (*Oncorhynchus masou*) complex identification. Aquacul Res 41:e316–e325

ICZN (The International Commission on Zoological Nomenclature) (1999) International code of zoological nomenclature, 4th edition. Adopted by the General Assembly of the International Union of Biological Sciences. The International Trust for Zoological Nomenclature, London

Iwatsuki Y, Ineno T, Tanaka F, Tanahara K (2019) The southernmost population of *Oncorhynchus masou masou* from Kyushu Island, Japan and gross genetic structure of the *O. masou* complex from the northwestern Pacific. In: Gwo JC, Shieh YH, Burridge CP (eds) The proceedings of International Symposium on the 100th Anniversary of the discovery of the Formosa landlocked salmon. National Taiwan Museum Press, Taipei, pp 101–118

Iwatsuki Y, Tanaka F, Ineno T, Seki S, Kawashima N (2020) Biological characteristics of the six genetic groups of complete cytochrome *b* genes (1141 bp) on four subspecies of the *Oncorhynchus masou* complex and biogeographical genetic strains from northwestern Pacific by Japanese explanation with notes (Iwatsuki et al., 2019). Nat Kagoshima 47:5–16 [in Japanese]

Jordan DS, Hubbs CL (1925) Record of fishes obtained by David Starr Jordan in Japan, 1922. Mem Carnegie Mus 10:93–346, pls 5–12

Jordan DS, Oshima M (1919) *Salmo formosanus*, a new trout from the mountain streams of Formosa. Proc Acad Nat Sci Phila 71:122–124

Jordan DS, Snyder JO (1902) A review of the salmonid fishes of Japan. Proc U S Nat Mus 24:567–593

Jordan DS, Tanaka S, Snyder JO (1913) A catalogue of the fishes of Japan. J Coll Sci Imper Univ Tokyo 33:1–497

Jordan DS, Thompson WF (1914) Record of the fishes obtained in Japan in 1911. Mem Carnegie Mus 4:205–313

Katayama M, Fujioka Y (1966) On the salmonid fishes and their distribution in the river in Yamaguchi Prefecture. Bull Fac Edu Yamaguchi Univ 15:65–76 [in Japanese with English summary]

Kato F (1968) On the salmon (*Oncorhynchus rhodurus*) of the River Nagara. Research report of the Kisosansen Survey Team 5:895–903 [in Japanese]

Kato F (1973a) On the sea-run form of *Oncorhynchus rhodurus* obtained in Ise Bay, Japan. Jpn J Ichthyol 20:107–112 [in Japanese with English summary]

Kato F (1973b) Ecological study on the sea-run form of *Oncorhynchus rhodurus*, found in Ise Bay, Japan. Jpn J Ichthyol 20:225–234 [in Japanese with English summary]

Kato F (1975) On the distribution of a sea-run form of the salmonid, *Oncorhynchus rhodurus* found in southwestern Japan. Jpn J Ichthyol 21:191–197 [in Japanese with English summary]

Kato F (1978a) Lepidological study on the sea-run form of *Oncorhynchus rhodurus*. Jpn J Ichthyol 25:51–57 [in Japanese with English summary]

Kato F (1978b) Morphological and ecological studies on two forms of *Oncorhynchus rhodurus* found in Lake Biwa and adjoining inlet. Jpn J Ichthyol 25:197–204 [in Japanese with English summary]

Kato F (1981) The amago salmon, *Oncorhynchus rhodurus*, collected in Lake Biwa. Jpn J Ichthyol 28:184–186 [in Japanese with English summary]

Kato F (1985) A note on the scientific name and phylogeny of the Amago salmon. Bull Fukui Municipal Mus Nat Hist 32:47–54 [in Japanese with English summary]

Kato F (1986) Notes on the meristic and morphometric characters of *Oncorhynchus masou* group. Fukui Rikusui Seibutsu Kaihou [Fukui Freshwater Biology] 6:1–10 [in Japanese with English summary]

Kato F (1987) Notes on the morphology of smolts in the Masu salmon group and *Oncorhynchus ishikawai*. Bull Fukui Municipal Mus Nat Hist 34:105–113 [in Japanese with English summary]

Kato F (1988) Morphological studies on the juveniles of three species of the Masu salmon group. Fukui Rikusui Seibutsu Kaihou [Fukui Freshwater Biology] 7:4–13 [in Japanese with English summary]

Kawase S (2015) Biwa-masu *Oncorhynchus* sp. In: Hosoya K (ed) Freshwater fishes of Japan. Yama-Kei, Tokyo, pp 252–253 [in Japanese]

Kimura S (1990) On the type specimens of *Salmo macrostoma*, *Oncorhynchus ishikawae* and *O. rhodurus*. Bull Inst Zool Academia Sinica Suppl 29:1–16

Kitanishi S, Mukai T, Yamamoto T, Tago Y, Oda M (2017) Genetic introgression between masu salmon *Oncorhynchus masou masou* and red spotted masu salmon *Oncorhynchus masou ishikawae*. Nippon Suisan Gakkaishi 83:400–402 [in Japanese]

Kubo T (1974) Notes on the phase differentiation and smolt transformation of juvenile masu salmon (*Oncorhynchus masou*). Sci Rep Hokkaido Salmon Hatch 28:9–26 [in Japanese with English summary]

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549

Kuwahara M (2013) Present status and conservation of the endemic subspecies of *Oncorhynchus masou* (Biwa salmon) in Lake Biwa. Jpn J Ichthyol 60:63–67 [in Japanese]

Kuwahara M, Iguchi K (1994) Occurrence of mature stream resident males of Biwa salmon, *Oncorhynchus masou* subsp. Jpn J Ichthyol 40:495–497 [in Japanese with English summary]

Kuwahara M, Iguchi K (2007) Occurrence of summer upstream migration in Biwa salmon (*Oncorhynchus masou* subsp.). Jpn J Ichthyol 54:15–20 [in Japanese with English summary]

Kuwahara M, Takahashi H, Kikko T, Kurumi S, Iguchi K (2012) Introgression of *Oncorhynchus masou* subsp. (Biwa salmon) genome into lake-run *O. m. ishikawae* (Amago salmon) introduced into Lake Biwa, Japan. Ichthyol Res 59:195–201

Kuwahara M, Takahashi H, Kikko T, Kurumi S, Iguchi K (2019) Trace of outbreeding between Biwa salmon (*Oncorhynchus masou* subsp.) and amago (*O. m. ishikawae*) detected from the upper reaches of inlet streams within Lake Biwa water system, Japan. Ichthyol Res 66:67–78

Matsubara K (1955) Fish morphology and hierarchy. Ishizeki Shoten, Tokyo [in Japanese]

Mayama H, Kimura S (1989) Sakuramasu and Yamame *Salmo* (*Oncorhynchus*) *masou masou*. In: Kawanabe H, Mizuno N (eds) Freshwater fishes of Japan. Yama-Kei, Tokyo, pp 156–179 [in Japanese]

Mayr E (1942) Systematics and the origin of species. Columbia University Press, New York

Miyadi D, Kawanabe H, Mizuno N (1963) Colored illustrations of the freshwater fishes of Japan. Hoikusha, Osaka [in Japanese]

Miyadi D, Kawanabe H, Mizuno N (1976) Colored illustrations of the freshwater fishes of Japan. New edition. Completely revised. Hoikusha, Osaka [in Japanese]

Murata S, Tanasaki N, Okazaki, Kobayashi T, Numachi K, Chang KH, Okada N (1998) Molecular evidence from short interspersed elements (SINEs) that *Oncorhynchus masou* (cherry salmon) is monophyletic. Can J Fish Aquat Sci 55:1864–1870

Nakabo T (2002) Introduction to Ichthyology. In: Nakabo T (ed) Fishes of Japan with pictorial keys to the species. English edition. Tokai University Press, Tokyo, pp xxi–xlii

Nakabo T (2009) Zoogeography of Taiwanese fishes. Korean J Ichthyol 21:311–321

Nakabo T (ed) (2018) The natural history of the fishes of Japan. Shogakukan, Tokyo [in Japanese]

Nakajima T, Shimura H, Yamazaki M, Fujioka Y, Ura K, Hara A, Shimizu M (2014) Lack of hormonal stimulation prevents the landlocked biwa salmon (*Oncorhynchus masou* subspecies) from adapting to seawater. Am J Physiol Regul Integr Comp Physiol 307:R414–R425

Nakamura M (1963) Key to the freshwater fishes of Japan fully illustrated in colors. Hokuryukan, Tokyo [in Japanese]

Ohno I, Ando J (1931) Sakezoku gryorui no uroko no kannsatsu [Observation of scales of *Oncorhynchus* fishes]. Suisangaku Zasshi [Journal of Fisheries] 34:14–23 [in Japanese]

Okada Y, Nakamura M (1948) Nippon no tansuigyorui [Freshwater fishes of Japan]. Nippon Shuppansha, Osaka [in Japanese]

Onuki K, Fuke Y (2022) Rediscovery of a native freshwater shrimp, *Neocaridina denticulate*, and expansion of an invasive species in and around Lake Biwa, Japan: genetic and morphological approach. Conserv Genet 23:967–980

Oohara I, Okazaki T (1996) Genetic relationship among three subspecies of *Oncorhynchus masou* determined by mitochondrial DNA sequence analysis. Zoolog Sci 13:189–198

Oshima M (1929) Yamame oyobi Amago no keitou narabini seikatsushi ni kansuru nisanno chiken [Notes on the phylogeny and life history of Yamame and Amago]. Shizenkagaku [Natural Science] 4:129–150 [in Japanese]

Oshima M (1930) Yamame oyobi Amago no bunpukyoukaisen ni tuite [Note on the biogeographical boundary of Yamame and Amago]. Chirigaku Hyoron [Geographical Review of Japan Series A] 6:1186–1208 [in Japanese]

Oshima M (1936) Sakuramasu narabini Biwamasu no gakumei ni tuite [Note on the scientific names of Sakuramasu and Biwamasu]. Sakemasu Iho [Report on salmonids] 8(29):7–17 [in Japanese]

Oshima M (1957) Studies on the dimorphic salmons, *Oncorhynchus masou* (Brevoort) and *Oncorhynchus rhodurus* Jordan & McGregor, found in Japan and adjacent territories. Nire Shobo, Sapporo [in Japanese]

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, De Bakker PIW, Daly MJ, Sham PC (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81:559–575

R Core Team (2023) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Rochette NC, Rivera-Colón AG, Catchen JM (2019) Stacks 2: analytical methods for paired-end sequencing improve RADseq-based population genomics. Mol Ecol 28:4737–4754

Sahlin K (2022) Strobealign: flexible seed size enables ultra-fast and accurate read alignment. Genome Biol 23:260

Suyama Y, Hirota SK, Matsuo A, Tsunamoto Y, Mitsuyuki C, Shimura A, Okano K (2022) Complementary combination of multiplex high-throughput DNA sequencing for molecular phylogeny. Ecol Res 37:171–181

Suyama Y, Matsuki Y (2015) MIG-seq: an effective PCR-based method for genome-wide single-nucleotide polymorphism genotyping using the next generation sequencing platforms. Sci Rep 5:16963

Tabata R, Kakioka R, Tominaga K, Komiya T, Watanabe K (2016) Phylogeny and historical demography of endemic fishes in Lake

Biwa: the ancient lake as a promoter of evolution and diversification of freshwater fishes in western Japan. Ecol Evol 6:2601–2623

Takasaki N, Murata S, Saitoh M, Kobayashi T, Park L, Okada N (1994) Species-specific amplification of tRNA-derived short interspersed repetitive elements (SINEs) by retroposition: a process of parasitization of entire genomes during the evolution of salmonids. Proc Natl Acad Sci U S A 91:10153–10157

Tega T, Noguchi D, Sano T, Tanaka H, Kuwada T, Mito T, Nakajima M, Taniguchi N (2012) Evaluation of genetic diversity and genetic relationship between red spotted masu trout *Oncorhynchus masou ishikawae* and Biwa salmon *O. m.* subsp. Fish Genet Breed Sci 41:51–61 [in Japanese with English summary]

Watanabe K, Tabata R, Nakajima J, Kobayakawa M, Mastuda M, Takaku K, Hosoya K, Ohara K, Takagi M, Jang-Liaw N (2020) Large-scale hybridization of Japanese populations of Hinamoroko, *Aphyocypris chinensis*, with *A. kikuchii* introduced from Taiwan. Ichthyol Res 67:361–374

Yamamoto S, Morita K, Kikko T, Kawamura K, Sato S, Gwo JC (2020) Phylogeography of a salmonid fish, masu salmon *Oncorhynchus masou* subspecies-complex, with disjunct distributions across the temperate northern Pacific. Freshw Biol 65:698–715

Yamazaki Y, Shimada N, Tago Y (2005) Detection of hybrids between masu salmon *Oncorhynchus masou masou* and amago salmon *O. m. ishikawae* occurred in the Jinzu river using a random amplified polymorphic DNA technique. Fish Sci 71:320–326

Yi X, Latch EK (2022) Nonrandom missing data can bias Principal Component Analysis inference of population genetic structure. Mol Ecol Resour 22:602–611