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Biological Notes on Immature Stages of *Laccophilus vagelineatus* Zimmermann, 1922 (Coleoptera, Dytiscidae)

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Abstract *Laccophilus vagelineatus* Zimmermann, 1922 (Coleoptera, Dytiscidae) is currently enumerated as "Endangered" in the national Red List for Japan. To determine the lengths of the immature stages of *L. vagelineatus*, *L. vagelineatus* individuals were reared at 26°C in the laboratory. The females laid eggs singly into the main stems of *Pogostemon* sp. (Lamiaceae). The complete development of *L. vagelineatus* was observed 33 to 35 days (n = 7) as follows: egg (duration: 7 days, n = 7); first instar larva (3 to 7 days, n = 15); second instar larva (2 to 7 days, n = 15); third instar larva (5 to 12 days, n = 15); and pupa (3 days, n = 1). The stadium from construction of a pupal chamber to pupation was 3 days (n = 1). That from construction of a pupal chamber to exiting of an adult from a pupal chamber was 8 to 11 days (n = 13). The presence of eggs and larvae in a rearing cup containing only females that were collected on July 6, 2020 indicates that at least one of these females had mated in the field. Given that the total length of the immature stages, excluding the egg stage, was 21 to 28 days (n = 15) and that the larvae have previously been found during June, the larvae and the new adults may emerge at least from June to July and from July to August, respectively in the field in Japan. The first and second instar larvae and a pupa of this species are illustrated for the first time.
Key words: conservation, diving beetle, endangered species, larvae, red list

Introduction

Laccophilus vagelineatus Zimmermann, 1922 (Coleoptera, Dytiscidae) (body length: 3.3–3.5 mm) (Fig. 1A) is a member of the *Laccophilus kobensis* species group inhabiting ponds with abundant aquatic vegetation (Kamite *et al*., 2005; Nakajima *et al*., 2020). This species has been recorded from Japan (Ibaraki and Shizuoka Prefectures), Russia, China, and Korea (Kamite *et al*., 2005; Lee & Ahn, 2015; Nakajima *et al*., 2020), and is currently enumerated as "Endangered" in the national Red List for Japan (Ministry of the Environment of Japan, 2015, 2020).

Information on the life history of the *L. kobensis* species group has been poorly known.

The habitats of most species of the group are 'plant-rich wetlands' (Nakajima *et al*., 2020) and females of *L. difficilis* Sharp, 1873 and *L. nakajimai* Kamite, Hikida et Satô, 2005 lay eggs into stems of aquatic plants (Watanabe, 2019; Yamasaki & Watanabe, 2020).

The third instar larva of *L. vagelineatus* has previously been illustrated and the larvae can be found during the month of June in its natural habitats in Japan (Mitamura *et al*., 2017). However, details of the life history of *L. vagelineatus* remain virtually unknown (Ministry of the Environment of Japan, 2015), particularly with respect to the immature stages, including oviposition sites and the stadia of different larval instars and pupa. Given its endangered status, elucidating the life history of *L. vagelineatus* will contribute to enhancing conservation of this species.

In this study, *L. vagelineatus* was reared in the laboratory with the aim of determining the developmental stadia for the immature stages.

Materials and Methods

Two male and four female adults of *Laccophilus vagelineatus* were collected from Ibaraki Prefecture, Japan, on July 6, 2020, by Kei HIRASAWA and Ryosuke MATSUSHIMA. In order to confirm the collected female adults had already mated, two (females only) and four (two males and two females) individuals were reared in two separate plastic rearing cups (13 cm in diameter and 10 cm in height, with a water depth of approximately 7 cm) in the Ishikawa Insect Museum, Hakusan, Ishikawa Prefecture, Japan. *Pogostemon* sp. 'dassen' (Lamiaceae) were placed on the cups as an oviposition substrate. The diving beetles were provisioned with frozen chironomid larvae as prey at intervals of 2–3 days. The cups were checked daily and the date and developmental stages of any individuals observed were recorded. With respect to eggs, I recorded the oviposition site by means of photography. Photographs were captured 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× lens.

Fifteen first instar larvae were transferred individually to plastic larval cups (8 cm in diameter and 4 cm in height, with a water depth of approx. 5 mm). Live chironomid larvae obtained from containers in the Ishikawa Insect Museum were provided daily as larval feed. Crushed and moistened peat moss (1 cm in depth) was placed on other plastic cups (the same size as larval cups) as a pupation medium (pupation cups). Third instar larvae were carefully transferred to the pupation cups individually when they ceased eating prey and began walking without stopping. The dates of molting, construction of pupal chambers, pupation, eclosion and emergence of new adults were recorded in detail.

Through the experiments, the beetles were reared at 26°C under a 9 L:15 D photoperiod (with illumination being provided between 08:15 and 17:15, JST).

Multiple digital photographs were combined by focus stacking using the digital image processing software Zerene Stacker version 1.04 for a pupa and a newly-eclosed adult.

Results

Eggs were laid singly into the main stems (1.48–3.48 mm in diameter; n = 3) of *Pogostemon* sp. (Fig. 1B). Egg-laying was observed from July 8 to October 1, 2020. The egg stadium was 7 days (n = 7). Eggs and larvae were found in each of the two plastic rearing cups (one containing two females and the other containing two males and two females). All larvae were observed feeding on live chironomid larvae on the hatching day. The stadia of the larval stages were as follows: first instar (Fig. 1C), 3 to 7 days (mean ± SD = 5.0 ± 1.2 days, range = ; n = 15); second instar (Fig. 1D), 2 to 7 days (4.3 ± 1.2 days; n = 15); third instar (Fig. 1E, F), 5 to 12 days (7.7 ± 1.5 days; n = 15); and all larval instars, 13 to 19 days (16.9 ± 1.6 days; n = 15). It was noted that 2 days prior to seeking a pupation site, the body coloration of third instar larvae turned from yellowish brown to green (Fig. 1F).

After transitioning to the pupation cups, the third instar larvae dug into the pupation medium, made pupal chambers, and thereafter pupated. One pupa was observed by dissecting a pupal chamber 3 days after it was constructed (Fig. 1G). Adult emergence was observed 6 days (n = 1) after construction of the pupal chamber. The stadium from construction of a pupal chamber to exiting of an adult (Fig. 1H) was 8 to 11 days (8.6 ± 0.9 days; n = 13). The stadium from hatching to adult's exiting was 21 to 28 days (25.5 ± 1.8 days; n = 13). All the newly-emerged adults began to feed on frozen chironomid larvae on the day they entered the water. The body lengths of the adults were within the range of those of the field-collected *L. vagelineatus*.

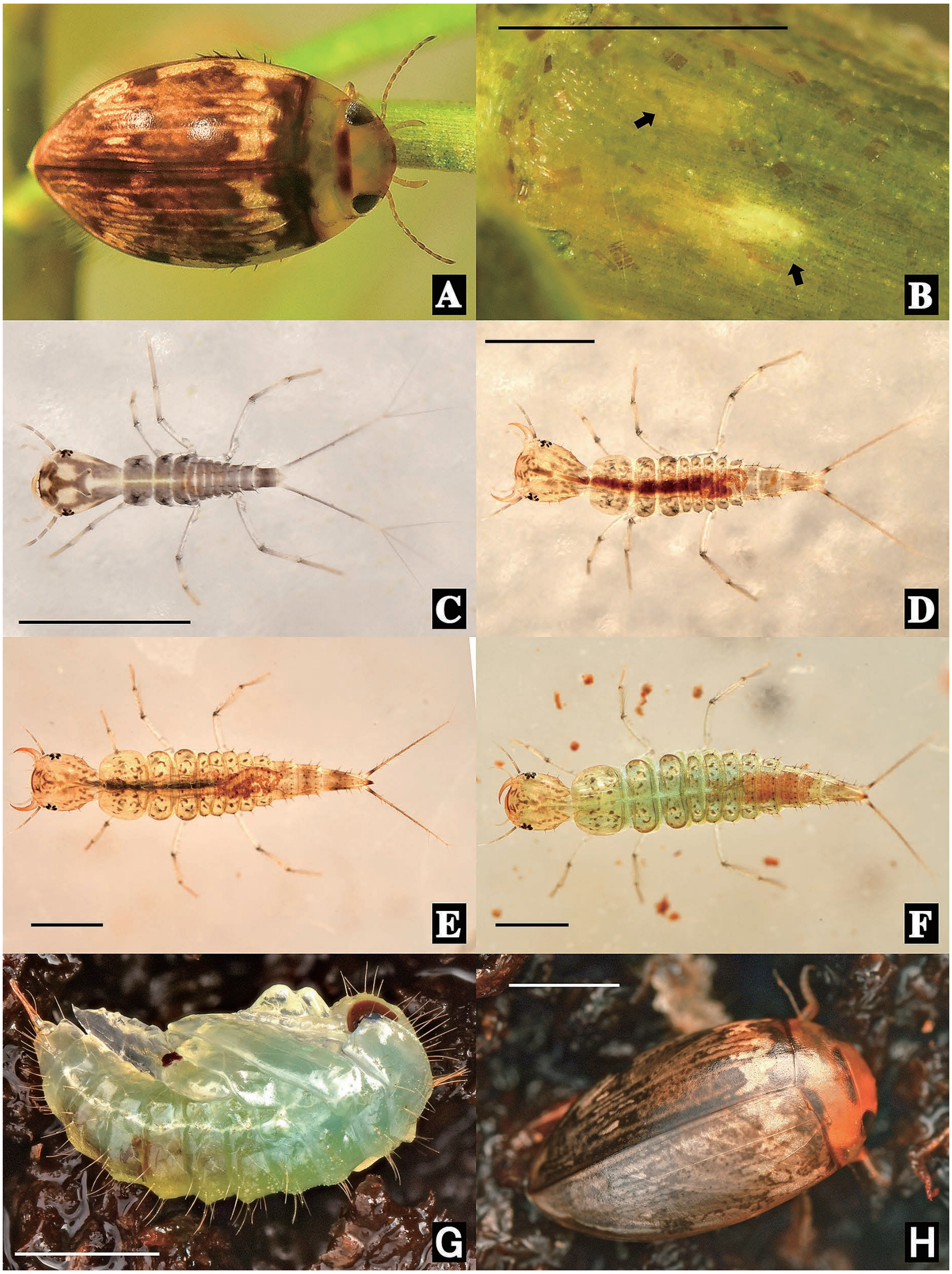

Fig. 1. *Laccophilus vagelineatus*. —— A, Adult collected from the field in Ibaraki Prefecture; B, eggs laid inside *Pogostemon* sp.; C, first instar larva; D, second instar larva; E, third instar larva; F, third instar larva immediately prior to seeking a pupation site; G, pupa; H, new adult on the peat moss surface. Arrows indicate eggs. Scale bars: 1.0 mm.

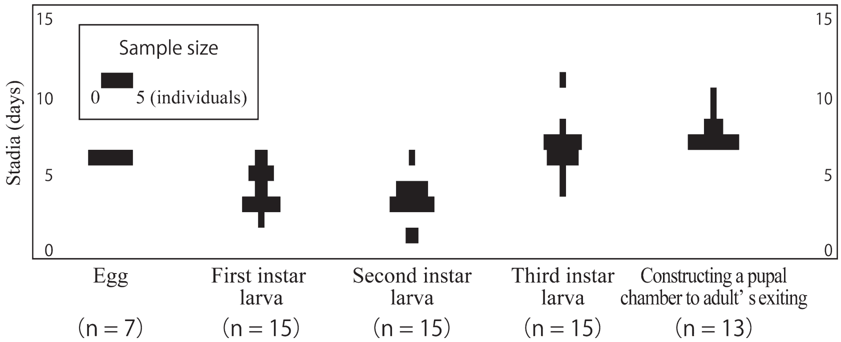

Fig. 2. The lengths of each developmental stage and from constructing a pupal chamber to adult's exiting from the chamber in *Laccophilus vagelineatus*.

Discussion

The observation of eggs and larvae in the plastic rearing cup containing only females indicates that at least one of these females had mated prior to the day of collection (July 6, 2020) in the field. Moreover, in the diving beetle's natural habitats, larvae have previously been found during June (MITAMURA *et al*., 2017). Given that the total stadium of the immature stages, excluding the egg stage was 21 to 28 days recorded in the present study, the larvae and the new adults may emerge at least from June to July and from July to August, respectively, in the field in Japan. The period of larval emergence may be much longer, since egg-laying was observed for three months when the adults were reared under 26°C. In the field, adults were observed all year round (MITAMURA *et al*., 2017), therefore, the new adults are then expected to reproduce before winter and/or overwinter and reproduce in the early summer of the following year. Future study is necessary to determine the period required for sexual maturation and the effect of temperature on reproduction.

On the basis of observations made in this study, it has been established that *Laccophilus vagelineatus* has at least some biological features in common with other species of *L. kobensis* species group in Japan. For example, *L. vagelineatus* laid eggs singly into the main stems of plant, which is similar to the oviposition behaviors of *L. difficilis* and *L. nakajimai* (WATANABE, 2019; YAMASAKI & WATANABE, 2020). Furthermore, the transition of the body color of third instar larvae of *L. vagelineatus* to green prior to pupation has also been observed in third instar larvae of *L. nakajimai* (WATANABE, 2019) and *L. difficilis* (WATANABE, unpublished data). The lengths from hatching to adult's exiting were almost identical between *L. vagelineatus* and *L. nakajimai* (25.0 ± 1.4 days; n = 3) (WATANABE, 2019). On the other hand, the egg stadium of *L. vagelineatus* reared at 26°C was shorter than that of *L. nakajimai* reared at 28°C (12.8 ± 0.4 days; n = 4) (YAMASAKI & WATANABE, 2020). Further research is necessary to determine whether the difference factor is due to environmental or genetical diferrences.

I conclude that it is important to maintain an environment rich in aquatic plants as egg-laying grounds for conservation of *L. vagelineatus*. Maintaining such environments may contribute to conservation of not only *L. vagelineatus* but also other *Laccophilus* species.

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要　　約

渡部晃平：キタノツブゲンゴロウ（鞘翅目ゲンゴロウ科）の卵，幼虫および蛹に関する生物学的知見．———— 環境省レッドリストにおいて絶滅危惧 IB 類に選定されているキタノツブゲンゴロウ *Laccophilus vagelineatus* ZIMMERMANN, 1922 の卵，幼虫および蛹期間の解明を目的として 26°C で飼育実験を行い，各発育期間を記載し，1，2 齢幼虫，蛹を初めて図示した．飼育下において，本種は *Pogostemon* sp.（シソ科）の主茎の組織内に 1 つずつ産卵した．各発育段階における発育期間は卵（7 日，n = 7），1 齢幼虫（3~7 日，n = 15），2 齢幼虫（2~7 日，n = 15），3 齢幼虫（5~12 日，n = 15），上陸〜蛹化（3 日，n = 1），蛹化 〜羽化（3 日，n = 1），上陸 ~ 羽化脱出（8~11 日，n = 13），卵〜羽化脱出（33〜35 日，n = 7）であった．2020 年 7 月 6 日に採集した雌成虫のみを飼育していた容器内で卵と幼虫が確認され，7 月上旬には野外で既に交尾済であったことが判明した．実験より得られた，孵化から新成虫が蛹室を脱出するまでの累計発育日数が 21~28 日（n = 15）であること，本種の幼虫は 6 月にも確認されていることから，国内の自然環境下において，幼虫の出現期間には少なくとも 6~7 月，新成虫の出現期間には少なくとも 7〜8 月を含むことが示唆された．新成虫は，羽化後越冬するまでの期間または翌年の初夏に繁殖すると考えられるが，性成熟に必要な期間や気温が繁殖に与える影響については今後の研究が必要である．

本研究の結果から，キタノツブゲンゴロウはツブゲンゴロウ *L. difficilis* SHARP, 1873 やナカジマツブゲンゴロウ *L. nakajimai* KAMITE，HIKIDA et SATÔ, 2005 と同様に，植物の組織内に産卵すること，上陸が近づいた 3 齢幼虫が体色を緑色に変化させることから，コウベツブゲンゴロウ種群の他種と複数の共通点が確認された．

本種は植物の茎を産卵基質として利用しており，保全には水生植物が豊富な環境の維持が重要であり，このような保全対策はツブゲンゴロウ属の他種の保全にも貢献する可能性がある．

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