Limnotrachelobdella okae (Hirudinida: Piscicolidae)
Parasitic on Big-scaled Redfin,
Pseudaspius hakonensis (Cypriniformes: Leuciscidae),
in Two Brackish Water Lakes, Hokkaido, Japan

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Specimens of the coastal marine fish leech Limnotrachelobdella okae (Moore, 1924) were collected from the pelvic fins and lateral body surface of big-scaled redfin, Pseudaspius hakonensis (Günther, 1877), in two brackish water lakes, Lake Tofutsu and Lake Abashiri, on the Sea of Okhotsk coast, Hokkaido, northern Japan. The external morphology of the specimens is briefly described, and the two lakes represent new locality records for the leech. Similarly to coastal marine waters, inshore brackish water lakes are suggested to be a habitat in which L. okae can complete its life cycle. Seasonal occurrence of the species may differ between subarctic Hokkaido and three temperate regions of Japan (Honshu, Shikoku, and Kyushu). The role of big-scaled redfin and its congener, Pacific redfin, Pseudaspius brandtii (Dybowski, 1872), in transporting L. okae alive from the coastal sea to brackish and fresh water bodies is discussed in terms of their diadromous migration ecology.

Key Words: marine fish parasite, fish leech, big-scaled redfin, Pacific redfin, brackish water lake, new locality records.

Introduction
The piscicolid leech Limnotrachelobdella okae (Moore, 1924) is a coastal marine species infesting various species of fishes in Far East Asia, including Japan (e.g., Blanchard 1896; Oka 1910a, b; Oka and Nagao 1965; Nagasawa et al. 2008a, b, 2009; Kuramochi and Takahashi 2010), Russia (Epstein 1962, 1964, 1987; Lukin 1976; Utevsky and Trontelj 2004; Utevsky et al. 2007; Utevsky 2008), and China (Yang 1987, 1996). When the species infests migratory fishes (e.g., salmonids), it can be transported to fresh waters (Nagasawa et al. 2010) and neritic marine waters (Nagasawa et al. 2008b). It also parazitizes marine fishes farmed in coastal waters of Japan, and is one of the important parasites in Japanese mariculture (Mizuno 1989, 2006; Nagasawa and Fukuoka 2008; Nagasawa and Hirai 2009; Nagasawa et al. 2009).

The big-scaled redfin, Pseudaspius hakonensis (Günther, 1877), previously known as Tribolodon hakonensis, is distributed in Far Eastern Asia, including Japan, Russia, China, and Korea (Froese and Pauly 2022). In Japan, this species has been well studied for its parasite fauna (Nagasawa and Katahira 2013, 2016), but information on leech infestation is limited: there are only two records of L. okae from big-scaled redfin (Nakano and Itoh 2011; Nagasawa et al. 2015). In 2018 and 2019, we collected specimens of L. okae from two brackish water lakes, Lake Tofutsu and Lake Abashiri, Hokkaido, northern Japan. We report here these collections with a brief description of L. okae using the specimens collected.
an parasites. Four leeches taken from Lake Tofutsu fish were relaxed in PBS (phosphate buffered saline) and fixed in 70% ethanol, whereas, of six leeches from Lake Abashiri fish, two were fixed in 70% ethanol under slight pressure between two slide glasses but the remaining four were not sampled.

Leech examination. Leech specimens were examined at the Aquaparasitology Laboratory, Shizuoka, and for descriptions, four and two specimens from Lake Tofutsu and Lake Abashiri, respectively, were observed with an Olympus SZX10 stereo microscope and measured for their total length (TL, from the anterior margin of the oral sucker to the posterior margin of the caudal sucker) and maximum body width (BW). Drawings were made with the aid of a drawing tube fitted on the microscope. Voucher leech specimens are deposited in the Annelida (An) collection of the National Museum of Science and Nature, Tsukuba, Ibaraki Prefecture (n = 4, NMST-An 1879 from Lake Tofutsu; n = 2, NSMT-An 1880 from Lake Abashiri). The scientific and common names of fishes mentioned in this paper follow Froese and Pauly (2022).

Results

Occurrence of leech on fish in two lakes. Two (225 and 270 mm BL) of the four big-scaled redfin (225–270 mm BL, n = 4) from Lake Tofutsu were infested each with two leeches on the pelvic fin and body surface. In particular, one leech was firmly attached by the caudal sucker to the pelvic fin, hemorrhage was evident at and around the attachment site (Fig. 1B). Moreover, one fish (230 mm BL) had no leech infestation, but there was a large scar (19 mm long and 9 mm wide) on the body surface near the pelvic fin (Fig. 1A).

Six leeches were found unattached in the plastic bag containing four individuals of big-scaled redfin (170–290 mm BL, n = 4) from Lake Abashiri.

External morphology of leech. Body distinctly divided into trachelosome and much larger urosome (Figs 1B, 2, 3A); trachelosome subcylindrical (when fresh) (Fig. 1B) or triangular (in 70% ethanol) (Figs 2, 3); urosome dorso-ventrally flattened (Figs 1B, 2, 3A); 56.1–87.0 (mean = 69.9, n = 4) mm and 51.5–61.2 (56.4, n = 2) mm TL in specimens from Lake Tofutsu and Lake Abashiri, respectively. Body widest around mid-point of urosome, tapering gradually posteriorly to caudal sucker; 8.1–13.7 (11.1) mm and 12.7–13.5 (13.1) mm BW in the above specimens. Color dark brown or black (when fresh) and whitish yellow (in 70% ethanol) (Figs 1, 2). Body surface wrinkled, lacking tubercles or papillae (Fig. 3A). Thirteen pairs of pulsatile vesicles present on lateral margins of urosome; the first pair smaller than the second; the second to eleventh pairs well developed; and the two posteriormost pairs indistinctly recognized (Figs 2B, C, 3A, F). Oral sucker eccentrically attached to trachelosome; no eyes found on dorsal surface of sucker (Fig. 3A–C). Urosome segments five-annulate (Fig. 3F). Caudal sucker much narrower than maximum body width, deeply cupped, eccentrically attached to urosome, lacking marginal ocelli (Fig. 3A, D, E). Bursa slightly everted and opens through male gonopore on ventral side of clitellum (Fig. 3A, B).

Remarks. The specimens of leech collected in this study correspond to the external morphology of *L. okae* reported from Japan (e.g., Oka 1927; Oka and Nagao 1965; Nagasawa et al. 2008b), China (e.g., Yang 1987) and Russia (e.g., Lukin 1976; Epshtein 1987; Utevsky 2008), and they are herein identified as *L. okae*. This species is readily identified by its large body which is distinctly divided into the anterior small trachelosome and the posterior large urosome, dark brown or black pigmentation of body (when fresh), and the presence of 11 pairs of large, lateral pulsatile vesicles. Due to the preservation of the specimens in 70% ethanol, their original pigmentation faded: the body color changed from dark brown or black to whitish yellow (Fig. 2), and no eyes were visible on the oral sucker (Fig. 3C), although two pairs of eyes have been reported from the species (Oka 1927; Epshtein 1987). Further, a small female gonopore is known to ventrally open near the posterior margin of the clitellum (Oka 1927; Oka and Nagao 1965; Utevsky 2008: pl. V, fig. 5), but it was invisible in the specimens examined in this study. The internal morphology of *L. okae* has been described in detail by Epshtein (1987) and Utevsky (2008).

Both Lake Tofutsu and Lake Abashiri, where we collected
the specimens of *L. okae*, represent new locality records for the species. Although the leech is a coastal marine species, it is very tolerant of low salinity: it has been reported to survive for at least three months in fresh waters (Nagasawa et al. 2010). Thus, it is likely that *L. okae* can survive for more than three months in the two lakes investigated in this study. Moreover, as discussed below, the species may be able to complete its life cycle in such brackish water lakes in Japan.

**Discussion**

Big-scaled redfin was found infested with *L. okae* in this study. This cyprinid spawns in freshwater streams, but it is so euryhaline that it can be found in coastal marine and brackish waters as well as in fresh waters (Tabeta and Tsukahara 1964; Sakai 1995; Ishizaki et al. 2009). In Hokkaido, its diadromous individuals migrate to the sea at age 2, grow there, and return to streams for reproduction at ages 4–10 (Sakai 1995). Based on a relationship of age and body size of the species (Sakai 1995), the fish individuals examined in this study are regarded as being at age 4 and older. Therefore, we infer that they became infested with *L. okae* during their residence at sea and then migrated to the lakes where they were caught. Since big-scaled redfin undertakes a further upstream migration to freshwater spawning areas, *L. okae* may be transported by big-scaled redfin to the streams flowing into the lakes.

*Limnotrachelobdella okae* has been recorded twice from big-scaled redfin. From a collection of leech specimens deposited for a long period in The University Museum of The University of Tokyo, Nakano and Itoh (2011) discovered eight specimens of *L. okae*, which were taken from the fins of big-scaled redfin in Tokyo in 1884. In this prefecture, the leech was reported in 1896 as "*Trachelobdella sinensis*" based on a specimen from Tokyo Bay without host information (Blanchard 1896: 317–318, fig. 2; see Moore 1924: 345). Another record of *L. okae* from big-scaled redfin was based on a collection in brackish Lake Shinji, Shimane Prefecture, western Japan (Nagasawa et al. 2015).

Pacific redfin, *Pseudaspius brandtii* (Dybowski, 1872), is a congener of big-scaled redfin and is one of the known hosts of *L. okae* (Epshtein 1964, 1987; Nagasawa et al. 2008a).
The leech was collected from Pacific redfin in the Khivanda River, a tributary of the lower Amur River, Russia (Epshtein 1964) and in Iwafune Fishing Port facing the Sea of Japan, Niigata Prefecture, Japan (Nagasawa et al. 2008a). Like big-scaled redfin, Pacific redfin spawns in fresh waters and migrates between coastal marine waters and inland streams (Sakai 1995). We thus believe that this diadromous fish species also can carry *L. okae* from the sea to brackish water bodies and then to freshwater streams.

There are two records of *L. okae* from Hokkaido: it was collected from a Japanese huchen, *Parahucho perryi* (Brevoort, 1852), near the mouth of the Sarufutsu River (Furiness et al. 2007) and from a sockeye salmon, *Oncorhynchus nerka* (Walbaum, 1792), in the middle reaches of the Bibi River (Nagasawa et al. 2010). Nagasawa et al. (2010: 37) suggested that the Japanese huchen became infested with *L. okae* at sea before migrating to the Sarufutsu River. Further, according to the same authors, the infested sockeye salmon was a precocious adult that had stayed in coastal waters of the Northwestern Pacific Ocean and, when it returned from the sea for spawning, *L. okae* was transported alive to the middle reaches of the Bibi River.

In this study, *L. okae* was found firmly attached to the pelvic fin of big-scaled redfin, and hemorrhage was found at and around the site of the caudal sucker attachment (Fig. 1B). A similar hemorrhagic lesion has been found at the attachment site of *L. okae* on Japanese eel, *Anguilla japonica* Temminck and Schlegel, 1846, from western Japan (Nagasawa and Utsumi 2015). Hemorrhage is one of the major symptoms found at the attachment sites of piscicolid and glossiphoniid leeches on fishes (e.g., Roubal 1986; Volantetrio et al. 2004; Schulz et al. 2011). Moreover, in this study, a large skin scar was found near the pelvic fin of big-scaled redfin without leech infestation (Fig. 1A). Nagasawa et al. (2008b) reported the presence of a large skin hemorrhagic erosion at the attachment site of *L. okae* on masu salmon, *Oncorhynchus masou* (Brevoort, 1856) (reported as cherry salmon, *O. m. masou*), from the Northwestern Pacific Ocean. Unlike this erosion, the scar found in this study was not hemorrhagic (Fig. 1A) but, based on its size, coloration, and location near the pelvic fin, it was most probably formed from a wound caused by *L. okae*. Mizuno (1989, 2006) suggested that a large skin erosion associated with leech infestation results in a secondary bacterial infection. To date, no pathological work has been made for fishes infested with *L. okae*, and it is necessary to assess the impact
of the species on a host.

In addition to Lake Tofutsu and Lake Abashiri, *L. okae* is also known to occur in two brackish water lakes, western Japan, i.e., Lake Shinji and Lake Nakauami (Yamauchi et al. 2008; Nagasawa and Nakano 2013; Nagasawa et al. 2015). Saline waters flow from the Sea of Japan into Lake Nakauami through a channel (ca. 7.5 km long) and then into Lake Shinji through a short river (ca. 7.6 km long); thus, Lake Shinji is less brackish than Lake Nakauami (Ishitobi et al. 2000). In Lake Shinji, *L. okae* was found infesting big-scaled redfin, gin-buna, *Carassius langdorffii* Temminck and Schlegel, 1846 (reported as *Carassius* sp.), and an unidentified crucian carp, *Carassius* sp. (Nagasawa et al. 2015). Crucian carps including gin-buna stay in Lake Shinji and do not move to Lake Nakauami (Ishitobi et al. 2000), which implies that *L. okae* can complete its life cycle in Lake Shinji using non-migratory fishes as its hosts. In order to understand this, more research is needed on the infestation of *L. okae* on migratory and non-migratory fishes in the two and other brackish water lakes, including Lake Tofutsu and Lake Abashiri.

Based on the collections of *L. okae* from four prefectures (Tokyo, Kanagawa, Yamagata, and Okayama) in Honshu, Nagasawa et al. (2009) report that fish-infesting individuals of the species were found from the winter to mid-spring (January to April, mostly in March). Although there are several records of small individuals collected in March from Shimane Prefecture (21 mm TL, Nagasawa et al. 2015) and in June from Mie and Okayama prefectures (13.2 and 12.0 mm TL, respectively, Nagasawa et al. 2013), large, fish-infesting individuals have been collected during the winter to mid-spring from four more prefectures in Honshu (from the northeast to the southwest), i.e., March from Iwate Prefecture (117 mm TL, Nagasawa et al. 2008b), March from Mie Prefecture (110.1–112.2 mm TL, Nagasawa and Sakaoka 2009), March and April from Shimane Prefecture (45–113 mm TL, Yamauchi et al. 2008; Nagasawa and Nakano 2013; Nagasawa et al. 2015), and April from Yamaguchi Prefecture (91–112 mm TL, Yamauchi et al. 2010). Moreover, similar large individuals have been sampled from fishes between December and April from Ehime Prefecture in Shikoku (55.7–150 mm TL, Mizuno 1989, 2006; Nagasawa and Hirai 2009) and, also in Kyushu, from January to March from Oita Prefecture (44.0–76.8 mm TL, Nagasawa and Fukuoka 2008; Yamauchi et al. 2010; Nagasawa and Utsumi 2015) and in February from Miyazaki Prefecture (56 mm TL, Yamauchi et al. 2010). Shikoku and Kyushu are located southwest of Honshu. Nonetheless, in contrast to these winter-spring collections from various prefectures of three regions (Honshu, Shikoku, and Kyushu), specimens of *L. okae* infesting fishes were all collected during the summer in Hokkaido, the northernmost region of Japan: August (no information on TL, Furiness et al. 2007), “summer” (68.5–94.8 mm TL, Nagasawa et al. 2010), and July (51.5–87.0 mm TL, this study). Hokkaido and the three other regions lie in the subarctic and temperate regions, respectively, and such climatic differences within the distribution range of *L. okae* may be related to the observed seasonal differences in its occurrence on fish hosts. In other words, the life cycle of *L. okae*, including its growth, maturation, and timing and duration of infestation, is likely to differ between subarctic Hokkaido and the three temperate regions. In order to clarify this, it is desirable to conduct a comparative study on the life cycle of *L. okae* in the subarctic and temperate regions.

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