

## Biological Notes on *Hyphydrus laeviventris* SHARP, 1882 (Coleoptera, Dytiscidae)

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**Abstract** *Hyphydrus laeviventris* SHARP, 1882 (Dytiscidae), collected from Ishikawa Prefecture, were reared in the laboratory. They laid eggs singly on the surface of the main axes and branches of Characeae, and seven larvae hatched. The developmental period for each stage was as follows: egg, within 5 days; 1st instar larva, 3–4 days; 2nd instar larva, 3–5 days; 3rd instar larva, 7–14 days; landing to pupation, 2 days; pupation to emergence, 4 days; and landing to escaping, 8–9 days. All the larvae that preyed on only seed shrimps were able to complete their larval development, which suggests that seed shrimps alone can provide the nutritional value needed for development.

**Key words:** conservation, diving beetle, endangered species, larvae, red list.

### Introduction

*Hyphydrus laeviventris* SHARP, 1882 (total length: 4.5–5.0 mm), is a Japanese member of the Dytiscidae and the endemic species is listed as “Vulnerable” in the national Red List for Japan (Ministry of the Environment of Japan, 2015, 2020). The larval morphology of this species is similar to *H. japonicus* SHARP, 1873, *H. lyratus* Swartz, 1808, and *H. pulchellus* CLARK, 1863 (NAKANISHI, 2001; MITAMURA *et al.*, 2017; HAYASHI, 2020). The life history of *H. laeviventris* is poorly understood; however, it is known that it overwinters at the adult stage, and the larvae are found between April and June (HAYASHI, 2015; MITAMURA *et al.*, 2017; NAKAJIMA *et al.*, 2020).

Information about the life history of a target species is indispensable if the endangered species is to be conserved. One of the most effective ways of investigating the life history of small species is to observe them under laboratory rearing conditions. Unlike in the field, larvae obtained under laboratory conditions can be identified as being from the research species. For example, WATANABE *et al.*, (2017) used laboratory rearing conditions to elucidate the reproductive ecology of *Copelatus parallelus* ZIMMERMANN, 1920, which is a dytiscid species listed as “Critically Endangered” in the national Red List for Japan. The eggs and 1st instar larvae were small, which meant that it would have been very difficult to initially locate them in the field. In addition, WATANABE (2019) and WATANABE and HAYASHI (2019) investigated the ecology of *Laccophilus nakajimai* KAMITE, HIKIDA et SATÔ, 2005 and *Copelatus masculinus* RÉGIMBART, 1899, which are also small dytiscid species, using observations obtained under laboratory rearing conditions.

In this study, I attempted to rear *H. laeviventris* in the laboratory to reveal the basic biology of this species. I report on its reproductive ecology and illustrate the egg and 1st to 2nd instar larval stages of *H. laeviventris* for the first time.

### Materials and Methods

More than twenty *Hyphydrus laeviventris* adults were collected from Awazu, Misaki-machi, Suzu-shi, Ishikawa Prefecture, Japan, on April 18, 2019. All the individuals were reared in a glass tank (95 cm × 45 cm × 45 cm, water depth: ca. 30 cm) at the Ishikawa Insect Museum, Hakusan, Ishikawa

Table 1. The period for each developmental stage of *Hyphydrus laevis*.

Sample No.	Period (days) of each developmental stage from egg to escaping							Note
	Egg	1st instar	2nd instar	3rd instar	Landing to pupation	Pupation to emergence	Landing to escaping	
1	4	3	5	10	2	4	8	
2	4	3	3	11	—	—	8	
3	1	4	4	14	—	—	9	
4	4	4	5	12	—	—	8	
5	5	4	5	7	—	—	8	
6	1	4	3	12	2	●	—	Surface was molded
7	2	3	4	9	—	●	—	Surface was molded
Mean $\pm$ SD	3.0 $\pm$ 1.5 (n = 7)	3.6 $\pm$ 0.5 (n = 7)	4.1 $\pm$ 0.8 (n = 7)	10.7 $\pm$ 2.1 (n = 7)	2.0 (n = 2)	4.0 (n = 2)	8.2 $\pm$ 0.4 (n = 2)	

Black circle (●) represents an individual died at the corresponding developmental stage.

Prefecture, Japan, on April 19, 2019. The tank had been planted with Characeae sp. and *Ceratophyllum demersum* (Ceratophyllaceae), and was maintained at 28°C with 9 h of light (from 8:15 am to 5:15 pm, JST) and 15 h of darkness (9 L : 15 D). Adequate quantities of frozen chironomid larvae were provided to the adults as prey every 2–3 days. The glass tank was checked every 1–3 days. The date and developmental stages were recorded for any eggs that were found. The eggs were then transferred to plastic cups (called rearing cups, 8 cm in diameter, 4 cm in height, with a water depth of ca. 5 mm), which contained Java moss. These cups were maintained at 26°C with 9 h of light (from 8:15 am to 5:15 pm, JST) and 15 h of darkness (9 L : 15 D). The larvae were then individually reared in the cups.

*Hyphydrus japonicus* SHARP, 1873, which is in the same genus as *H. laevis*, is known to eat seed shrimps (HAYASHI & OHBA, 2018). Therefore, living seed shrimps that had been bred in the laboratory were provided as prey during the larval stage. Each individual larva was provided with about 30 live seed shrimps a day. I carefully placed 3rd instar larvae in pupation cups (the same size as the larval cups but containing a ca. 1 cm deep layer of crushed and moistened peat moss) when they stopped eating prey items and walked without stopping.

The dates of hatching, moulting, transition to the soil, and when newly emerged adults escaped to the soil surface were recorded in detail for each individual. The rearing experiment was terminated on May 31, 2019.

Photographs were taken using a Nikon D500 digital camera equipped with a Nikon AF-S VR MICRO-NIKKOR 105 mm f/2.8G ED and a Laowa ULTRA MACRO 25 mm f/2.8 2.5–5 $\times$  lens.

## Results

The first egg was found on April 23, four days after adult rearing. The females laid eggs until April 29, 2019, and a total of seven eggs were found over the ten days. The eggs were laid singly on the surfaces of the main Characeae sp. axes and branches during this period (Fig. 1A). All seven eggs were fertile. The egg period was 1–5 days (3.0  $\pm$  1.5 days, n = 7) (Table 1). The egg size was 0.7 mm long and 0.4 mm wide (n = 1).

All larvae started to prey on living seed shrimps from the hatching day without destroying their shells (Fig. 1E–F). The period of each developmental stage was as follows: 1st instar (Fig. 1B, E), 3–4 days (mean  $\pm$  SD = 3.6  $\pm$  0.5 days, n = 7); 2nd instar (Fig. 1C), 3–5 days (4.1  $\pm$  0.8 days, n = 7); 3rd

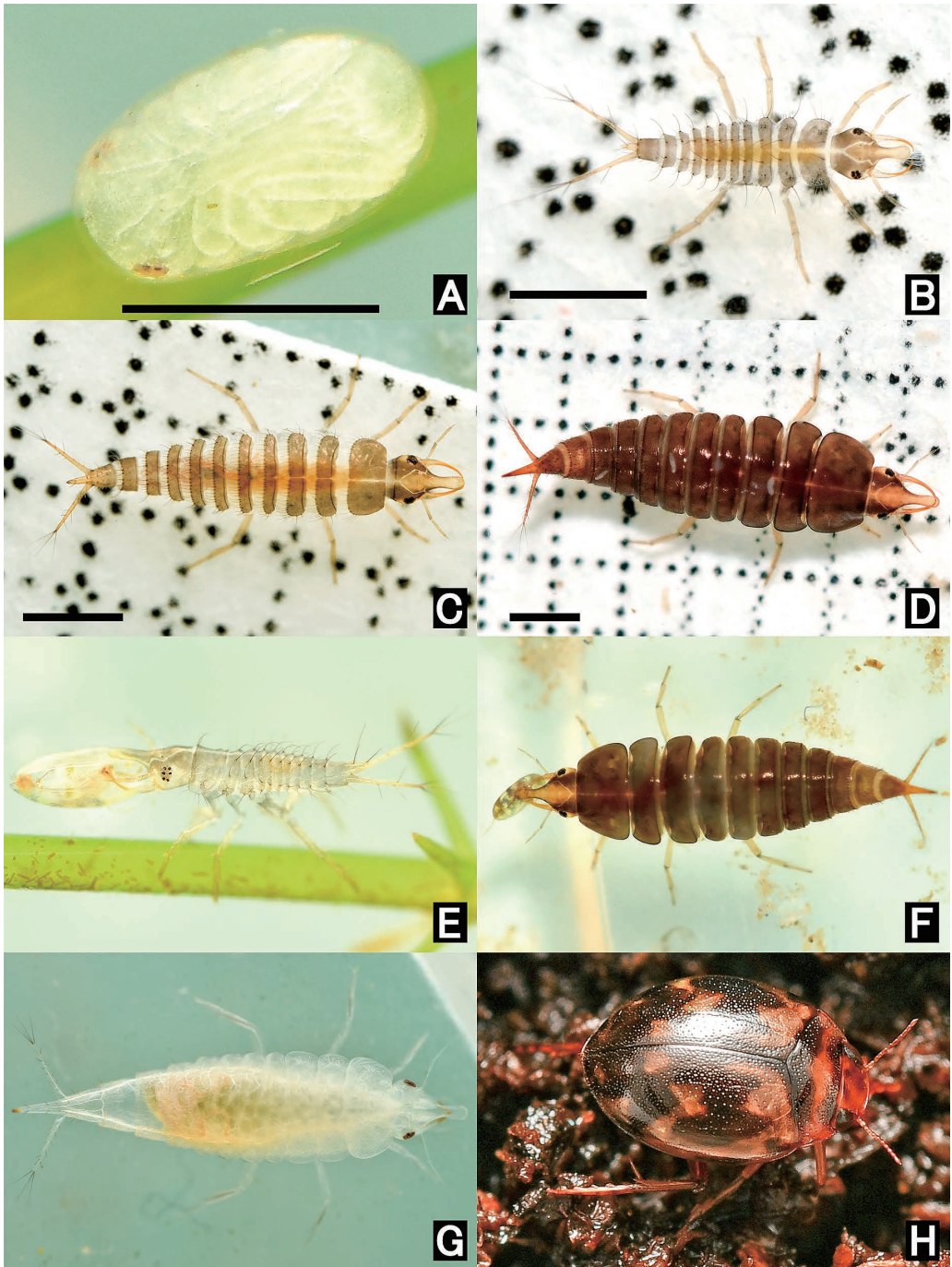


Fig. 1. *Hyphydrus laeiventris*. — A, An egg laid on Characeae sp.; B, 1st instar larva; C, 2nd instar larva; D, 3rd instar larva; E, 1st instar larva preying on a living seed shrimp; F, 3rd instar larva preying on a living seed shrimp; G, 3rd instar larva immediately after molting; H, new adult escaping to the soil surface. Scale bars: 0.5 mm for A; 1.0 mm for B–D.

instar (Fig. 1D, F–G), 7–14 days ( $10.7 \pm 2.1$  days,  $n = 7$ ); and total larval period, 16–22 days ( $18.4 \pm 2.2$  days,  $n = 7$ ) (Table 1).

After transitioning to land, the larvae dug into the soil and made a pupal chamber. The period of each stage after landing was as follows: landing to pupation, 2 days ( $n = 2$ ); pupation to emergence, 4 days ( $n = 1$ ); and landing to escaping, 8–9 days ( $8.2 \pm 0.4$  days,  $n = 5$ ) (Table 1). All newly emerged adults escaped from the pupal chambers to the soil surface. These adults began to feed on frozen chironomid larvae from the day they escaped from the pupal chambers. The total period of the immature stages was 29–33 days ( $30.6 \pm 1.6$  days,  $n = 5$ ). The larval survival rate was 100% ( $n = 7$ ), the pupation rate was 100% ( $n = 7$ ), and the emergence rate was 71% ( $n = 7$ ). The body length of the newly emerged adults was almost the same as that of the parental generation adults collected in the field.

### Discussion

The egg periods observed in this experiment ranged from 1–5 days. However, not all the eggs were found on the laying day. Most eggs were confirmed during their development. This study was based on a small sample size, and it is reasonable to conclude that the egg period within five days. In the field, larvae are found from April to June (HAYASHI, 2015; MITAMURA *et al.*, 2017; NAKAJIMA *et al.*, 2020). As the total larval period was 16 to 22 days in this study, it is possible that the new adults emerge from May to July in the field. In the pupal stage, two dead pupae grew mold on their surface, which suggested that these deaths were due to excessive soil humidity.

The prey consumed by aquatic Coleoptera larvae varies depending on the species. For example, *Hydrophilus acuminatus* MOTSCHULSKY, 1854 larvae (Hydrophilidae), known as specialist predators of snails, died during the 1st instar when only *Palaemon* (Decapoda, Palaemonidae), *Prosilocerus* larvae (Diptera, Chironomidae), and *Asellus* (Isopoda, Asellidae) were provided as prey (INODA *et al.*, 2015). OHBA (2009) reported that all *Cybister brevis* AUBÉ, 1838 larvae (Dytiscidae) provided with only tadpoles died in the rearing experiment. In this study, although the number of samples was small, all the *Hyphydrus laevis* larvae that were provided with only seed shrimps were able to complete their larval development. This suggests that seed shrimps can provide the nutritional value needed for the development of this species. These results support the unique predatory behavior reported by HAYASHI and OHBA (2018). *Hyphydrus japonicus* larvae also use a projection on the head to attack seed shrimps without destroying their shells (HAYASHI & OHBA, 2018). Therefore, I can conclude that *H. laevis* larvae prey on seed shrimps as one of the prey animals in the field. Larvae in the genus *Hyphydrus*, including this species, generally have head appendages (e.g., NAKANISHI, 2001; MITAMURA *et al.*, 2017), which suggests that the other larvae in the genus *Hyphydrus*, including some endangered species in Japan, also prey on seed shrimps in the field.

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## 要 約

渡部晃平：ヒメケシゲンゴロウ（鞘翅目ゲンゴロウ科）の生態的知見。——絶滅が危惧されている日本固有種のヒメケシゲンゴロウ *Hyphydrus laeiventris* SHARP, 1882 の繁殖生態を解明することを目的として飼育実験を行った。飼育下において7つの卵が得られ、本種はシャジクモ科植物の表面に卵を1つずつ産卵した。各発育段階における成育期間は卵（5日以内）、1齢幼虫（3~4日）、2齢幼虫（3~5日）、3齢幼虫（7~14日）、上陸~蛹化（2日）、蛹化~羽化（4日）、上陸~羽化脱出（8~9日）であった。カイミジンコだけを捕食した全ての幼虫が上陸に至るまで成育し、カイミジンコは本種の成育に十分な栄養を備えていることが判明した。この実験結果と、同属種ケシゲンゴロウ *H. japonicus* SHARP, 1873 の幼虫がカイミジンコを効率良く捕食することを示した先行研究の結果から推測すると、本種の幼虫は自然環境下においても餌の一つとしてカイミジンコを利用しているものと考えられる。

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