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Salmincola markewitschi or *S. carpionis* (Copepoda: Lernaeopodidae)? A requirement for taxonomic revision due to their high morphological variations

Ryota Hasegawa[1], **Hirotaka Katahira**[2] and **Itsuro Koizumi**[1, 3]

[1] Graduate School of Environmental Science, Hokkaido University, Sapporo, Hokkaido, Japan;

[2] Department of Environmental Science, School of Life and Environmental Science, Azabu University, Fuchinobe, Chuo-ku, Sagamihara, Kanagawa, Japan;

[3] Faculty of Environmental Earth Science, Hokkaido University, Sapporo, Hokkaido, Japan

Abstract: *Salmincola markewitschi* Shedko et Shedko, 2002 (Copepoda: Lernaeopodidae) is an ectoparasitic copepod mainly infecting the buccal cavities of white-spotted charr *Salvelinus leucomaenis* (Pallas) (Salmonidae). This species has only been recorded from Northeast Asia, where a morphologically similar congener *Salmincola carpionis* (Krøyer, 1837) is also distributed, using the same host species. These copepods are hard to distinguish from each other because of their similarities. We thus examined the newly collected specimens morphologically and genetically from five populations of white-spotted charr in Japan. Most of the specimens were morphologically consistent with *S. markewitschi* but showed great variations in the numbers of spines on the exopods of the antennae, shape of the maxilliped myxal palps, and the bulla diameter. Consequently, some specimens shared characteristics with *S. carpionis*. In addition to the mophological continuities, genetic analyses of 28S rDNA and COI mitochondrial DNA confirmed that all specimens belong to a single species. Further taxonomic revisions are required to draw conclusions of whether *S. markewitschi* is a valid species different from *S. carpionis*, by collecting samples from across their wide distributional ranges, such as Europe, North America, and Northeast Asia. A key to identification of species of *Salmincola* Wilson, 1915 occurring in Japan is also provided.

Keywords: Taxonomy, parasitic copepod, Salmonidae, 28S rDNA, COI, Japan

The genus *Salmincola* Wilson, 1915 is a group of ectoparasitic copepods commonly infecting salmonid fishes (Kabata 1969). Some of the species cause histopathological impact on their hosts and have been regarded as harmful parasites in hatcheries and fish farms (Gall et al. 1972, Sutherland and Wittrock 1985, Roberts et al. 2004, Ruiz et al. 2017, Neal et al. 2021). To date, 22 valid species have been recorded from the genus (Walter and Boxshall 2018) and most members of the genus have circumpolar distribution (Kabata 1969).

In Japan, the following five species have been recorded: *Salmincola californiensis* (Dana, 1852) (reported as *Salmincola yamame* Hoshina et Suenaga, 1954 in Hoshina and Suenaga 1954, Hoshina and Nishimura 1976, Nagasawa and Urawa 2002), *S. carpionis* (Krøyer, 1837) (Nagasawa et al. 1995, 1998, Nagasawa and Sakaki 2019), *S. stellata* Markevich, 1936 (Nagasawa and Urawa 1991, Nagasawa et al. 1994, Hiramatsu et al. 2001, Nagasawa et al. 2021), *S. edwardsii* (Olsson, 1869) (Nagasawa 2020a, Nagasawa and Kawai 2020, Nagasawa 2021, Hasegawa et al. 2022) and *S. markewitschi* Shedko et Shedko, 2002 (Shedko and Shedko 2002, Nagasawa 2020b, Nagasawa 2021, Nagasawa and Ishiyama 2021) (see below for the key to the species of the genus *Salmincola* in Japan, provided in this study).

Salmincola markewitschi was described in 2002 using the specimens recovered from the buccal cavities of white-spotted charr *Salvelinus leucomaenis* (Pallas) in the Kuril Islands, Northeast Asia (Shedko and Shedko 2002). This copepod has recently been found on the same host species in Japan (Nagasawa 2020b, Nagasawa and Ishiyama 2021), but the morphologically similar congener, *S. carpionis*, which has a circumpolar distribution (Kabata 1969) attaching to the same infection site of the same host species, is also known to occur in this region (Nagasawa et al. 1995, Nagasawa 2020b).

Due to their morphological and ecological similarities, these two species might have been previously mixed, as indicated by Nagasawa (2020b). According to Shedko and Shedko (2002), *S. markewitschi* can be distinguished from *S. carpionis* and other congeners by three main characters: (1) the distal end of the exopod of the antenna (as second

Address for correspondence: Ryota Hasegawa, Graduate School of Environmental Science, Hokkaido University, N10W5 Sapporo, Hokkaido, 060 0810, Japan. E-mail: ryotahase344922@gmail.com.

Table 1. Sampling sites and date of the copepods parasitic on *Salvelinus leucomaenis* (Pallas) in Japan.

Sampling date	Prefecture	Sites	No. of fish examined	Fork length range (mm)	Prevalence (%)	Intensity (mean)
September 2020	Hokkaido Island	Toraibetsu Brook, Bekanbe-ushi River, Akkeshi	13	141–347	31	1–2 (1.3)
July 2020		Sapporo Salmon Museum, Sapporo, Makomanai, Sapporo	53	310–414	93	1–11 (4.7)
June, July and October 2020		Shiodomari River, Hakodate	754	69–528	34	1–11 (1.6)
May 2020	Fukushima Pref.	Tagokura-high dam, Tadami River, Minamiaizu	1	501	100	4
June 2020	Toyama Pref.	Jo-gan-zi River, Toyama	1	458	100	13

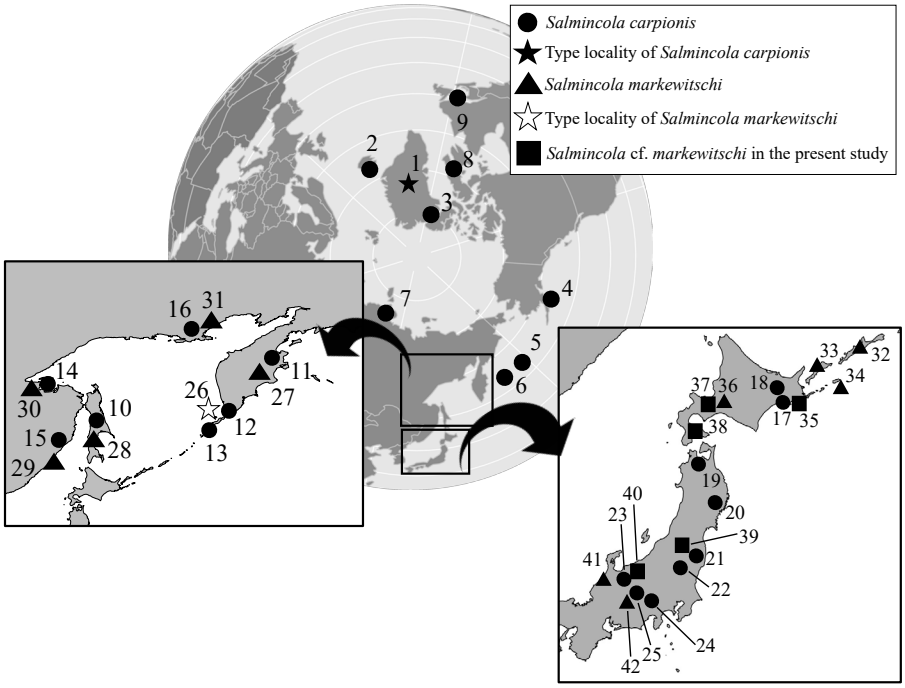

Fig. 1. Map of the known localities of *Salmincola carpionis* (Krøyer, 1837) and *S. markewitschi* Shedko et Shedko 2002. 1–25; *S. carpionis*. Based on Yamaguti (1939; reported as *Salmincola faculata*), Kabata (1969), Kumagai (1985; reported as *Salmincola* sp.), Nagasawa et al. (1995, 1997, 1998), Wakabayashi (1997), Yamamoto and Nagasawa (1999, 2001), Watanabe and Ishii (2000), Nagasawa and Urawa (2002), Shedko and Shedko (2002), Shedko et al. (2005a,b), Sokolov et al. (2012), Nagasawa and Ishikawa (2017), Kawanobe (2018, 2020), Nagasawa and Sakaki (2019). 26–42 – *S. markewitschi*. Based on Nishimura and Hoshina (1977; reported as *Salmincola californiensis*), Shedko and Shedko (2002), Shedko et al. (2005a, b), Denda and Ogawa (2011; reported as *Salmincola californiensis*), Sokolov et al. (2012), Nagasawa (2020b), Nagasawa and Ishiyama (2021), Nagasawa (2021), present study.
Salmincola carpionis: 1. Greenland (type locality); 2. Hrúta Fjord (described as Hrutafjordara in Kabata 1969), Iceland; 3. Etah, Greenland; 4. Alitak Bay, Alaska; 5. Attu, Alaska; 6. Bering Island; 7. Lake Taymyr (described as Lake Taimyr in Kabata 1969); 8. Baffin Island; 9. Quebec; 10. Sakhalin; 11. Kamchatka Peninsula; 12. Shumushu Island; 13. Onekotan Island; 14. Shantar Islands; 15. Primorye; 16. Magadan Region; 17. Toraibetsu Brook, Bekanbe-ushi River, Hokkaido; 18. Lake Panke, Hokkaido; 19. Aomori Prefecture; 20. Iwate Prefecture; 21. Fukushima Prefecture; 22. Tochigi Prefecture; 23. Toyama Prefecture; 24. Yamanashi Prefecture; 25. Nagano Prefecture.
Salmincola markewitschi: 26. Shumshu Island (type locality); 27. Kamchatka Peninsula; 28. Sakhalin Island; 29. Primorye; 30. Shantar Islands; 31. Magadan region; 32. Iturup Island; 33. Kunashir Island; 34. Shikotan Island; 35. Toraibetsu Brook, Bekanbe-ushi River, Hokkaido; 36, 37. Sapporo Salmon Museum (SSM), Hokkaido; 38. Shiodomari River, Hokkaido; 39. Fukushima Prefecture; 40. Toyama Prefecture; 41. Ishikawa Prefecture; 42. Nagano Prefecture.

antenna in Kabata 1969) has numerous small spines in addition to the two large papillae, whereas *S. carpionis* has no spines, (2) maxilliped palp has two overhanging outgrowths, whereas *S. carpionis* has only a single outgrowth on the maxilliped palp, and (3) bulla diameter is larger than those of other *Salmincola* spp. (Shedko and Shedko 2002). However, considering the high morphological variations in this genus (Kabata 1969, Fryer 1981), careful identification using a comprehensive approach with combining morphological and genetic analyses is required.

Here, we evaluated *S. markewitschi* and *S. carpionis* by examining morphological variations of newly collected specimens from various localities around Japan, together with a comparison of the nuclear 28S ribosomal DNA and mitochondrial cytochrome oxidase gene subunit I (COI) sequences. We also discuss the need for taxonomic reassessment of the genus using samples collected from all over the world to solve this taxonomic complexity, especially in the Northeast Asia.

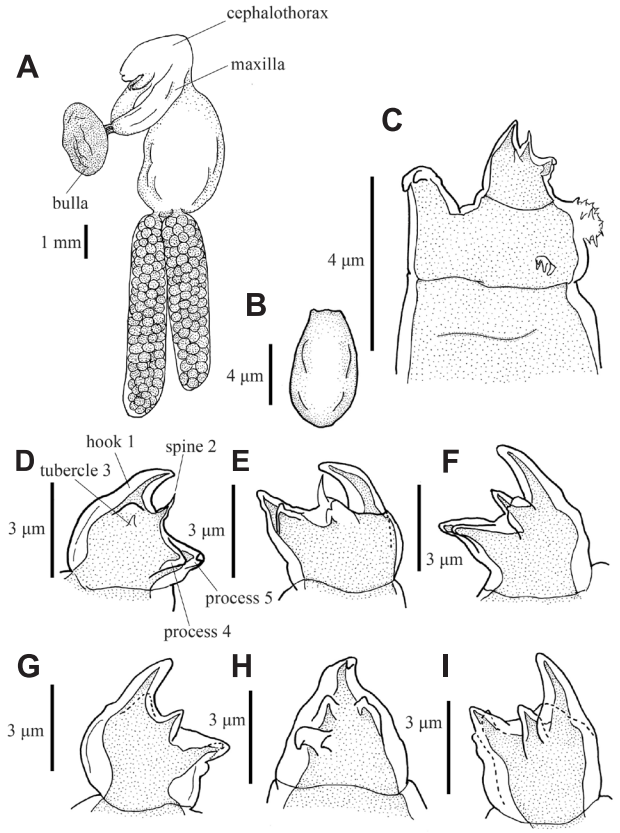

Fig. 2. Female *Salmincola* cf. *markewitschi* Shedko et Shedko 2002 from white-spotted charr *Salvelinus leucomaenis* (Pallas) at five sites in Japan. Abbreviations in parentheses represent places where specimens were collected as follows; Shiodomari River, southern Hokkaido (Shiodomari), Sapporo Salmon Museum, central Hokkaido (SSM), Bekanbe-ushi River, eastern Hokkaido (Bekanbe-ushi), Tadami River, Fukushima Prefecture (Fukushima), Jo-gan-zi River, Toyama Prefecture (Toyama).
A – entire, lateral (Shiodomari, ID17); **B** – cephalothorax, dorsal (Shiodomari, ID16); **C** – antenna, entire, lateral (Shiodomari, ID7); **D** – antenna, tip of endopod, lateral (Shiodomari, ID7); **E** – same, lateral (Shiodomari, ID1); **F** – same, lateral (SSM, ID15); **G** – same, lateral (Bekanbe-ushi, ID12); **H** – same, ventral (Fukushima, ID13); **I** – same, lateral (Toyama, ID10).

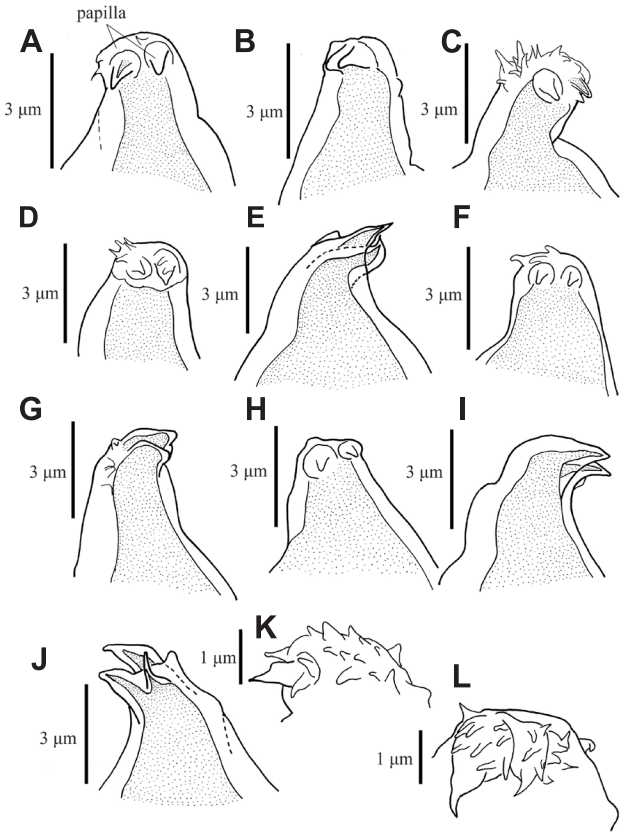

Fig. 3. Female *Salmincola* cf. *markewitschi* Shedko et Shedko 2002 from white-spotted charr *Salvelinus leucomaenis* (Pallas) at five sites in Japan. Abbreviations in parentheses represent places where specimens were collected as follows; Shiodomari River, southern Hokkaido (Shiodomari), Sapporo Salmon Museum, central Hokkaido (SSM), Bekanbe-ushi River, eastern Hokkaido (Bekanbe-ushi), Tadami River, Fukushima Prefecture (Fukushima), Jo-gan-zi River, Toyama Prefecture (Toyama).
A – antenna, tip of exopod, lateral (Shiodomari, ID4); **B** – same, lateral (Shiodomari, ID7); **C** – same, lateral (SSM, ID8); **D** – same, lateral (SSM, ID15); **E** – same, ventral (Bekanbe-ushi, ID12); **F** – same, lateral (Bekanbe-ushi, ID11); **G** – same, ventral (Fukushima, ID13); **H** – same, lateral (Fukushima, ID14); **I** – same, ventral (Toyama, ID9); **J** – same, dorsal (Toyama, ID10); **K** – spiny pad of endopod, lateral (Shiodomari, ID4); **L** – same, lateral (SSM, ID15).

MATERIALS AND METHODS

Fish and copepods collection

Host fish were caught from five sites (four rivers and one aquarium) from three prefectures (Hokkaido, Fukushima and Toyama) in Japan (Table 1, Fig. 1). At the four rivers, wild fish were caught by angling and electrofishing. Found copepods were carefully removed by forceps and preserved in vials filled with 70% ethanol. The aquarium samples were collected from the Sapporo Salmon Museum (hereafter, SSM following Nagasawa 2021), Makomanai, Sapporo. In this aquarium, infections of *Salmincola markewitschi* in the buccal cavity of white-spotted charr have been reported since 1985 (reported as *Salmincola californiensis* in Anonymous 1989, Takayama et al. 1999, Nagasawa 2021). White-spotted charr originated from the Toyohira River, Hokkaido and Miya River, Gifu (Anonymous 2006), and has been reared at SSM. The source populations of *S. markewitschi* have been unknown, but they were supposedly from some rivers in Hokkaido (Takayama et al. 1999, Nagasawa 2021). The buccal cavities of white-spotted charr were checked, and collected copepods were preserved in the same manner as at other sites.

Morphological description

Morphological examination of the parasite specimens was conducted using light microscopes (BX53 and BH2, Olympus Inc., Shinjuku, Japan) and stereo microscopes (SZX16 and SZX10, Olympus Inc., Shinjuku, Japan). The number of specimens examined from each site is as follows; Bekanbe-ushi (two), SSM (two), Shiodomari (four), Fukushima (two), Toyama (two). Before the morphological examination, specimens were soaked in lactophenol. Dissection and morphological examination were conducted using the wooden slide method following Humes and Gooding (1964). Drawings of each copepod specimen were made with the aid of drawing tubes attached to the light microscopes.

Table 2. Summary of previous identifications of *Salmincola markewitschi* Shedko et Shedko, 2002 and *Salmincola carpionis* (Krøyer, 1837) and morphological variation of parasitic copepods recovered from *Salvelinus leucomaenis* (Pallas) in the present study. ID indicates the specimen's ID. (also shown in Fig. 2–6).

Species and Sites	Specimen's ID	The ration of cephalothorax long / bulla diameter	The number of spines of exopod	The maxilliped palp	28S r analysis	COI analysis	Reference
Salmincola markewitschi							
Russian Far East	-	0.74–1.17 (n = 16, mean = 0.91)	4–5 (at least 3, n = 45)	two outgrowths, but some had three (n = 45)	-	-	Shedko and Shedko 2002
Magadan Region, Russia	-	0.58–1.32 (n = 86, mean = 0.84)	3–4 (n = 491)	two outgrowths, but some humps (n = 491)	-	-	Shedko et al. 2005a
Sakhalin Island		0.67–1.13 (n=22, mean = 0.84)	No description	No description	-	-	Shedko et al. 2005b
Nagano Prefecture, Japan	-	0.72–1.13 (n = 6, mean = 0.90)	3–4 (n = 1)	two outgrowths (n = 1)	-	-	Nagasawa 2020b
Ishikawa Prefecture, Japan	-	0.56–0.80 (n= 9, mean = 0.66)	several number (n = 1)	two outgrowths (n = 1)	-	-	Nagasawa and Ishiyama 2021
SSM, Hokkaido, Japan	-	No description	several number (n = 1)	two outgrowths (n = 1)	-	-	Nagasawa 2021
Salmincola carpionis							
Some countries in circumpolar region	-	No description	0 (n > 78)	quite irregular shape	-	-	Kabata 1969
Aquarium in Aomori Prefecture, Japan	-	No description	0 (n = 10)	quite irregular shape	-	-	Nagasawa et al. 1995
Russian Far East	-	0.34–0.80 (n = 31, mean = 0.56)	0 (n = 102)	one outgrowth (n = 102)	-	-	Shedko and Shedko 2002
Magadan Region, Russian	-	0.27–0.68 (n = 47, mean = 0.48)	0 (n = 110)	one outgrowth (n = 110)	-	-	Shedko et al. 2005a
Salmincola cf. *markewitschi*							
Toraibetsu (Bekanbe-ushi)	ID11	Lost bulla	3	two outgrowths	-	-	-
	ID12	0,738	0	two outgrowths	○	○	-
Sapporo Salmon museum (SSM)	ID8	0,747	14	two outgrowths and one hump near its base	○	○	-
	ID15	0,670	7	two outgrowths	○	○	-
Shiodomari River	ID1	1,020	0	two outgrowths	○	○	-
	ID3	-	-	-	○	○	-
	ID4	0,722	3	two outgrowths	○	○	-
	ID5	-	-	-	○	○	-
	ID6	0,741	1	two outgrowths	○	-	-
	ID7	0,730	0	two outgrowths	○	○	-
	ID16	0,402	-	-	-	-	-
	ID17	-	-	-	-	-	-
Fukushima Prefecture	ID13	0,707	4	two outgrowths (not prominent) and one hump near its base	○	○	-
	ID14	0,786	0	two outgrowths	○	○	-
Toyama Prefecture	ID9	0,924	0	two outgrowths (not prominent)	○	○	-
	ID10	0,683	2	two outgrowths	○	○	-

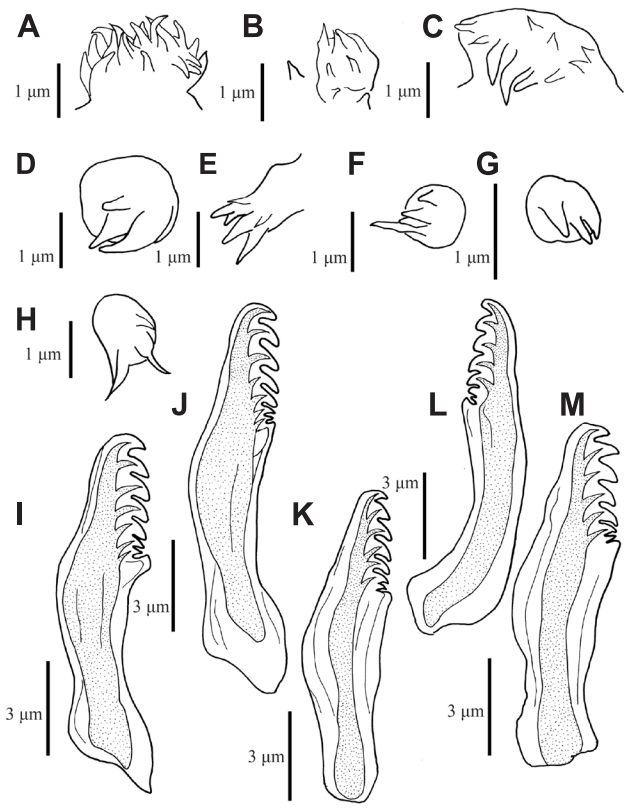

Fig. 4. Female *Salmincola* cf. *markewitschi* Shedko et Shedko 2002 from white-spotted charr *Salvelinus leucomaenis* (Pallas) at five sites in Japan. Abbreviations in parentheses represent places where specimens were collected as follows; Shiodomari River, southern Hokkaido (Shiodomari), Sapporo Salmon Museum, central Hokkaido (SSM), Bekanbe-ushi River, eastern Hokkaido (Bekanbe-ushi), Tadami River, Fukushima Prefecture (Fukushima), Jo-gan-zi River, Toyama Prefecture (Toyama).
A – spiny pad of endopod, lateral (Bekanbe-ushi, ID11); **B** – same, lateral (Fukushima, ID13); **C** – same, lateral (Toyama, ID10); **D** – spiny pad of sympod, lateral (Shiodomari, ID4); **E** – same, lateral (SSM, ID15); **F** – same, lateral (Bekanbe-ushi, ID11); **G** – same, lateral (Fukushima, ID13); **H** – same, lateral (Toyama, ID10); **I** – mandible, lateral (Shiodomari, ID1); **J** – same, lateral (SSM, ID8); **K** – same, lateral (Bekanbe-ushi, ID11); **L** – same, lateral (Fukushima, ID14); **M** – same, lateral (Toyama, ID9).

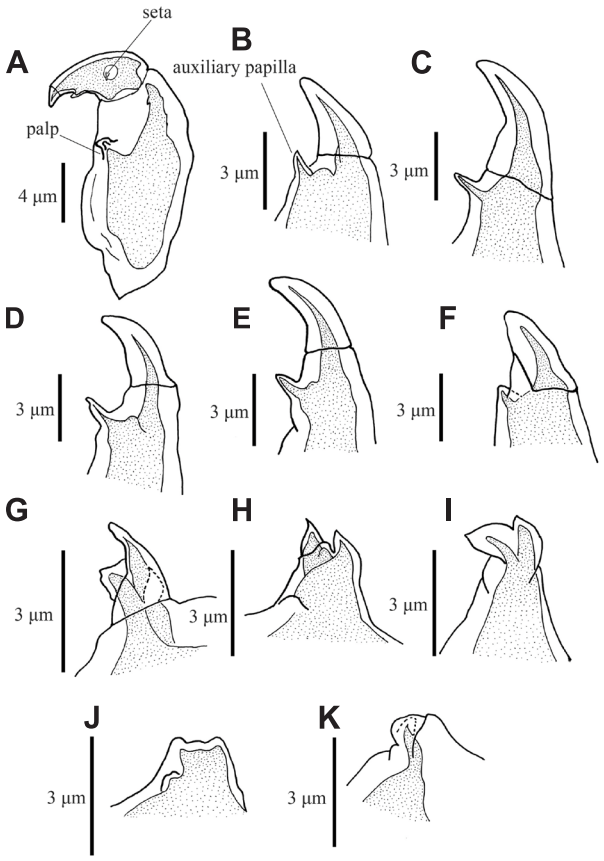

Fig. 5. Female *Salmincola* cf. *markewitschi* Shedko et Shedko 2002 from white-spotted charr *Salvelinus leucomaenis* (Pallas) at five sites in Japan. Abbreviations in parentheses represent places where specimens were collected as follows; Shiodomari River, southern Hokkaido (Shiodomari), Sapporo Salmon Museum, central Hokkaido (SSM), Bekanbe-ushi River, eastern Hokkaido (Bekanbe-ushi), Tadami River, Fukushima Prefecture (Fukushima), Jo-gan-zi River, Toyama Prefecture (Toyama).
A – maxilliped, entire, ventral (Shiodomari, ID1); **B** – tip of maxilliped, ventral (Shiodomari, ID1); **C** – same, ventral (SSM, ID8), **D** – same, ventral (Bekanbe-ushi, ID11); **E** – same, ventral (Fukushima, ID13); **F** – same, ventral (Toyama, ID9); **G** – maxilliped palp, ventral (Shiodomari, ID1); **H** – same, ventral (SSM, ID8); **I** – same, ventral (Bekanbe-ushi, ID11); **J** – same, ventral (Fukushima, ID13); **K** – same, ventral (Toyama, ID9).

All specimens we examined were deposited in the Invertebrates collection of the Hokkaido University Museum (ICHUM 8333–8337), Sapporo, Japan. The morphological terminologies were used following Huys and Boxshall (1991); antennule (as first antenna in Kabata 1969), antenna (as second antenna in Kabata 1969), maxillule (as first maxilla in Kabata 1969), maxilla (as second maxilla in Kabata 1969), maxilliped and mandible. For the armatures on the endopod of antenna (i.e., hook 1, spine 2, tubercle 3, process 4 and 5), we followed the terminologies used in Kabata (1969). The morphological identifications were made by using Kabata (1969), Shedko and Shedko (2002) and Nagasawa (2020b).

Genetic analysis

In total 13 and 12 specimens (Table 2) were used for the 28S rDNA and COI analyses, respectively. Total genomic DNA was extracted from whole parasites using a PureGene DNA isolation kit (Applied Biosystems, Foster, USA). A part of the egg sac was used for DNA extraction, lysed in 20 µL of 0.02 N NaOH at 98°C for 30 min (Nakao et al. 2018). The PCR was performed using primers D1a (5'-CCC(C/G)CGTAA(T/C)TTAAGCATAT-3') and D3b (5'-TCCGGAAGGAACCAGCTACTA-3') for 28S rDNA (von Reumont et al. 2009) and the primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) for COI (Folmer et al. 1994). The PCR reactions for 28S rDNA were performed in 25 µl volumes with thermocycling protocol for gene amplification as follows: initial denaturation at 95ºC for 2 min, 35 cycles of 95ºC for 30 sec, annealing at 55ºC for 40 sec and extension at 72ºC for 90 sec, followed by a further extension at 72°C for 8 min. For COI, the PCR reactions were carried out in 10µl volumes following protocol; 94°C for 5 min, 35 cycles of 94°C for 60 sec, 50°C for 60 sec and 72°C for 60 sec, and 5 min of final hold at 72°C. Purified products were cycle sequenced with the

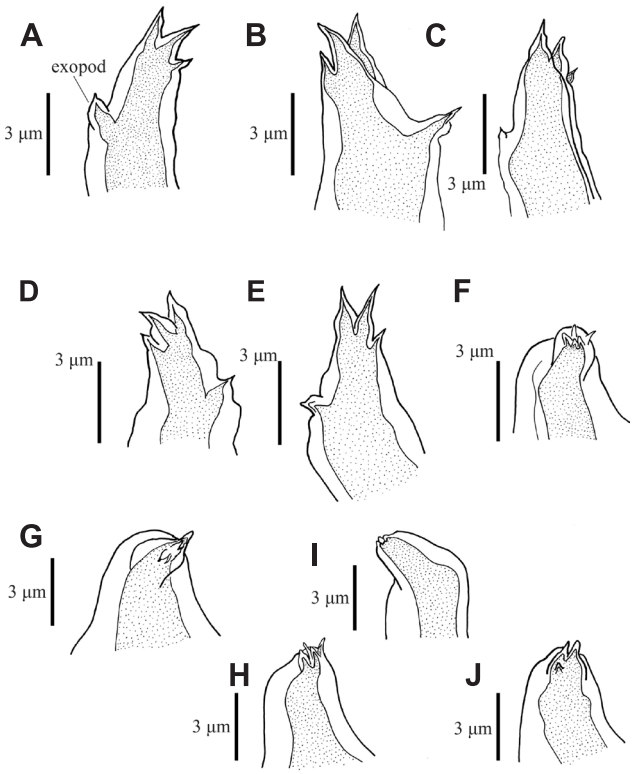

Fig. 6. Female *Salmincola* cf. *markewitschi* Shedko et Shedko 2002 from white-spotted charr *Salvelinus leucomaenis* (Pallas) at five sites in Japan. Abbreviations in parentheses represent places where specimens were collected as follows; Shiodomari River, southern Hokkaido (Shiodomari), Sapporo Salmon Museum, central Hokkaido (SSM), Bekanbe-ushi River, eastern Hokkaido (Bekanbe-ushi), Tadami River, Fukushima Prefecture (Fukushima), Jo-gan-zi River, Toyama Prefecture (Toyama).
A – maxillule, lateral (Shiodomari, ID1); B – same, lateral (SSM, ID15); C – same, lateral (Bekanbe-ushi, ID11); D – same, lateral (Fukushima, ID14); E – same, lateral (Toyama, ID9); F – antennule, ventral (Shiodomari, ID6); G – same, lateral (SSM, ID15); H – same, ventral (Bekanbe-ushi, ID11); I – same, ventral (Fukushima, ID14); J – same, ventral (Toyama, ID10).

forward and reverse primer (i.e., D1a and D3b for 28S rDNA and LCO1490 and HCO2198 for COI).

Sequence alignment and calculation of genetic distance were performed with the software MEGA ver. 10.0.4 (Kumar et al. 2018). The sequences of 28S rDNA were compared with known sequences of *Salmincola edwardsii* from Norway and North America (DQ180346.2, KY113080.1, KY113081.1) and *S. californiensis* (KY113082.1, KY113083.1) (Ruiz et al. 2017) from the GenBank database. No inter-specific comparison was made for COI because of the lack of reference for species of the genus *Salmincola*.

RESULTS

The numbers of the copepod specimens and inspected fish are summarised in Tables 1 and 2. All copepods were found in buccal cavities of fish examined.

Morphological description

Adult female composed of three major body parts, cephalothorax, maxilla and trunk with egg sacs (Fig. 2A). Oval-shaped cephalothorax, distinguished from trunk by deep constriction (Fig. 2A). Cylindrical maxilla extending towards ventral side distally with bulla (Fig. 2A). Brownish bulla, mushroom shaped with short manubrium (Fig. 2A). Ratio of bulla diameter/cephalothorax length 0.40–1.02 (mean 0.74, *n* = 12; Table 2), almost consistent with description of *Salmincola markewitschi* (Shedko and Shedko 2002, Table 2). Trunk almost ovoid, 2.46–4.82 long (mean 3.84 mm, *n* = 13). Two egg sacs, generally in equal size, attaching on posterior trunk (Fig. 2A).

Antennule not segmented, three to five short setae at their tips (Fig. 6F–J). Antenna, composed of biramous sympod; spiny pad generally with more than three spines on lateral side of sympod (Fig. 2C). Biramous sympod, composed of two-segmented endopods with spiny pad on basal segment and unsegmented exopods (Fig. 2C). Five apical armatures on distal end of endopod: dorsal hook 1, spine 2, tubercle 3, process 4 and 5 (Fig. 2D–I). Exopod, with large variations, generally having two papillae and numerous small spines (Fig. 3A–J); specimens from the Shiodomari River in Hokkaido (ID7, Fig. 3B) and Fukushima Prefecture (ID14, Fig. 3H) possess no spines which, corresponds to *Salmincola carpionis*; specimens from SSM possessing more than 10 spines (ID8, Fig. 3C), corresponding to *S. markewitschi*. Mandible in buccal apparatus with seven teeth; the distal five teeth noticeably larger than the proximal two (Fig. 4I–M).

Maxillule with three papillae extending ventrally from its tips and small exopod near its basal area (Fig. 6A–E). Maxilliped, two-segmented, comprised with subchela with short curved claw and corpus (Fig. 5A); claw positioned at distal end of subchela; subchela elongating from distal end of corpus; one short ventral seta and one auxiliary palp extending from basal and distal area of subchela; palp with two outgrowths (Fig. 5G–K) positioned at medial area of corpus with variations in its shapes; most having two prominent outgrowths as reported from *S. markewitschi* (e.g., ID1, Fig. 5G) others not prominent (e.g., ID13, Fig. 5J) and some having humps or protrusions (e.g., ID8, Fig. 5H).

Genetic analysis

A total of 884 bp of the partial 28S rDNA region showed a 100 % match amongst all specimens in the present study (*n* = 13). These sequences had a 99.55% match with *Salmincola edwardsii* from Norway (GenBank accession numbers is DQ180346.2) and a 99.43% and 99.32% match with *S. edwardsii* caught in North America (GenBank accession numbers are KY113080.1 and KY113081.1; Ruiz et al. 2017). All specimens also showed a 98.76% and 98.65% match with *Salmincola californiensis* from North America (GenBank accession numbers are KY113082.1 and KY113083.1; Ruiz et al. 2017). For COI, in total of 601 bp were obtained. Four haplotypes were detected from different regions in Japan with only 0–4 base pair differences (mean genetic differences: 0.28%, range: 0–0.67 %, *n* = 12). GenBank accession numbers are LC713076–LC713088 for 28S rDNA and LC713314–LC713325 for COI.

DISCUSSION

Our genetic analysis of both nuclear and mitochondrial DNA confirmed that the examined specimens from five sites in Japan contained only a single species of the genus *Salmincola*: 28S rDNA was monomorphic and genetic distance of COI fell within the range of intraspecific variation, as shown in other parasitic copepods (Montes et al. 2017). In addition, based on morphological observations, most of the specimens were consistent with *Salmincola markewitschi* described by Shedko and Shedko (2002): (1) the distal end of the exopod had some small spines (mainly three to five) in addition to two papillae, (2) two outgrowths were present on the palp extending from the base of maxilliped, and (3) the ratio of the bulla diameter/cephalothorax length in the present study (range 0.40–1.02, mean 0.74) was similar to the original description (range 0.74–1.17, mean 0.91; see Shedko and Shedko 2002).

However, morphological variations of our specimens were high and some specimens partly had characteristics consistent with *Salmincola carpionis*. While all 12 specimens had two outgrowths at the maxilliped palp, five (ID 1, 7, 9, 12, 14) had no spines at the distal end of the exopod of the antenna (Table 2). In addition, nine specimens (ID 4, 6, 7, 8, 10, 12, 13, 14, 15) had a small bulla diameter, which fits the range of *S. carpionis* (range 0.34–0.80, mean 0.56; Shedko and Shedko 2002). This makes a reliable morphological species identification difficult.

To discriminate between the two species, we have to examine the validity of each morphological trait (i.e., spines at the distal end of the exopod of antenna, shapes of maxilliped palp, bulla diameter). The number or shape of spines on the antenna's exopod has been widely used as a key character to discriminate *Salmincola* spp. (Kabata 1969). For instance, *S. californiensis* is distinguished by its cluster of very strong and large spines and this characteristic was consistently observed in all populations examined so far (Kabata 1969, Hoshina and Nishimura 1976, Ruiz et al. 2017). However, caution is still needed in using this feature, because of some variations. In *Salmincola thymalli* (Kessler, 1868), the specimens from Nearctic had long prominent spines, but those from the Palearctic had very small and scattered spines (Kabata 1969). As is this unreliable case, the present individuals represented both patterns with and without spines on the antenna's exopod even in the same site with identical 28S rDNA and COI sequences.

The shape of maxilliped palps is less reliable for discriminating *S. markewitschi* from *S. carpionis*. While some species such as *S. thymalli* are characterised by their long and slender palps, considerable variability was recognised in some species (Kabata 1969). For instance, *Salmincola salmoneus* (Linnaeus, 1758) and *S. californiensis* showed large intraspecific variations for the number and shape of the outgrowths on their palps. In our case, whereas most of the specimens two prominent outgrowths on the maxilliped palps, their length and shape showed high variation within and among populations as well.

The remaining key trait, the bulla diameter, is also a presumably unreliable character in our case. Although bulla shape is widely used identification of species of the genus *Salmincola*, its diameter can be easily changed by its attachment sites and host characteristics (Kabata 1969). In other siphonostomatoid copepods, it is also reported that the attachment organs are affected by host body parts, as well as ambient environmental factors like temperature (Fryer 1961, Hogans 1987, Abaunza et al. 2001, Hua et al. 2019, Suyama et al. 2019, González et al. 2021). The ratio of the bulla diameter/cephalothorax length of *S. markewitschi* was highly variable even within a small geographic range (Shedko and Shedko 2002, Nagasawa 2020b, Nagasawa and Ishiyama 2021) and the high variation may be due to host characteristics and/or physical environmental factors, which affect the parasite's development.

Taken together, while we can tentatively identify our specimens as *S.* cf. *markewitschi* because of the overall morphological consistency, we should be careful of the possibility that *S. markewitschi* is a regional type of *S. carpionis* and the former is a synonym of the latter or its subspecies. Because *Salmincola* spp. often have large morphological variations (Kabata 1969) and even the same species can show distinct morphological traits among local populations, especially considering the wide circumpolar distribution (Fig. 1). Genetic analysis is particularly useful to delineate species with high morphological variations (Nadler and Pérez-Ponce de León 2011), but surprisingly very few studies have conducted genetic analysis for *Salmincola* spp. (Ruiz et al. 2017, Hasegawa et al. 2022). It is necessary to compare specimens collected from throughout their distributional range, including the Northeast Asia, North America and Europe, using both genetic and morphological traits. In particular, Shedko and Shedko (2002) reported the morphological distinction between sympatric *S. markewitschi* and *S. carpionis* in Kuril Islands where the type collection of the former was obtained. Genetic analysis of this population will be the priority.

Key to the species of the genus *Salmincola* in Japan

1. Ventral side of the basal segment of endopod of antenna with large smooth tapering outgrowth; bulla stellate; common parasite of Sakhalin taimen *Parahucho perryi* (Brevoort) ..... *Salmincola stellata* Markevich, 1936
- Ventral side of the basal segment of endopod of antenna with spiny pad; bulla not stellate (commonly round or mushroom shape); not commonly parasitic on Sakhalin taimen *Parahucho perryi* 2

2. One process as large as, or larger, than dorsal hook and other much smaller processes present at ventral side of the terminal segment of endopod of antenna; the exopod and sympod of antenna highly inflated .... *Salmincola edwardsii* (Olsson, 1869)
- Two processes generally smaller than dorsal hook and other much smaller process present at ventral side of the terminal segment of endopod of antenna; exopod and sympod of antenna are not inflated 3

3. Maxilliped palp large; distal end of exopod of antenna with more than five huge spines; common parasite of

species of *Oncorhynchus* Suckley .... *Salmincola californiensis* (Dana, 1852)

- Maxilliped palp small; distal end of exopod of antenna had no or several small spines; commonly parasitic on fish of the genus *Salvelinus* Richardson 4

4. Maxilliped palp with two outgrowths but no hump (protrusion) near its base; distal end of exopod of antenna with numerous small spines in addition to two papillae; bulla diameter large (ratio of bulla diameter/cephalothorax length range 0.74–1.17, mean = 0.91 reported in original description by Shedko and Shedko 2002) .... *Salmincola* cf. *markewitschi* Shedko et Shedko, 2002

- Maxilliped palp with single outgrowth with one hump (protrusion) near its base; distal end of exopod of antenna with only two papillae, no spines; bulla diameter small (ratio of bulla diameter / cephalothorax length range 0.34–0.80, mean = 0.56 reported in original description by Shedko and Shedko 2002) .... *Salmincola carpionis* (Krøyer, 1837) (suspected as *Salmincola* cf. *markewitschi* Shedko et Shedko, 2002)

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Data accessibility. The sequence data are available from GenBank (accession numbers are LC713076–LC713088 for 28S rDNA and LC713314–LC713325 for COI) and figshare (link: 10.6084/m9.figshare.20000441).

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