**Two Copepods Salmincola edwardsii and Salmincola markewitschi (Lernaeopodidae) Parasitic on Char (Salvelinus spp.) Reared in a Salmon Museum, Northern Japan**

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Specimens of two species of lernaeopodid copepods, *Salmincola edwardsii* (Olsson, 1869) and *Salmincola markewitschi* Schedko and Shedko, 2002, were collected from chars (*Salvelinus spp.*) reared in exhibition tanks of the Sapporo Salmon Museum, Hokkaido, Japan. Descriptions of these copepods are provided using the specimens. In 1988, adult females of *S. edwardsii* were found on the gills of southern Asian Dolly Varden, *Salvelinus malma* kracheninnikova Taranetz, 1933, and those of *S. markewitschi* in the buccal cavity of whitespotted char, *Salvelinus leucomaenis* pluvius (Hilgendorf, 1876), and brook trout, *Salvelinus fontinalis* Mitchill, 1814. These copepods are considered to have been introduced to the museum in the late 1980s along with wild chars transported live from natural waters of Hokkaido. Adult copepods have been manually removed using forceps once or twice a year from reared chars, but such infrequent practices have not been effective to eliminate *S. markewitschi*, which was thus collected again in 2020. In contrast, *S. edwardsii* was completely eliminated by stopping rearing an infected population of southern Asian Dolly Varden, to which this parasite is host-specific.

**Key Words:** Copepoda, fish parasite, control, southern Asian Dolly Varden, whitespotted char, Nikko char, brook trout.

**Introduction**

Up to 19 species of salmonids have been reared for public exhibition in the Sapporo Salmon Museum (SSM), Hokkaido, Japan, since 1984 when it was opened. Various bacterial and parasitic diseases have been detected from salmonids held in exhibition tanks, and copepod infection is one of the major diseases of chars (the genus *Salvelinus* Richardson, 1836) (Anonymous 1989). Copepods were first observed in 1985 and have been found to date (Takayama et al. 1999; Nozomi Aruga, SSM, personal communication). In the late 1980s, two species of copepods were recognized, and one of them was reported as *Salmincola californiensis* (Dana, 1852) (Anonymous 1989). However, this identification is doubtful because *S. californiensis* is not a parasite of chars but of Pacific salmon (the genus *Oncorhynchus* Suckley, 1861) (Kabata 1969). Moreover, another species of copepod was not identified. This paper aims to identify copepods using specimens collected from chars in 1988 and 2020, to review how the copepods were introduced to the SSM, and to discuss control measures for those copepods.

**Materials and Methods**

Copepods were manually collected using forceps by the staff from chars (*Salvelinus spp.*) in the SSM, Makomanai (43°00′04″ N, 141°20′36″ E), Sapporo, Hokkaido, northern Japan, in November 1988 and July 2020 (Table 1). Copepods taken in 1988 were first kept in 10% formalin and much later transferred into in 70% ethanol, but in 2020 copepods were fixed and preserved in 70% ethanol. The SSM staff took no data on the prevalence and intensity of copepods on the chars examined. Copepod specimens collected in both years were sent to the author for identification. They were first observed using an Olympus SZX10 stereo microscope, and two specimens collected in 1988 (each from southern Asian Dolly Varden and whitespotted char) were soaked in lactophenol for 2–3 h, dissected, and observed using the wooden slide procedure of Humes and Gooding (1964). All drawings were made with the aid of drawing tubes fitted on the stereo microscope (for the habitus) and an Olympus BX51 compound microscope (for the second antenna, mandible, first maxilla, and maxillipedes). Morphological terminology follows Kabata (1979) and that for the armature of the endopod of the second antenna is based on Kabata (1969). Voucher copepod specimens have been deposited in the Crustacea collection of the National Museum of Nature and Science, Tsukuba, Ibaraki Prefecture [NSMT-Cr 28445 (n=5), 28446 (n=7), 28447 (n=5), and 28448 (n=3), respectively, from southern Asian Dolly Varden, whitespotted char, Nikko char, and brook trout], and the remaining specimens are retained by the author. The scientific and common names of fishes mentioned in this paper follow FishBase (Froese and Pauly 2020). Nonetheless, *Salvelinus malma* and *Salvelinus leucomaenis* have been synonymized as *Salvelinus fontinalis* Mitchill, 1814. Descriptions of these copepods are provided using the specimens.
krascheninnikova Taranetz, 1933 and “southern Asian Dolly Varden” are used for species based on WoRMS Editorial Board (2020) and Dunham et al. (2008: 539), respectively (see Nagasawa 2020a). Since “Nikko char” has been used at the SSM for a subspecies of whitespotted char, Salvelinus leucomaenis (Pallas, 1814), these names are adopted in this paper.

**Results**

Two species of lernaeopodid copepods are identified: Salmincola edwardsii (Olsson, 1869) (Figs 1A, 2) and Salmincola markewitschi Shedko and Shedko, 2002 (Figs 1B–D, 3). In 1988, adult females of *S. edwardsii* were collected from the gills of southern Asian Dolly Varden, and those of *S. markewitschi* from the buccal cavity of three species of chars [whitespotted char, Salvelinus leucomaenis (Pallas, 1814), Nikko char, and brook trout, Salvelinus fontinalis (Mitchill, 1814)] (Table 1). In 2020, *S. markewitschi* was found again, but no specimen of *S. edwardsii* was collected.

Some of the specimens of both *S. edwardsii* and *S. markewitschi* collected in 1988 had contracted due to fixation and preservation in 10% formalin (Fig. 1C) but no such contraction was found for the specimens of *S. markewitschi* fixed and preserved in 70% ethanol in 2020 (Fig. 1D). Morphological features of adult females of *S. edwardsii* and *S. markewitschi* are given below.

**Adult female of *S. edwardsii*.** Cephalothorax (Figs 1A, 2A) subtriangular, shorter than trunk, and separated from trunk by shallow constriction. Trunk (Figs 1A, 2A) nearly oval with rounded lateral margins. Second antenna (Fig. 2B) biramous with spiny pod bearing many spines on sympod; exopod equipped with two papillae and scattered spines; endopod two-segmented; basal segment with spiny pod with many spines; distal segment armed with projecting hook 1, small spine 2, prominent process 4, and smaller process 5. Mandible (Fig. 2C) with six teeth; four distal teeth larger than two proximal teeth. First maxilla (Fig. 2D) with three terminal papillae on endopod; central papilla largest; setule on lateral exopod. Second maxilla (Figs 1A, 2A) cylindrical, slightly longer than trunk; bulla (Figs 1A, 2A) consisting of subconical anchor and short manubrium. Maxilliped (Fig. 2E) tapering towards tip; corpus with prominent palp surmounted by two (bulbous and slender) outgrowths; former outgrowth divided into two tips (Fig. 2F); subchela with seta near base and patch of spines near base of claw.

Total body length (excluding egg sacs) and trunk length of formalin-fixed females from southern Asian Dolly Varden are 2.1–3.4 (mean, 2.7) mm (n=7) and 1.6–2.0 (1.9) mm (n=7), respectively.

**Adult female of *S. markewitschi*.** Cephalothorax (Figs 1B–D, 3A) oval with dorsal prominence, shorter than trunk, and separated from trunk by short constriction. Trunk (Figs 1B–D, 3A) roughly ovoid with rounded lateral margins; shallow transverse grooves on ventral side. Second antenna

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**Table 1.** Specimens of *Salmincola* spp. collected from chars (*Salvelinus* spp.) reared in the Sapporo Salmon Museum, Hokkaido, Japan.

| Species               | Host                          | Infection site | Number of specimens | Date of collection |
|-----------------------|-------------------------------|----------------|---------------------|--------------------|
| Salmincola edwardsii  | Southern Asian Dolly Varden   | Gills*         | 7                   | 30 November 1988   |
| Salmincola markewitschi| Whitespotted char              | Buccal cavity  | 14                  | 12 November 1988   |
|                        | Nikko char                    | Buccal cavity  | 9                   | 12 November 1988   |
|                        |                               |                | 1                   | 30 July 2020       |
|                        | Brook trout                    | Buccal cavity  | 4                   | 12 November 1988   |
|                        |                               |                | 4                   | 30 July 2020       |

* See the Remarks for this infection site.
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(Fig. 3B) biramous with small spiny pad with few spines on sympod; exopod equipped with two papillae and several small spines; endopod two-segmented; basal segment with spiny pad; distal segment armed with powerful hook 1, small spine 2, tubercle 3, conical process 4, and small process 5. Mandible (Fig. 3C) with six teeth. First maxilla (Fig. 3D) with three terminal papillae on endopod; central papilla largest; setule on lateral exopod. Second maxilla (Figs 1B–D, 3A) varying in shape and length from cylindrical and long to thick and short in differently fixed and preserved specimens; bulla (Figs 1B–D, 3A) with mushroom-shaped anchor and long manubrium. Maxilliped (Fig. 3E) tapering towards tip; corpus with small palp surmounted by two outgrowths (Fig. 3F); subchela with small seta near base and barb near base of claw.

Total body length (excluding egg sacs) and trunk length of formalin-fixed females from whitespotted char are 2.1–4.1 (mean, 2.9) mm (n=14) and 1.8–2.6 (2.1) mm (n=14), respectively. Those lengths of formalin-fixed females from Nikko char are 2.2–3.8 (mean, 2.8) mm (n=9) and 1.7–2.8 (2.0) mm (n=9), respectively. Both lengths of ethanol-fixed females from brook trout are slightly larger [3.8–4.8 (mean, 4.4) mm, 2.6–3.4 (3.0) mm, n=4] than those of formalin-fixed females from the same host species [2.9–4.0 (3.4) mm, 2.1–3.0 (2.6) mm, n=4].

Remarks. The specimens of Salmincola C. B. Wilson, 1915 collected from the SSM-reared chars are herein identified as two species, S. edwardsii and S. markewitschi. The morphology of the specimens of each species is identical to that reported for S. edwardsii (Shedko and Shedko 2002; Nagasawa 2020a) and S. markewitschi (Shedko and Shedko 2002; Shedko et al. 2005a; Nagasawa 2020c; Nagasawa and Ishiyama 2021). The two species can be distinguished by the following characters: 1) the shape and size of the bulla, i.e., a small subconical anchor with a short manubrium in S. edwardsii (Figs 1A, 2A) but a relatively large mushroom-shaped anchor with a long manubrium in S. markewitschi (Figs 1B–D, 3A); 2) the apical armature of the distal segment of the second antenna endopod, i.e., the presence of a prominent process 4 and a powerful hook 1, respectively, in S. edwardsii (Fig. 2B) and S. markewitschi (Fig. 3B); 3) the size and spinulation of the spiny pad on the second antenna...
sympod, i.e., a large spiny pad bearing many spines in *S. edwardsii* (Fig. 2B) but a small spiny pad with a few spines in *S. markewitschi* (Fig. 3B); and 4) a patch of spines and a barb found near the base of the maxilliped claw, respectively, in *S. edwardsii* (Fig. 2E) and *S. markewitschi* (Fig. 3E).

*Salmincola edwardsii* has a wide distribution in the holartic region and is a parasite of various species of chars (Kabata 1969; Nagasawa 2020a). In Fast East Asia, the species has been reported from southern Asian Dolly Varden in Russia (e.g., Shedko and Shedko 2002; Shedko 2005; Shedko et al. 2005a, b; Sokolov et al. 2012) and Japan (Shedko and Shedko 2002). It was later reported from whitespotted char from the Kamchatka Peninsula and Magadan District (Shedko 2005; Shedko et al. 2005a) and also from whitespotted char, southern Asian Dolly Varden [as *Salvelinus curilus* (Pallas, 1814)], and Japanese huchen, *Parahucho perryi* (Brevoort, 1856), from Sakhalin Island, Russia (Shedko et al. 2005b; Sokolov et al. 2012). In Japan, in addition to whitespotted char from the southern Kuril Islands (Shedko and Shedko 2002), it was reported from Nikko char (Nishimura and Hoshina 1977) and whitespotted char (Denda and Ogawa 2011; Nagasawa 2020c), both of which were collected from the Zako River, Nagano Pre-

*Salmincola markewitschi* was originally described in 2002 using specimens from the buccal cavity of whitespotted char in the Russian Far East and the southern Kuril Islands, Japan (Shedko and Shedko 2002). It was later reported from whitespotted char from the Kamchatka Peninsula and Magadan District (Shedko 2005; Shedko et al. 2005a) and also from whitespotted char, southern Asian Dolly Varden [as *Salvelinus curilus* (Pallas, 1814)], and Japanese huchen, *Parahucho perryi* (Brevoort, 1856), from Sakhalin Island, Russia (Shedko et al. 2005b; Sokolov et al. 2012). In Japan, in addition to whitespotted char from the southern Kuril Islands (Shedko and Shedko 2002), it was reported from Nikko char (Nishimura and Hoshina 1977) and whitespotted char (Denda and Ogawa 2011; Nagasawa 2020c), both of which were collected from the Zako River, Nagano Pre-

Fig. 3. *Salmincola markewitschi*, female, NSMT-Cr 28446, from whitespotted char, *Salvelinus leucomaenis*, reared in the Sapporo Salmon Museum, Hokkaido, Japan. Formalin-fixed and preserved specimen. A, Habitus, ventrolateral view; B, second antenna, distal half, ventral view; C, mandible, lateral view; D, first maxilla, lateral view; E, maxilliped, lateral view; F, palp of maxilliped. Abbreviations: ex, exopod; h1, hook 1; p, palp; pap, papilla; p4, process 4; p5, process 5; s2, spine 2; t3, tubercle 3. Scale bars: A, 1 mm; B, 50 µm; C, 20 µm; D, 30 µm; E, 100 µm; F, 20 µm.
fecture, Honshu Island, the main island of Japan. It was also found on whitespotted char reared at fisheries research institute in Ishikawa Prefecture, central Honshu Island (Nagasawa and Ishiyama 2021). In the present study, *S. markewitschi* was found to parasitize brook trout, which is herein regarded as its new host.

A species of *Salmincola* found in the buccal cavity of whitespotted char was previously reported by the SSM staff (Anonymous 1989) as "*Salmincola californiensis*” without providing morphological information. This identification is herein found to be wrong because the species in question is identified as *S. markewitschi* using the specimens collected in 1988. The reason why the SSM staff reported it as "*S. californiensis*” is because in the late 1980s this species was the only parasitic copepod of the genus known from Japanese chars (Nishimura and Hoshina 1977). *Salmincola californiensis* is characterized by having two papillae and a cluster of very strong and large spines on the second antenna exopod (Kabata 1969) and a large palp on the maxilliped corpus (Hoshina and Suenaga 1954; Hoshina and Nishimura 1976). In contrast, as reported above, *S. markewitschi* has two papillae and several small spines on the second antenna exopod (Fig. 3B) and a small palp surmounted by two outgrowths on the maxilliped corpus (Fig. 3E, F). Therefore, both species can be separated using a combination of these characters.

A lernaeopodid copepod reported as "*Salmincola carpionis* (Krøyer, 1837)” is known to infect whitespotted char on Hokkaido Island, where the SSM is located (Yamaguti 1939; Nagasawa and Urawa 2002: appendix, footnote). Although Yamaguti (1939) reported the copepod as "*Salmincola falculata* (Wilson, 1908)” from land-locked sockeye salmon, "*Onchorhyncus nerka* (Walbaum)", these species were referred to as "*S. carpionis*” and whitespotted char, respectively, by Nagasawa et al. (1995). However, because "*S. carpionis*” resembles *S. markewitschi* (Shedko and Shedko 2002) and also because "*S. carpionis*” had been reported from various regions of Japan including Hokkaido Island before *S. markewitschi* was described as a new species (Nagasawa et al. 1995; see Nagasawa 2020c and Nagasawa and Ishiyama 2021 for the other literature), Nagasawa (2020c) has recently suggested that specimens reported as "*S. carpionis*” from Japan including Hokkaido Island should be re-identified, based on Shedko and Shedko (2002).

The infection site of *S. edwardsii* is herein reported as the "gills", which is based on information provided by the SSM staff and also on their reports (Anonymous 1989; Takayama et al. 1999). However, *S. edwardsii* is known to use three microhabitats (the wall of the branchial cavity, the gill filaments, and the inner surface of the operculum) in the host’s gill region (Nagasawa 2020a), and it is thus better to regard the "gills" as a general infection site of the species.

The specimens of *S. edwardsii* and *S. markewitschi* fixed and preserved in 10% formalin were contracted. In particular, the second maxillae were highly contracted (Fig. 1C). A similar observation has been made for *S. markewitschi* (Nagasawa 2020c).

**Discussion**

It has been suggested that both *S. edwardsii* and *S. markewitschi* were introduced into the SSM along with wild hosts (Anonymous 1989; Takayama et al. 1999). According to SSM staff (Takayama et al. 1999), a species of copepod, herein identified as *S. edwardsii*, was constantly found only on southern Asian Dolly Varden during the 1990s after individuals of the fish species were transported alive in 1989 from the Shari River to the SSM. The Shari River is one of the four Hokkaido rivers where *S. edwardsii* is found (Nagasawa 2020a; Nagasawa and Kawai 2020). Thus, it is most likely that *S. edwardsii* was brought into the SSM along with southern Asian Dolly Varden from the Shari River and thereafter maintained its population using this fish species during the 1990s. However, it has not been found since 2003 (see the last paragraph in this Discussion), which was confirmed in July 2020 (Table 1).

The SSM staff (Anonymous 1989; Takayama et al. 1999) also observed that Nikko char and brook trout acquired infection by another species of copepod when wild whitespotted char were transported in the late 1980s from several sites of Hokkaido Island to the SSM. This copepod was previously reported as "*Salmincola californiensis*” (Anonymous 1989) but is herein identified as *S. markewitschi*, which is even now found to parasitize the three species of chars reared in the museum. Although this parasite has not yet been recorded from Hokkaido Island, the SSM staff’s observation indicates that it occurs on the island. In Japan, it has been reported from whitespotted char from the southern Kuril Islands and Honshu Island (Shedko and Shedko 2002; Nagasawa 2020c; Nagasawa and Ishiyama 2021). As stated in the above Remarks, copepods reported as "*S. carpionis*”, which is morphologically similar to *S. markewitschi*, are known to infect whitespotted char on Hokkaido Island (Yamaguti 1939; Nagasawa and Urawa 2002), thus it is desirable to conduct taxonomic work using specimens previously reported as "*S. carpionis*” and plus newly collected material from the island in order to confirm whether *S. markewitschi* actually occurs there.

It is interesting to note that both *S. edwardsii* and *S. markewitschi* were collected in 1988 but *S. edwardsii* was not found in 2020 (Table 1). As stated above, *S. edwardsii* was recognized during the 1990s (Takayama et al. 1999), but the species disappeared in 2003. This is because rearing southern Asian Dolly Varden of the Shari River origin was discontinued in that year (Anonymous 2004, 2005). Since *S. edwardsii* is host-specific to southern Asian Dolly Varden (Shedko and Shedko 2002), removal of this host species from an exhibition tank (2.15 m long, 2.5 m wide, 1.0 m deep) resulted in elimination of the parasite. However, despite the fact that adult copepods have been manually removed once or twice a year using forceps from infected chars (Takayama et al. 1999; Nozumi Aruga, SSM, personal communication), *S. markewitschi* has not been eliminated to date (Table 1). This failure of control is definitely due to the extended intervals between episodes of manual removal and
also because all copepods could not be removed from chars held in multiple exhibition tanks within a limited amount of time. This leads to the inference that infective copepodids of *S. markewitschi* are released from ovigerous females that were not removed and can become a new source of infection to chars within the exhibition tanks. Manual removal practiced at even 3-week intervals has been reported to be insufficient for elimination of *S. californiensis* from captive broodstocks of chinook salmon, *Oncorhyncus tsawytscha* (Walbaum, 1792), in the U.S.A. (Johnson and Heindel 2001). For eliminating *S. markewitschi* in the SS, it is highly recommended to repeat manual removal of copepods from infected chars, not once or twice a year but at much shorter intervals, until no new infection is observed.

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