Occurrence of *Salmincola edwardsii* (Olsson, 1869) and *Salmincola markewitschi* Shedko & Shedko, 2002 (Copepoda: Lernaeopodidae) on stream-dwelling salmonids in eastern Hokkaido, Japan, with observations on the morphology of the copepods

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**Abstract.**—Two copepod parasites, *Salmincola edwardsii* (Olsson, 1869) and *S. markewitschi* Shedko & Shedko, 2002, were collected from salmonids in eastern Hokkaido, Japan: these copepods were taken from whitespotted char, *Salvelinus leucomaenis* (Pallas, 1814), and brook trout, *S. fontinalis* (Mitchill, 1814) in the Nishibetsu River, and *Salmincola edwardsii* from southern Asian Dolly Varden, *Salvelinus malma krascheninnikova* Taranetz, 1933, in the Ichani River. Both whitespotted char and brook trout are new Japanese hosts for *Salmincola edwardsii*, and the two rivers represent new locality records for *S. edwardsii* and *S. markewitschi*. The Nishibetsu River is regarded as a new southernmost distribution limit of *S. edwardsii* in Asia and is also recorded as the first locality of *S. markewitschi* infecting wild salmonids in Hokkaido. The morphology of *S. edwardsii* and *S. markewitschi* is reported based on adult female specimens collected from the salmonids.

**Key words:** fish parasite, parasitic copepod, new distribution record, whitespotted char, southern Asian Dolly Varden, brook trout

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**Introduction**

Copepods of the lernaeopodid genus *Salmincola* are parasites of freshwater teleost fishes (mostly salmoniform fishes) in the Northern Hemisphere (Kabata, 1969). Five nominal species in the genus are known to infect salmonids in Hokkaido, Japan, including *Salmincola californiensis* (Dana, 1852), *S. stellata* Markevich, 1936, *S. carpio*is (Krøyer, 1837), *S. edwardsii* (Olsson, 1869), and *S. markewitschi* Shedko & Shedko, 2002 (see Nagasawa & Urawa, 2017, for the literature on the first three species). The last two species were reported from chars (*Salvelinus* spp.) in the past few years (Nagasawa, 2020a, 2021).

Southern Asian Dolly Varden, *Salvelinus malma krascheninnikova* Taranetz, 1933, and whitespotted char, *S. leucomaenis* (Pallas, 1814), are common in eastern Hokkaido (Ishigaki, 1984; Fausch et al., 1994). *Salmincola edwardsii* infecting southern Asian Dolly Varden in streams of the Shiretoko Peninsula was studied for its morphology, distribution (Fig. 1), prevalence and intensity, and effects on the host fish (Nagasawa, 2020a, b; Nagasawa & Kawai, 2020; Hasegawa et al., 2022).

Whitespotted char is also widespread in streams of the Shibetsu Region south of the Shiretoko Peninsula, where the species occasionally cohabits with southern Asian Dolly Varden and some other salmonids (Ishigaki, 1984; Mayama, 1991). Between 1987 and 2011, specimens of two *Salmincola* species, *S. edwardsii* and *S. markewitschi*, were collected during several surveys of the salmonid para-
sites in two streams of the region. Many aspects of the biology of these copepods remain poorly studied in Hokkaido, and this paper reports on their occurrence on the salmonids and briefly redescribes them.

Materials and Methods

Study area and fish sampling

Salmonids were collected by cast nets in two streams, the Nishibetsu River and the Ichani River, from 1987 to 2011 (Fig. 1). The Nishibetsu River is about 80 km long, and the Ichani River is a tributary of the Po River and is shorter (about 13 km long). The salmonids collected comprised five species: whitespotted char; southern Asian Dolly Varden; brook trout, Salvelinus fontinalis (Mitchill, 1814); masu salmon, Oncorhynchus masou (Brevoort, 1856); and rainbow trout, O. mykiss (Walbaum, 1792). Of these species, both brook trout and rainbow trout are not native to Japan, being of North American origin. Since their introduction to the river around 1935 and in the 1920s, respectively, they have established self-sustaining populations (Mayama, 1991). Whitespotted
char and southern Asian Dolly Varden have river-resident and anadromous individuals (Ishigaki, 1984). Masu salmon migrates to the ocean after its juveniles stay in the rivers, but both brook trout and rainbow trout stay in the rivers without migrating to the sea (Mayama, 1991).

In the Nishibetsu River salmonids were collected at five locations: the spring-fed headwater reaches near the Nijibetsu Salmon Hatchery Station (43°30′47.0″N, 144°37′19.2″E; locality 1 in Fig. 1), the upper-reaches at Nijibetsu (43°27′20.1″N, 144°41′34.4″E), the middle-reaches at Nishi-shunbetsu (43°23′44.4″N, 144°48′47.2″E), the lower-reaches at Betsukai (43°24′24.7″N, 145°12′50.8″E), and near the river mouth (43°22′58.8″N, 145°17′16.8″E). In addition to these collections, southern Asian Dolly Varden was collected in the lower reaches of the Ichani River at Kawakita (43°41′29.6″N, 145°05′36.2″E; locality 2 in Fig. 1). All salmonids were fixed in 10% formalin immediately after capture and transported to the laboratory of the Hokkaido Salmon Hatchery (currently, Salmon Research Department, Fisheries Resources Institute), Sapporo, central Hokkaido, where they were individually measured for fork length (FL, mm) and examined for parasites. Copepods were taken from the fishes, re-fixed in 10% neutral buffered formalin, and later preserved in 70% ethanol. The number of copepods and their attachment sites were recorded for each infected fish, but such data were not taken in 2011.

**Copepod examination**

Copepod specimens were examined at the Aquaparasitology Laboratory, Shizuoka, with an Olympus SZX10 stereo microscope and an Olympus BX51 phase-contrast compound microscope. For descriptions, specimens of *S. edwardsii* and *S. markewitschi*, from whitespotted char and brook trout, respectively, were observed in detail and measured for their total body length and trunk length. Further, to study their cephalothoracic appendages, two specimens (one *S. edwardsii* and one *S. markewitschi*, respectively, from whitespotted char and brook trout) were soaked for 30 min. in lactophenol and dissected using the wooden slide method of Humes & Gooding (1964). Illustrations were made with the aid of drawing tubes fitted on the stereo microscope (for the habitus) and on the compound microscope (for the cephalothoracic appendages). Morphological terminology follows Kabata (1979) and that for the armature of the endopod of the second antenna is based on Kabata (1969). Voucher specimens have been deposited in the Crustacea collection of the National Museum of Nature and Science, Tsukuba, Ibaraki Prefecture [*S. edwardsii*, NSMT-Cr 30862 (n = 5) and NSMT-Cr 30863 (n = 1) from whitespotted char and brook trout, respectively, from the Nishibetsu River; NSMT-Cr 30864 (n = 5) from southern Asian Dolly Varden from the Ichani River; *S. markewitschi*, NSMT-Cr 30865 (n = 1) and NSMT-Cr 30866 (n = 10) from whitespotted char and brook trout, respectively, from the Nishibetsu River]. The remaining specimens are retained by the senior author. The scientific and common names of fishes are based on FishBase (Froese & Pauly, 2022). As in Nagasawa (2020a, 2021), *Salvelinus malma krascheninnikova* and its common name “southern Asian Dolly Varden” follow WoRMS Editorial Board (2022) and Dunham *et al.* (2008), respectively. Use of prevalence, intensity, and mean intensity follows Bush *et al.* (1997).

**Results**

**Occurrence of Salmincola spp. on salmonids**

**Nishibetsu River:** Four species of salmonids (whitespotted char, brook trout, masu salmon, and rainbow trout) were collected. Most of the whitespotted char and all brook trout examined in this study were collected in the spring-fed headwater reaches, and some individuals of these species were infected with *Salmincola*...
No copepod infection was found on whitespotted char from the upper- and lower reaches, and no masu salmon and rainbow trout were infected in any location (Table 1).

The intensity of infection by *S. edwardsii* ranged from five to nine on whitespotted char but remained low (two) on brook trout. Conversely, the intensity of *S. markewitschi* infection on brook trout was higher (up to eight) than on whitespotted char (one). Although the number of fish examined was only 19, there was a trend that, in brook trout, large fish were more frequently and more heavily infected with *S. markewitschi* than small fish (Table 2).

**edwardsii** and/or *S. markewitschi* (Table 1). No copepod infection was found on whitespotted char from the upper- and lower reaches, and no masu salmon and rainbow trout were infected in any location (Table 1).

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**Table 1.** Occurrence of *Salmincola edwardsii* and *S. markewitschi* on salmonids in the Nishibetsu River, eastern Hokkaido, Japan.

| Location          | Salmonid            | Fork length in mm (mean) | No. of fish infected/examined (% prevalence) | Intensity (mean) | Total no. of copepods | Date       |
|-------------------|---------------------|--------------------------|---------------------------------------------|-----------------|-----------------------|------------|
|                   | Species             |                          |                                             | *S. edwardsii* | *S. markewitschi*     |            |
| Headwater reaches | Whitespotted char   | 167–237 (205)            | 2/5 (40.0)                                  | 5–9 (7.0)       | 1 (1.0)               | 15         |
|                   |                     | 164–247 (231)            | 1/8 (12.5)                                  | 0               | 1 (1.0)               | 18 Apr. 2011 |
|                   | Brook trout         | 88–225 (169)             | 10/19 (52.6)                                | 0               | 1–6 (3.0)             | 30         |
|                   |                     | 180–202 (192)            | 3*                                          | 0               | 2–8 (6.0)             | 18         |
|                   |                     | 82–235 (130)             | 1/15 (6.7)                                  | 2 (2.0)         | 0                     |         |
|                   | Masu salmon         | 64–82 (72)               | 0/10 (0)                                    | 0               | 0                     |            |
|                   |                     | 94–145 (120)             | 0/2 (0)                                     | 0               | 0                     |            |
|                   |                     | 72–178 (169)             | 0/5 (0)                                     | 0               | 0                     |            |
|                   | Rainbow trout       | 66–212 (107)             | 0/8 (0)                                     | 0               | 0                     |            |
|                   |                     | 84–149 (117)             | 0/3 (0)                                     | 0               | 0                     |            |
|                   |                     | 162–210 (190)            | 0/3 (0)                                     | 0               | 0                     |            |
| Upper reaches     | Whitespotted char   | 164 (164)                | 0/1 (0)                                     | 0               | 0                     | 0          |
|                   | Rainbow trout       | 84–113 (96)              | 0/5 (0)                                     | 0               | 0                     | 0          |
| Middle reaches    | Whitespotted char   | 112 (112)                | 0/1 (0)                                     | 0               | 0                     | 0          |
|                   | Masu salmon         | 119–122 (121)            | 0/3 (0)                                     | 0               | 0                     | 0          |
| Lower reaches     | Masu salmon         | 115–142 (129)            | 0/38 (0)                                    | 0               | 0                     | 0          |
| Near the river mouth | Masu salmon       | 97–142 (118)             | 0/11 (0)                                    | 0               | 0                     | 0          |

*Only infected fish were examined.*

**Table 2.** Occurrence of *Salmincola markewitschi* on brook trout of four fork-length classes in the Nishibetsu River, eastern Hokkaido, Japan.*

| Fork-length class in mm | No. of fish infected/examined (% prevalence) | Intensity (mean) |
|------------------------|---------------------------------------------|-----------------|
| 50–99                  | 0/3 (0)                                     | 0 (—)           |
| 100–149                | 1/3 (33.3)                                  | 1 (1.0)         |
| 150–199                | 3/6 (50.0)                                  | 1–6 (3.0)       |
| 200–249                | 6/7 (85.7)                                  | 1–6 (3.3)       |
| Total                  | 10/19 (52.6)                                | 1–6 (3.0)       |

*The fish were collected in the spring-fed headwater reaches on 2 October 1987.
49) on the gill arches (n = 31, 63.3%, mostly on the first and second gill arches) and on the roof and floor of the buccal cavity (n = 18, 36.7%).

**Ichani River:** Four of the six Southern Asian Dolly Varden examined [115–205 (mean, 162) mm FL] were infected with a total of eight *S. edwardsii* (prevalence, 66.7%; intensity, 1–4; mean intensity, 2.0). Specimens of *S. edwardsii* (n = 8) were found on the wall of branchial cavity (n = 1, 12.5%), the inner surface of the operculum (n = 6, 75.0%), and the gill filament (n = 1, 12.5%).

**Adult female of *Salmincola edwardsii***

Cephalothorax (Fig. 2A) oval in lateral view, with shallow constriction between anterior and posterior portions, and separated from trunk by deeper constriction. Trunk (Fig. 2A) suboval, 1.1–1.7 (mean, 1.4) mm long (n = 5). Total body length (excluding egg sacs) 2.0–2.6 (2.2) mm (n = 5).

Second antenna (Fig. 2B) biramous with conspicuous spiny pad on sympod; exopod anteriorly bulbous, unsegmented, with scattered spinules on anterior surface; endopod two-segmented; basal segment with spiny pad; distal

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*[Fig. 2. *Salmincola edwardsii*, female, NSMT-Cr 30862, from whitespotted char, Salvelinus leucomaenis, in the Nishibetsu River, eastern Hokkaido, Japan. A, specimen attached to gill filament, lateral view; B, second antenna, lateral view; C, distal half of mandible, lateral view; D, mouth cone and first maxillae, ventral view; E, bulla, posterior view; F, maxilliped, ventral view; G, palp of maxilliped, ventral view. Abbreviations: ant2, second antenna; b, bulla; c, cephalothorax; es, egg sac; ex, exopod; gf, gill filament; h1, hook 1; mc, mouth cone; mx2, second maxilla; mxp, maxilliped; p, palp; p4, process 4; p5, process 5; s, spiny pad on sympod; s2, spine 2; t, trunk. Scale bars: A, 0.5 mm; B, 50 μm; C, 10 μm; D, F, 100 μm; E, 300 μm; G, 20 μm.]*
segment with armature consisting of large process 4, small process 5, projecting hook 1, and small spine 2. Mandible (Fig. 2C) with six teeth; four distal teeth larger than two proximal teeth. First maxillae (Fig. 2D) located beside mouth cone, each biramous with three terminal papillae on endopod; central papilla largest; exopod lateral with small setule on tip. Second maxilla (Fig. 2A) cylindrical, originating from base of cephalothorax at junction with trunk; bulla (Fig. 2E) with anchor being almost circular in posterior view. Maxilliped (Fig. 2A, F, G) positioned in ventral region of cephalothorax, tapering towards tip; corpus with prominent myxal palp on medial margin (three outgrowths on anterior extremity of palp); subchela with small seta near base and patch of denticles near base of claw, ending in small claw.

Adult female of *Salmincola markewitschi*

Cephalothorax (Fig. 3A) oval with dorsal posterior swelling, shorter than trunk, and separated from trunk by short constriction. Trunk (Fig. 3A) ovoid with rounded lateral margins; shallow and deep transverse grooves on dorsal and ventral surfaces, respectively, 1.6–2.3 (mean, 2.0) mm long (n = 10). Total body length (excluding egg sacs) 2.6–3.8 (3.1) mm (n = 10).

Second antenna (Fig. 3B) biramous with small spiny pad on sympod; exopod one-segmented, with two large papillae and three or four small spines on tip; endopod two-segmented; basal segment with large spiny pad on ventral surface; distal segment armed with large hook 1, small spine 2 and tubercle 3 near base of hook 1, conical process 4, and small process 5. Mandible (Fig. 3C) with six teeth; four distal teeth larger than fifth tooth; proximal tooth smallest. First maxillae (Fig. 3D) with three terminal papillae on endopod; central papilla largest; exopod represented by lateral swelling with small setule on tip. Second maxilla (Fig. 3A) short and thick, originating from lateral side of posterior portion of cephalothorax; bulla (Fig. 3A) mushroom-shaped with stout manubrium. Maxilliped (Fig. 3E, F) positioned in ventral region of cephalothorax, tapering towards tip; corpus robust with palp.
of two outgrowths; subchela with small seta near base and barb near distal margin, ending in hooked claw.

**Discussion**

In the present study, *S. edwardsii* was collected from three species of the genus *Salvelinus*: whitespotted char and brook trout from the Nishibetsu River, and southern Asian Dolly Varden from the Ichani River. In Japan, *S. edwardsii* has been reported only from southern Asian Dolly Varden (Shedko & Shedko, 2002; Nagasawa, 2020a, b, 2021; Nagasawa & Kawai, 2020; Hasegawa *et al.*, 2022). Both whitespotted char and brook trout are reported herein as new Japanese hosts for *S. edwardsii*, and these two rivers represent new locality records for the species.

There are many records of *S. edwardsii* from brook trout in North America (Kabata, 1969, 1988; Hoffman, 1998), where this trout serves as an important host for the copepod (e.g., Mitro, 2016; Mitro & Griffin, 2018; White *et al.*, 2020). Nevertheless, in the Nishibetsu River, *S. edwardsii* was not found on brook trout in 1987 and 1997, and only one of the 15 brook trout examined in 2011 was infected with this copepod (Table 1). Two whitespotted char examined in 1987 were each infected with five and nine *S. edwardsii*. These data indicate that whitespotted char is more important than brook trout for this copepod in the river.

*Salmincola markewitschi* was originally described in 2002 using specimens from whitespotted char from northeastern Russia (Shumshu Island, Bolshoy Shantar Island, Sakhalin, and Primorye) and northern Japan (Kunashiri Island and Etorofu Island) (Shedko & Shedko, 2002). Later in Japan, the species was reported from whitespotted char from a mountain stream (Nagasawa, 2020c) and a fisheries research center, Honshu (Nagasawa & Ishiyama, 2021), and from whitespotted char, its subspecies, and brook trout reared at a salmon museum, Hokkaido (Nagasawa, 2021). Thus, the collections of *S. markewitschi* from whitespotted char and brook trout in the Nishibetsu River represent its first records from wild salmonids in Hokkaido, and the river is the first locality of its natural distribution in Hokkaido.

Whitespotted char and brook trout had *Salmincola* infection in the spring-fed headwater reaches of the Nishibetsu River, but no copepods were found on the same host species from downstream locations (Table 1). As these salmonids were easily collected and common in the headwater reaches, both *S. edwardsii* and *S. markewitschi* are inferred to have maintained their populations utilizing those fishes. Further, although the sample size of fish examined was low, prevalence and mean intensity of *S. markewitschi* showed an increasing trend with fork length of brook trout from the headwater (Table 2). This is possibly because larger (older) fish might have had more chances to get infected for a longer period.

Both masu salmon and rainbow trout are known to host *Salmincola californiensis* in the Pacific rim countries including Japan (Kabata, 1969). Nonetheless, these salmonids from the Nishibetsu River had no *Salmincola* infection, which indicates that *S. californiensis* does not occur there. In Japan, several records exist of *S. californiensis* from central Honshu (Hoshina & Suenaga, 1954; Hoshina & Nishimura, 1976; Nagasawa *et al.*, 2018), but there is only a single record of the species from Hokkaido, where it was collected in the Bekanbeushi River (Nagasawa & Urawa, 2002). It may be that *S. californiensis* has a restricted distribution range in Hokkaido.

Based on the recent findings of *S. edwardsii* on southern Asian Dolly Varden in several streams of eastern Hokkaido, the Shibetsu River has been regarded as the southernmost Asian locality of the species (Nagasawa, 2020a, locality 3 in Fig. 1). However, in this study, *S. edwardsii* was collected from whitespotted char and brook trout in the Nishibetsu River.
(locality 1 in Fig. 1), and the river is located south of the Shibetsu River. Thus, the southernmost distribution limit of *S. edwardsii* in Asia moves herein from the Shibetsu River to the Nishibetsu River.

Whitespotted char is distributed in Far East Asia from northeastern Russian to central Japan (Dunham *et al.*, 2008). Both *S. edwardsii* and *S. markewitschi* are known to use this salmonid as their host: *S. edwardsii* was collected from whitespotted char in several locations in Russia (Shedko & Shedko, 2002; Shedko *et al.*, 2005b) and in the Nishibetsu River, Japan (this paper), and *S. markewitschi* infecting whitespotted char was reported from many locations in Russia (Shedko & Shedko, 2002; Shedko, 2005; Shedko *et al.*, 2005a, b; Sokolov *et al.*, 2012; see a distribution map in Nagasawa, 2020c) and several locations in Japan (Shedko & Shedko, 2002; Nagasawa, 2020c, 2021; Nagasawa & Ishiyama, 2021; this paper). The present study did not examine whitespotted char from the Ichani River, but the species actually occurs in the river (Ishigaki, 1984). Further, it is widely distributed in Hokkaido (Fausch *et al.*, 1994). Therefore, in order to clarify the geographical distributions of *S. edwardsii* and *S. markewitschi* in Hokkaido, it is necessary to study their infection of whitespotted char over a wide area of Hokkaido including the Ichani River.

The copepod specimens collected in this study are identified as *S. edwardsii* and *S. markewitschi*. This identification was based on Kabata (1969) for *S. edwardsii* and on Shedko & Shedko (2002) for *S. markewitschi*. There are no significant differences in the morphology of the species reported by these authors and herein. The copepod specimens also correspond to the recent descriptions of each species from other localities of Japan (Nagasawa, 2020a; Nagasawa & Kawai, 2020; Hasegawa *et al.*, 2022, for *S. edwardsii*; Nagasawa, 2020c, 2021; Nagasawa & Ishiyama, 2021, for *S. markewitschi*). However, for identification of *S. markewitschi*, there is an issue that needs to be solved, because, from Hokkaido, there are records of *S. carpionis* from salmonids (Yamaguti, 1939, see Nagasawa *et al.*, 1995, for copepod identification; Nagasawa & Urawa, 2002, appendix table, footnote) and *Salmincola* sp. from whitespotted char (Hasegawa & Koizumi, 2021; Ayer *et al.*, 2022). The latter authors did not report the morphology of *Salmincola* sp. but stated that their specimens show marked morphological variations and possess the features that are found in both *S. carpionis* and *S. markewitschi*. When *S. markewitschi* was originally described by Shedko & Shedko (2002), it was differentiated from *S. carpionis*, and the present study follows these authors in identification of *S. markewitschi*. Sokolov *et al.* (2012) also regarded *S. carpionis* and *S. markewitschi* from salmonids in Sakhalin north of Hokkaido as independent species. Nonetheless, based on Hasegawa & Koizumi (2021) and Ayer *et al.* (2022), it is necessary to make a detailed examination of morphological variations in *S. markewitschi* and *S. carpionis* from Russia and Japan and to conduct a molecular analysis of these species.

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