

## ***Limnotrachelobdella sinensis* (Hirudinida: Piscicolidae) parasitic on Japanese crucian carp (*Carassius cuvieri*) in game-fishing ponds in central Japan**

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**Abstract.** *Limnotrachelobdella sinensis* (Blanchard, 1896) was found infesting Japanese crucian carp (*Carassius cuvieri*) held in game-fishing ponds in Osaka Prefecture, central Japan. This finding constitutes the first record of *L. sinensis* from non-wild fish in Japan. Leeches occurring on the inner surface of the host's operculum were large and their attachment sites were close each other, while those found in other parts of the host were small and not aggregated. Leeches are estimated to grow at a rate of 0.45 mm per day from early February to mid-April.

**Key words:** *Limnotrachelobdella sinensis*, Piscicolidae, leech, fish parasite, Japanese crucian carp, *Carassius cuvieri*, game-fishing ponds

### **Introduction**

The piscicolid leech *Limnotrachelobdella sinensis* (Blanchard, 1896) is a parasite of cyprinid fishes in Far East Asia (Epstein, 1987; Yang, 1996; Nagasawa *et al.*, 2008). In Japan, this leech so far has been reported only from wild populations of three species of the cyprinid subfamily Cyprininae (Japanese crucian carp *Carassius cuvieri* Temminck & Schlegel, 1846; silver crucian carp *Carassius auratus langsdorfii* Temminck & Schlegel, 1846; and common carp *Cyprinus carpio carpio* Linnaeus, 1758) in the Yodo River and its tributaries in Osaka and Kyoto Prefectures (Ogawa *et al.*, 2007; Nagasawa & Tanaka, 2009, 2011; Ogawa, 2011).

In December 2010, we were informed by the manager of game-fishing ponds in Osaka Prefecture that many individuals of *C. cuvieri* held in the ponds

harbored large parasites in the gill cavities and the swimming of such fish was very inactive. Thus, we collected the parasites in the game-fishing ponds and identified them as *L. sinensis*. This paper reports on the infestation of *L. sinensis* on *C. cuvieri* in the game-fishing ponds.

### **Materials and Methods**

Fifteen individuals of *C. cuvieri* collected in the game-fishing ponds in Yao City, Osaka Prefecture, on 8 and 14 February 2011 were transported alive to Hiroshima University, Higashi-Hiroshima City, Hiroshima Prefecture, where they were held in an outdoor concrete tank (2.1 × 1 × 1 m, water depth = 0.6 m) without feeding until 14 April 2011. These fish were sampled, measured for standard length (SL, cm) and examined for parasites on 10 February (*N* = 9), 23 February (*N* = 1), 5 March (*N* = 4), and 14 April (*N* = 1). Leeches were removed from the fish, relaxed with the gradual addition of ethanol, and fixed in 5% formalin. Their attachment sites were

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recorded in February and March 2011. Leeches were identified based on Ogawa *et al.* (2007) and Nagasawa & Tanaka (2009), and measured for total length (TL, mm, including the suckers). An equation of a straight line to fit the growth of leeches was obtained using TL data, based on a least squares method. Representative specimens are deposited in the Annelida collection at the National Museum of Nature and Science, Tsukuba (NSMT–An 417). The scientific and English names of fishes follow Froese & Pauly (2012), except for the English name of *C. auratus langsdorfii*.

### Results

All of the 15 fish examined (20.5–30.0 [mean 23.6] cm SL) were found infested by a total of 140 leeches, and the number of leeches found on an infested fish ranged from 1–21 (mean = 9.3). Large leeches were attached to the inner surface of the host's operculum, and their anterior body was extruded from the gill cavity (Fig. 1)

Of the 135 leeches collected in February and March 2011, 113 (83.7%) occurred on the inner surface of the host's operculum, but 22 (16.3%) were found in other parts: 9 (6.7%) on the body surface near the pectoral fins, 7 (5.2%) on the outer surface of the operculum, 5 (3.7%) around the lips, and 1 (0.7%) on the wall of the branchial cavity. There was no difference in total number of leeches between left ( $N = 56$ , 41.5%) and right ( $N = 57$ , 42.2%) inner surfaces of the operculum. Leeches occurring on the inner surface of the operculum were large, and their attachment sites were close each other (Fig. 2). However, leeches found in other parts were usually small and were not aggregated.

The leeches collected from 10 February to 14 April 2011 measured 12–54 (mean = 31.0) mm TL ( $N = 135$ ): they steadily grew during this period (25.4 mm in mean TL on 10 February; 37.8 mm on 23 February; 34.3 mm on 5 March; and 56.0 mm on 14 April) (Fig. 3). A linear regression was well fitted to TL data of leeches, and the equation is  $y = 0.45x + 27.19$  ( $R^2 = 0.93$ ), where  $y$  is the TL (mm) and  $x$  the

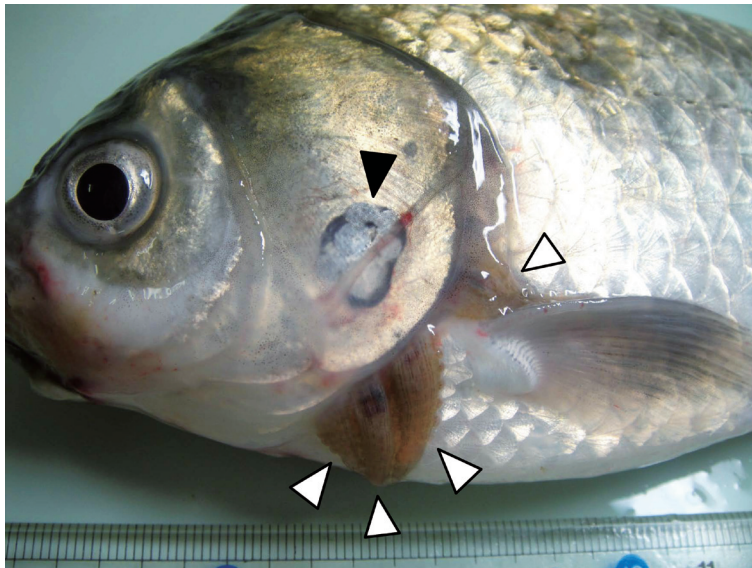


Fig. 1. *Limnotrachelobdella sinensis* (Blanchard, 1896) infesting a Japanese crucian carp (*Carassius cuvieri* Temminck & Schlegel, 1846) from the game-fishing pond in Osaka Prefecture, central Japan. The anterior bodies of four leeches (white arrowheads) were extruded from the host's gill cavity. Their attachment sites are seen as a mark (black arrowhead). Scale in mm.

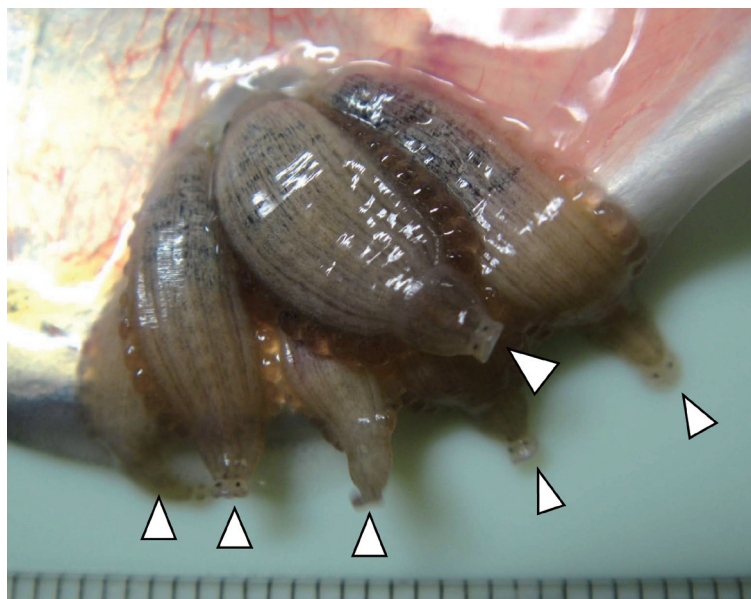


Fig. 2. Six individuals (white arrowheads) of *Limnotrachelobdella sinensis* (Blanchard, 1896) on the inner surface of the operculum of a Japanese crucian carp (*Carassius cuvieri* Temminck & Schlegel, 1846). Note leeches occurring close together at attachment sites. Scale in mm.

age (days,  $x = 0$  on 10 February 2011). This equation indicates that leeches grow at a rate of 0.45 mm per day and that, if the growth of leeches fits the equation, they hatch in mid-December although the size of hatchlings is unknown.

### Discussion

*Limnotrachelobdella sinensis* is a recently discovered parasite of cyprinid fishes in Japan: it was first found in the Yodo River, Osaka Prefecture, in January 2000 (Ogawa *et al.*, 2007). Since then, its distribution has been expanding to several tributaries of the river in Osaka and Kyoto Prefectures (Nagasawa & Tanaka, 2009, 2011). In Japan, this leech has been reported only from wild cyprinids, and the present finding in the game-fishing ponds represents the first record of the species from non-wild fish in this country. The leech is known to infest farmed *C. carpio carpio* in Korea and China (Rhee, 1986; Yang, 1996).

The route of introduction or invasion by *L. sin-*

*ensis* to the game-fishing ponds is unknown. According to the manager of the game-fishing ponds, leeches were first recognized on *C. cuvieri* in November 2009 and have since occurred in every cold-water season. Because the geographical distribution of *L. sinensis* in Japan is restricted to Osaka and Kyoto Prefectures, it is very likely that some infested fish were transported to the ponds from somewhere in these prefectures. The manager told us that several individuals of *C. carpio carpio* were introduced to the game-fishing ponds from a private pond in Osaka Prefecture in 2007 or 2008 and these fish might have been infested. On the other hand, live individuals of *C. cuvieri* are supplemented to the game-fishing ponds several times per year from reservoirs from around Takamatsu City, Kagawa Prefecture, Shikoku, but the manager said that these fish had no leech infestation when they arrived in the ponds. Moreover, it is unlikely that wild infested fish invaded to the ponds, because the water used in the ponds is taken from the ground and there is a big filter to clean the water before discharge.

Ogawa *et al.* (2009) stated that *L. sinensis* has a one-year life cycle in the Yodo River, central Japan: eggs hatch in late autumn or early winter, hatched leeches begin infesting fish hosts in December, and mature leeches leave the hosts by April for laying eggs. The manager of the game-fishing ponds mentioned that leeches are found from November to April ever year. In the present study, from February to April 2011, we collected leeches from individuals of *C. cuvieri* kept in Hiroshima University after transportation from the game-fishing ponds. Based on the equation ( $y = 0.45x + 27.19$ ,  $x = 0$  on 10 February 2011) obtained in this study, leeches are estimated to hatch in mid-December and grow from early February to mid-April at a rate of 0.45 mm per day. This estimation and the observations by the manager and us suggest that *L. sinensis* has a similar pattern of the life cycle in the game-fishing ponds as in the Yodo River. Nonetheless, since the sample size of leeches collected in April 2011 was quite small ( $N = 5$ ), it is desirable to collect more leech specimens in order to obtain a more reliable

equation. If leeches are smaller in April than those (56.0 mm in mean TL) collected in this study, they are considered to hatch earlier than in mid-December, which is consistent with the observations by the manager.

According to Sawyer (1986), the growth of leeches is of two basic types (continuous and saltatory), and continuous growth is the most generalized growth pattern in leeches. Based on the growth pattern found in this study, *L. sinensis* is regarded as a species that feeds frequently on the host for its continuous growth.

In this study, we did not measure water temperature (WT) in the outdoor concrete tank in which the infested fish were kept, but WT should have remained low because the water was often ice-covered in February 2011. Also, the manager of the game-fishing ponds said that WT decreases to around 3°C there in February. Thus, it is clear that *L. sinensis* actively feeds and grows well under such low WT conditions.

Various aspects of clinical and pathological fea-

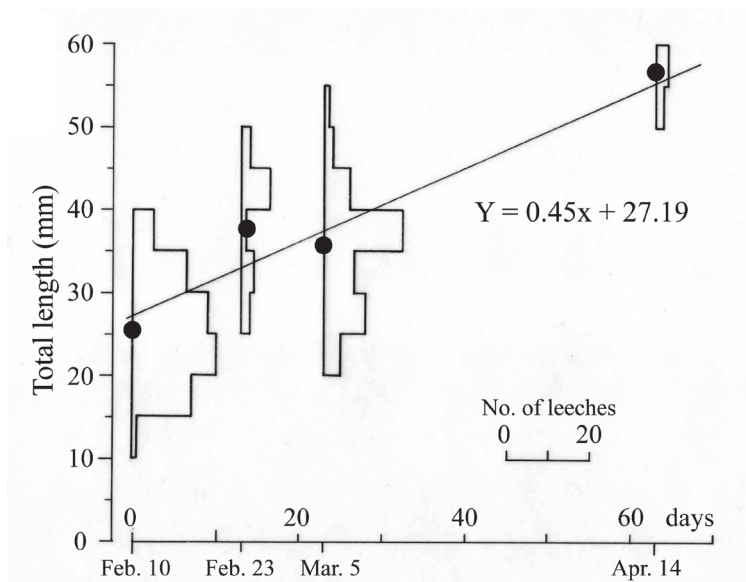


Fig. 3. Changes in total length frequency distribution of specimens of *Limnotrachelobdella sinensis* (Blanchard, 1896) from Japanese crucian carp (*Carassius cuvieri* Temminck & Schlegel, 1846) from 10 February to 14 April 2011. Closed circles show mean total lengths. A straight line ( $y = 0.45x + 27.19$ ) to fit the growth of the leech is shown.

tures of fishes infested by *L. sinensis* have been reported (Park & Kim, 2002; Ogawa & Tanaka, 2007; Nagasawa *et al.*, 2009; Park *et al.*, 2010, 2011). The blood feeding by this parasite can cause anemia in the infested host, as reported by Park & Kim (2002). In particular, leeches are large in March and April, and their feeding may result in severe anemia when their numbers are high.

A considerably large variation in size of leeches was observed for each sampling (Fig. 3). A small specimen (12 mm TL) was collected even on 10 February. This may indicate that, although the main infestation appears to occur in November or December, leeches infest the hosts over a lengthy period, as earlier suggested by Nagasawa & Tanaka (2009, 2011).

It is interesting to note that large and small leeches used different parts of the host's body surface. Two explanations may be possible for this observation. One explanation is that small leeches first attach to various parts of the host and then move to the latter region with growth. Another explanation is that small leeches are excluded by large leeches from the inner surface of the operculum and do not grow well because such small leeches cannot take enough blood meals from the host's gills. If the latter explanation is the case, the small leeches found in mid-February, unlike the suggestion in the above paragraph, may not be individuals that have newly infested.

Large leeches were observed to occur close together at their attachment sites on the inner surface of the host's operculum. A similar observation is found in Park & Kim (2002: fig. 2), Ogawa *et al.* (2007: fig. 2) and Park *et al.* (2010: fig. 2D), where five, two and three individuals of *L. sinensis* were attached to a narrow area of the operculum, respectively. Moreover, Park *et al.* (2011: table 1, fig. 2B) found as many as 14 leeches on the inner surface of the operculum. Several piscicolid species are known to aggregate in certain sites of the host's body surface (e.g., Paperna & Zwerner, 1974; Cruz-Lacierda *et al.*, 2000). Such aggregation appears to be beneficial to cross-fertilization between mature individu-

als of *L. sinensis*.

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