

## Descriptions of Larva and Pupa of *Graphelmis shirahatai* (NOMURA) (Coleoptera, Elmidae)

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**Abstract** Morphology of mature larva of *Graphelmis shirahatai* (NOMURA, 1958) is described based on specimens collected from Shimane Prefecture, west Honshu, Japan. The larva of this species is easily distinguished from other Japanese elmids by the body coloration and the shape of abdominal segment IX. Prothorax possesses three ventral sclerites and procoxae closed posteriorly. Mesothorax possesses three ventral sclerites. The pupae of *G. shirahatai* were obtained from laboratory rearing which is also described in this paper.

**Key words:** aquatic beetle, Elminae, lotic insects, immature stage.

### Introduction

Genus *Graphelmis* DELÈVE, 1968 is medium-sized riffle beetles (Elmidae) which includes more than 80 species of the world (ČIAMPOR, 2002). Among 22 species from Asia have been reviewed taxonomically by ČIAMPOR (2001, 2002, 2003, 2004, 2005 a, b, 2006) and ČIAMPOR & KODADA (2004).

In Japan, one member of the genus, *G. shirahatai* (NOMURA, 1958) is recorded from a main island, Honshu: Yamagata (type locality), Miyagi, Niigata, Aichi, Hyogo, Shimane Prefectures and Tokyo Metropolis (NOMURA, 1958; YOSHITOMI *et al.*, 1999; HAYASHI, 2007; IWATA, 2011; IWATA *et al.*, 2012). This species is one of the most rare elmids in Japan because recent inhabit areas are limited several river systems, Shinano-gawa in Niigata Pref., Yahagi-gawa in Aichi Pref., Hii-kawa and Kando-gawa in Shimane Pref. (YOSHITOMI *et al.*, 1999; HAYASHI, 2007; IWATA, 2011; HAYASHI *et al.*, 2011). The larva and pupa of this species were reported by HAYASHI (2008: figs. 25–33) and HAYASHI & SOTA (2010: fig. 6) but its detail morphology has not been described.

In this paper, morphology of the mature larva and pupa of *G. shirahatai* are described by a light microscope observation and its biology is reviewed by its observations from the field survey and the laboratory rearing.

### Materials and Methods

Larvae were collected from lower stream of Hii-kawa River in Shimane Prefecture, west Honshu, Japan. The larvae were fixed in 70% ethanol, and are deposited at the Hoshizaki Institute of Wildlife Protection, Izumo, Shimane Prefecture, Japan.

The larvae were examined and photographed for the external structure under a light microscope, Nikon Eclipse E600 with a CCD camera, Nikon Digital Sight (DS-L2). About 10 to 30 digital photographs were made by focus stacking, using a digital image processing software, Adobe Photoshop CS4 for Macintosh. The larval specimens were prepared for observation after ultrasonic cleaning (25°C, 5–10 minutes), soaking 2.5 to 3.5 w/v% H<sub>2</sub>O<sub>2</sub> (oxydol: 50°C, 5 hours) and 3% KOH solution (50°C, 5 hours or more hours). Muscles and internal organs were removed by tweezers under a stereoscopic microscope.

Terminology for morphological features of elmid larva and pupa is followed after KODADA & JÄCH (2005).

### Descriptions of larva and pupa

#### *Graphelmis shirahatai* (NOMURA, 1958)

(Figs. 1–44)

##### *Description of larva* (Figs. 1–41).

Body (Figs. 1–3) elongate, thorax as broad as abdominal segments I to VIII, abdominal segment IX (last segment) gradually narrowing middle to apex, apex rounded in dorsal view; thorax and abdominal segments I to VIII convex dorsally and flattened ventrally, segment IX with a large slope (slanting flat part) dorsally; spiracles on mesothorax (Fig. 28) and abdominal segment I to VII; middle suture present on thorax and abdominal segments I to IV; body color dark brown dorsally and cream ventrally, with right spots on head, pro- and meso-thoracies, dark spots on side of each abdominal segments I to VII; both side of head, surrounding stemmata white (Fig. 2). Setae on body surface include various types (Figs. 24–31, 35).

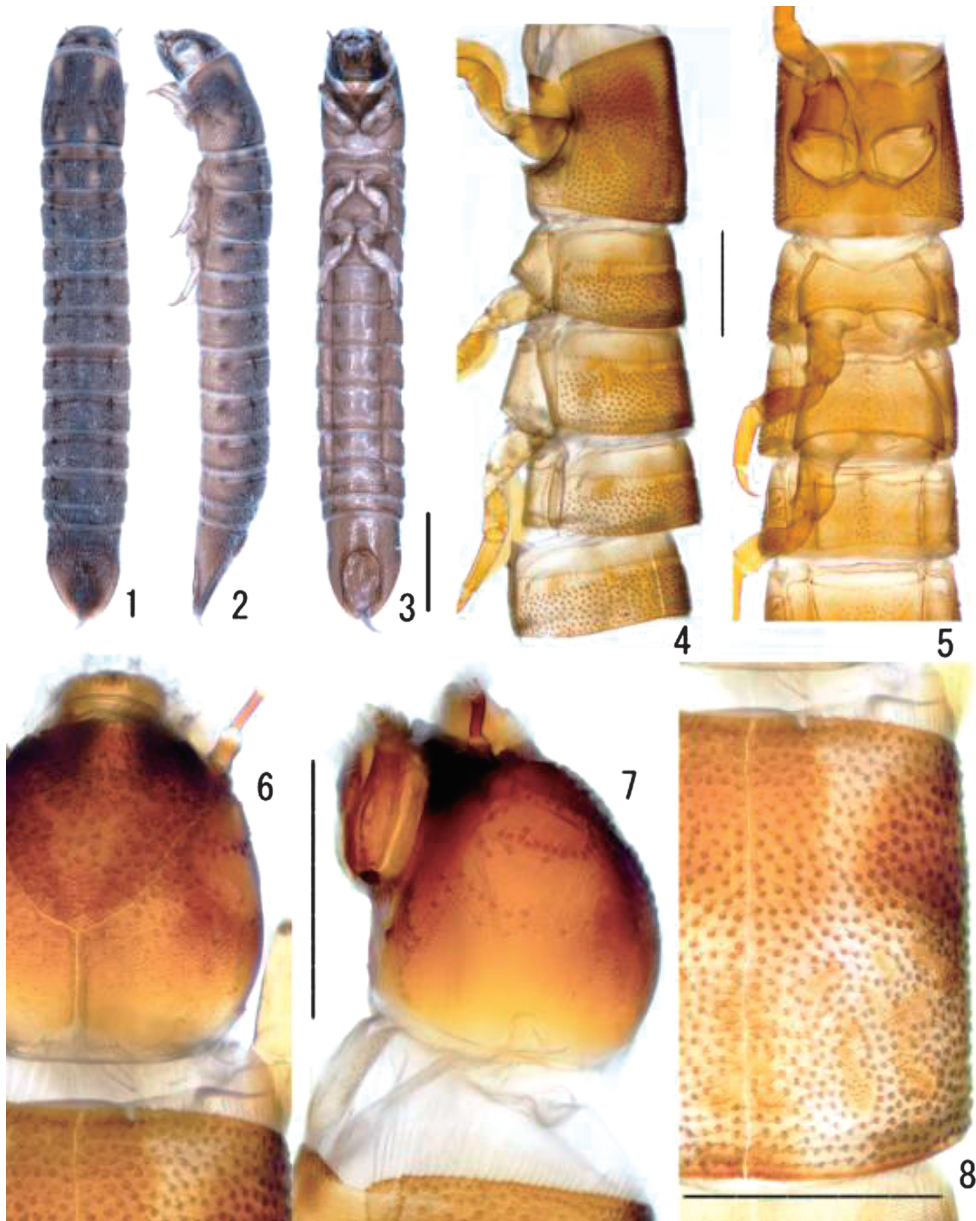
Head visible from above, well exposed from prothorax (Fig. 1); entirely rounded in dorsal and lateral views, width as long as length; clypeus (Fig. 9) transverse, with dense, branched setae on front (Fig. 10); frons with granules and spiky setae; vertex with granules on front; posterior edge not emarginate (Fig. 6); epicranial stem present and contiguous at base; frontoclypeal suture indistinct (Fig. 9). Antenna (Fig. 10) 3-segmented; 1st shorter than 2nd; 1st with long branched setae; 2nd with sensorial appendage; 3rd slender and short. Labrum (Figs. 14, 15) transverse and short, with dense, long, pectinate setae along frontal margin (Fig. 14); epipharynx with dense setae (Fig. 15). Mandible (Figs. 11–13) tridentate at apex (Fig. 12), articulated pubescent process in inner margin (Fig. 13), long, pectinate setae in outer margin (Fig. 11). Maxilla and labium forming an unit (maxillolabial unit: Figs. 16, 17). Maxilla with 4-segmented palpus; cardo small; stipes large; galea and lacinia separate, setose apically. Labium narrow with 2-segmented palpus; ligula short and transverse; mentum long; submentum short and transverse.

Thorax not serrated on lateral sides; dorsum covered by fine granules (Figs. 4, 8); granule with a pair of seta: one type is two spiky setae (Fig. 35a), another type is spiky seta and straight spatula-like seta (Fig. 35b). Prothorax longer than width, 1.8 times as long as mesothorax; glabrous spots on baso-lateral part; with 3 ventral sclerites, procoxae closed posteriorly: two lateral and one postero-medial sclerites; long, pectinate setae on front and hind margins. Meso- and metathoracies transverse, with granulate, pairs of spiky and straight spatula-like setae on dorsum and lateral parts; glabrous spots on both sides. Mesothorax with three ventral sclerites (Fig. 21). Legs (Figs. 36–38) 5-segmented, short and stout; 2nd and 3rd segments with long setae in all legs.

Abdomen 9-segmented (Figs. 1–3). Segments I to VIII transverse, with granules, pairs of spiky and straight spatula-like setae on dorsum and lateral parts; segments I to VII with glabrous spots on both sides. Last segment (segment IX: Figs. 22, 23) longer than width, base sub-parallelled and middle gradually narrowing to apex, apex rounded; large slope with granules and lateral ridges (Fig. 22); ventral operculum, opercular claws, and anal gills present (Fig. 23).

Body length: ca. 5.5 mm in expanded specimen preserved in 70% ethanol; ca. 5.0 mm in dry condition.

Comparisons: It is easily distinguished from the Japanese members of Elmidae in the body coloration and the shape of abdominal segment IX. Larva of this species is similar to that of *Hedyselmis*



Figs. 1–8. Larva of *G. shirahatai*. — 1–3, Habitus and body coloration; 4–5, thorax and abdominal segments I to II; 6, 7, head; 8, prothorax. 1, 6, 8, dorsal view; 2, 4, 7, lateral view; 3, 5, ventral view. Scale bars, 1–3 in 1.0 mm, others in 0.05 mm. Photographs by zoom microscope in Figs. 1–3; light microscope in other figures.

*opsis* Hinton, 1976 (ČIAMPOR & RIBERA, 2006): rounded head; dense, long pectinate setae on labrum and outer margin of mandible; abdominal segment IX with widely, slanting flat on dorsum. However, *H. opis* is distinguished from *G. shirahatai* in dense setae on body surface.

*Description of Pupa* (Figs. 42–45).



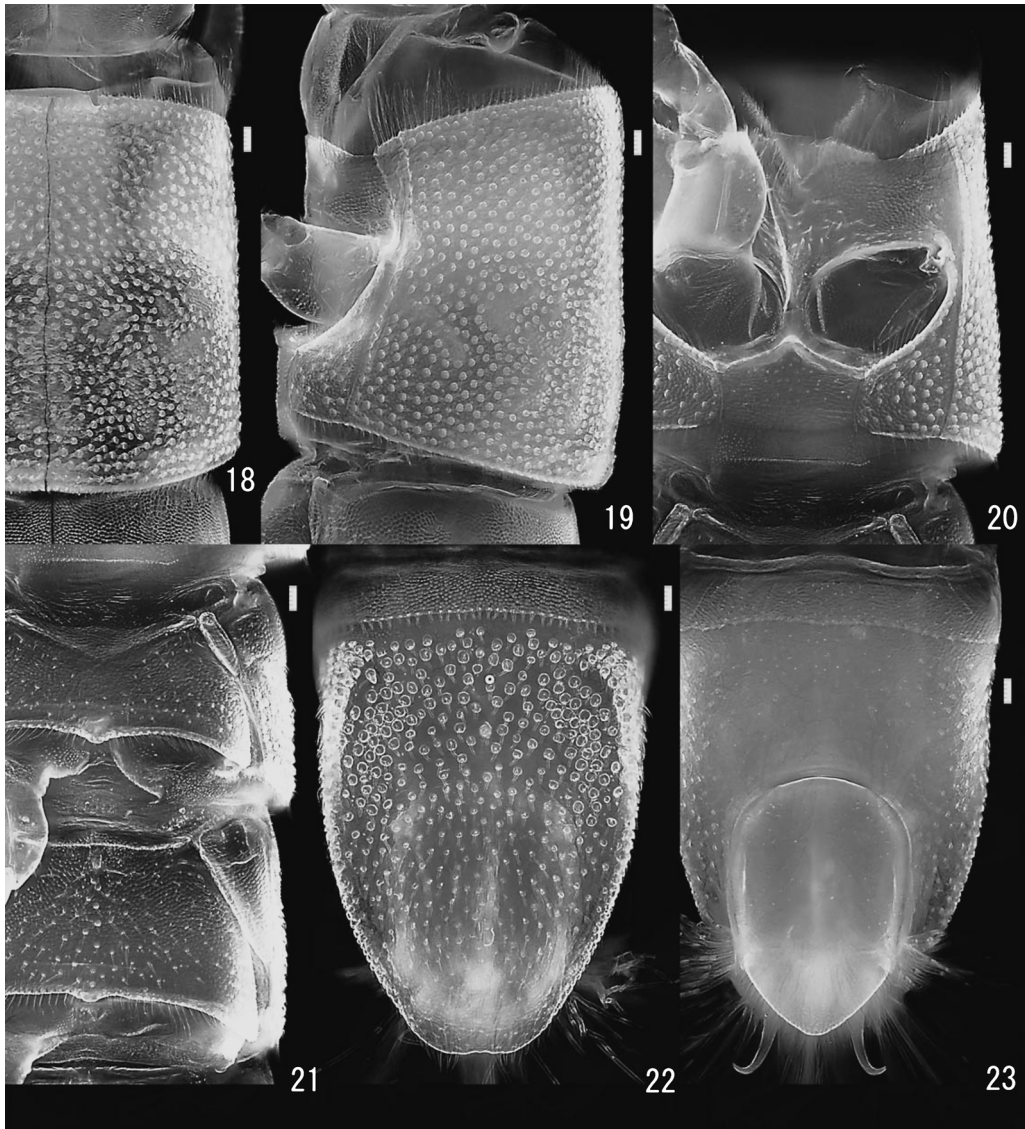


Figs. 9–17. Larva of *G. shirahatai*. — 9, Head capsule; 10, antenna and frons; 11, 13, left mandible in ventral view; 12, inner side of right mandible; 14, labrum; 15, epipharynx; 16, 17, maxillolabial unit. Scale bars are 0.05 mm. Photographs by right microscope.

Exarate type, feebly sclerotized, with appendage sheaths free, elongate, adecticous, and setose. Head strongly retracted into prothorax; thoracic segments setose; prothorax with two anterior and posterior filaments; abdomen elongate, posterior most segment with a pair of postero-lateral projections.

Length: ca. 3.8–4.3 mm preserved in 70% ethanol.

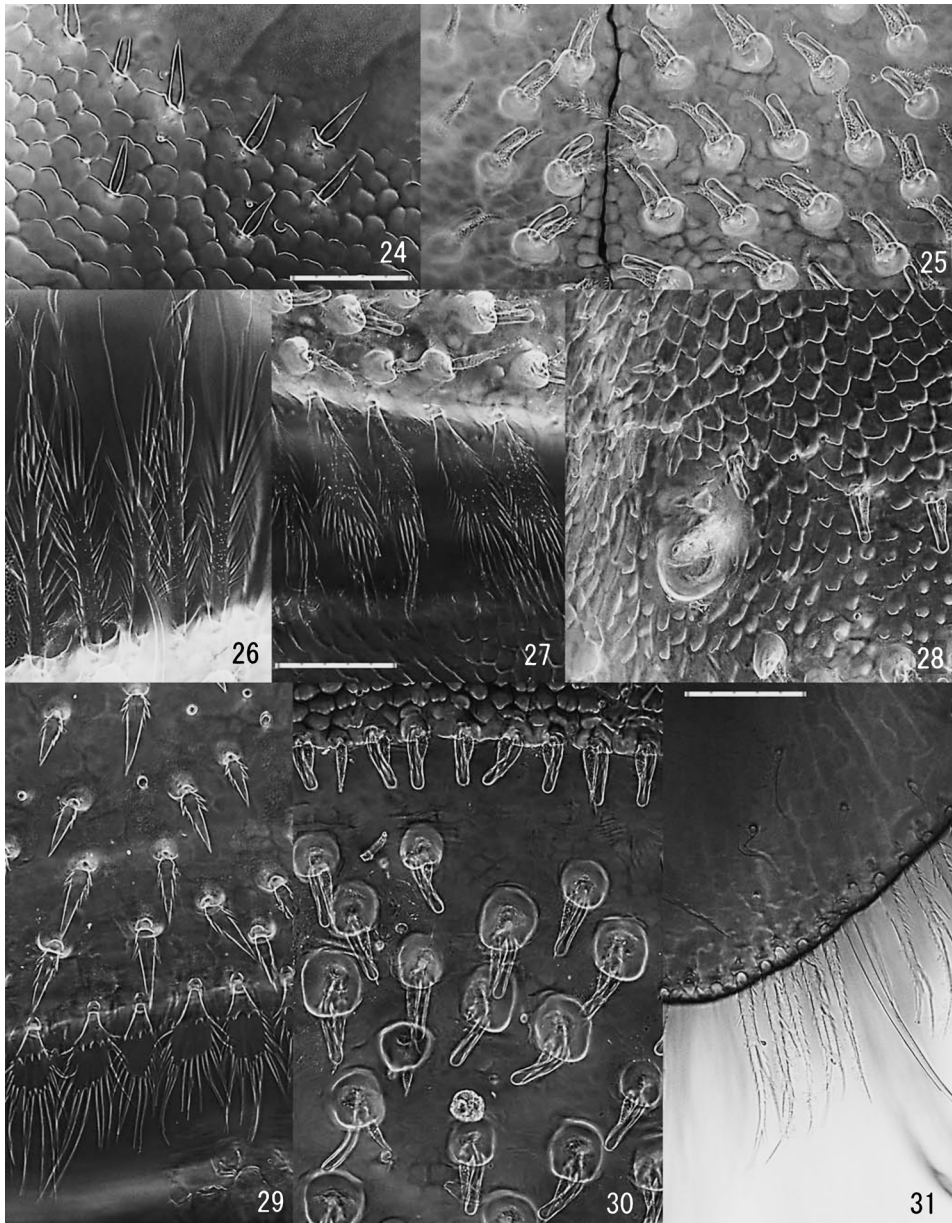




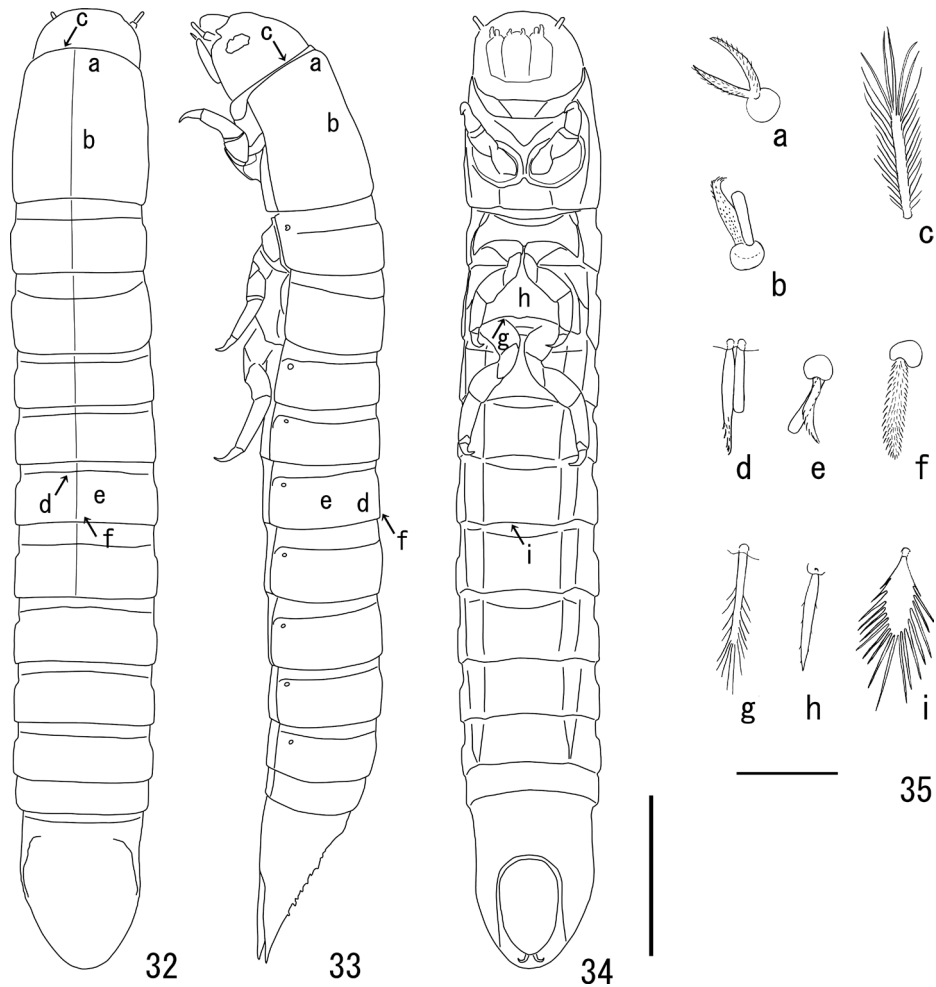
Figs. 18–23. Larva of *G. shirahatai*. — 18–20, Prothorax; 21, meso- and metathoraxies; 22, 23, abdominal segment IX; 18, 22, dorsal view; 19, lateral view; 20, 21, 23, ventral view. Scale bars are 0.05 mm. Photographs by right microscope.

*Specimens examined.*

Larvae: 3 exs., Mizuho-ôhashi, Hii-kawa River, Hikawa-chô, Shimane Pref., 11–VIII–2006, MH (M. HAYASHI) leg.; 30 exs., same locality, 4–IV–2007, MH leg.; 7 exs., same locality, 3–X–2007, MH leg. 4 exs., Nishidai-bashi, Hii-kawa River, Izumo-shi, Shimane Pref., 23–IX–2006, MH leg.; 3 exs., same locality, 31–VII–2007, MH leg. 41 exs., Kitakandachi-bashi, Hiikawa-River, Izumo-shi, Shimane Pref., 13–V–2006, MH leg.



Figs. 24–31. Various types of setae on larva of *G. shirahatai*. — 24, Lateral part of vertex; 25, middle part of prothorax; 26, front margin of prothorax; 27, hind margin of prothorax; 28, spiracle on mesothorax; 29, hind margin of ventral sclerite of metathorax; 30, dorso-basal part of abdominal segment IX; apical margin of segment IX. Scale bars are 0.05 mm in all photographs. Photographs by right microscope.



Figs. 32–35. Larva of *G. shirahatai*. — 32–34, Habitus (“a” to “i” showing position of each type of seta, see Fig. 35); 35, various types of setae (35a to 35i). Scale bars, 1.0 mm in 32–34; 0.05 mm in 35.

Pupae: 10 exs. (laboratory rearing): larvae were collected from Hii-kawa River, Shimane Pref. in 2006 to 2007 by the author.

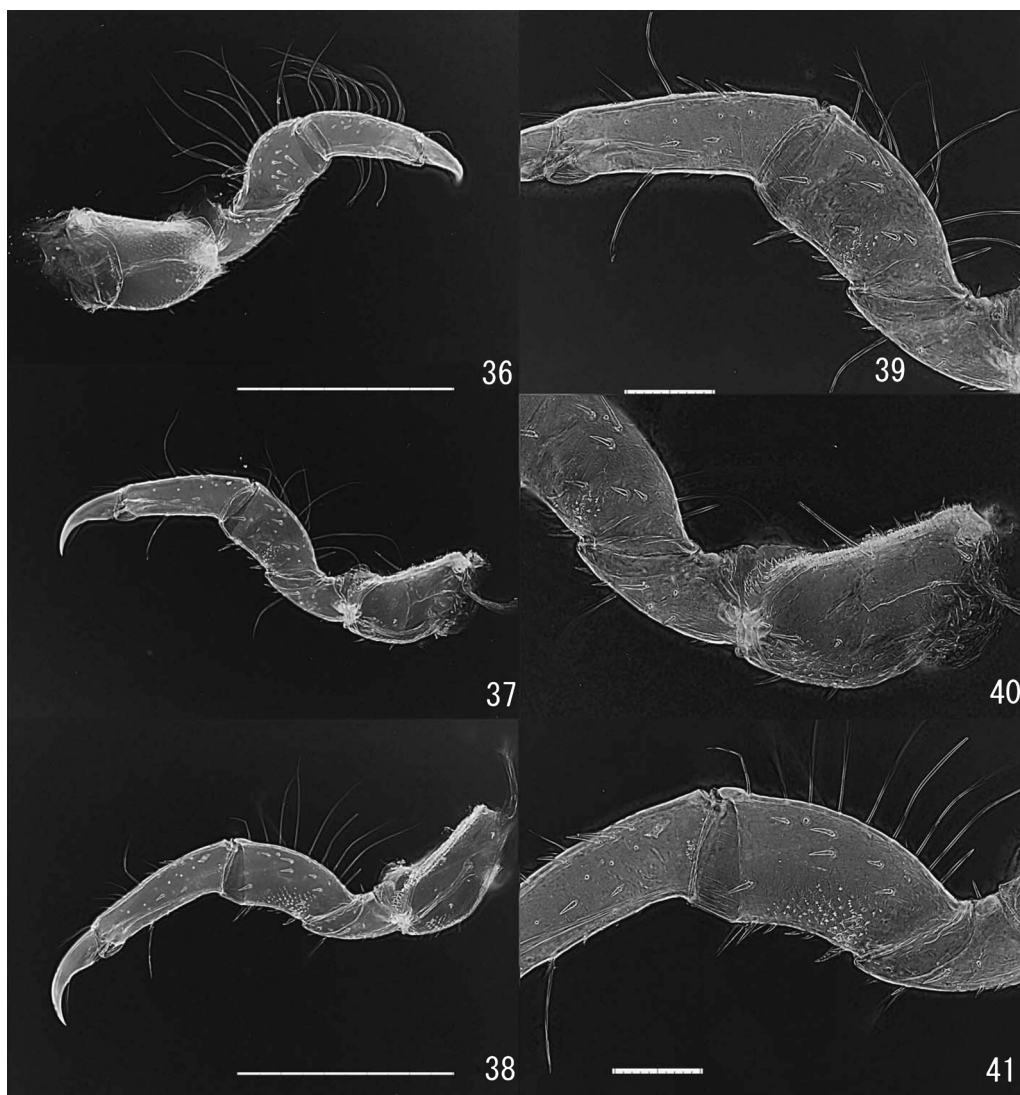
### Biology of *G. shirahatai*

Ecological observations of *G. shirahatai* have been reported by HAYASHI (2007, 2008) based on the field survey and the laboratory rearing.

In the field, adults are found from decaying woods in running water every summer, mid July to late September and the larvae from same habitat of the adult during April to October (Fig. 46). Most of the woods are *Salix* sp., which are most common riparian trees of the habitat.

In the laboratory rearing, adults survived several months in a water tank (fish tank) with decaying woods of the habitat and aeration system, air-stones attached air pumps. All adults dead until winter

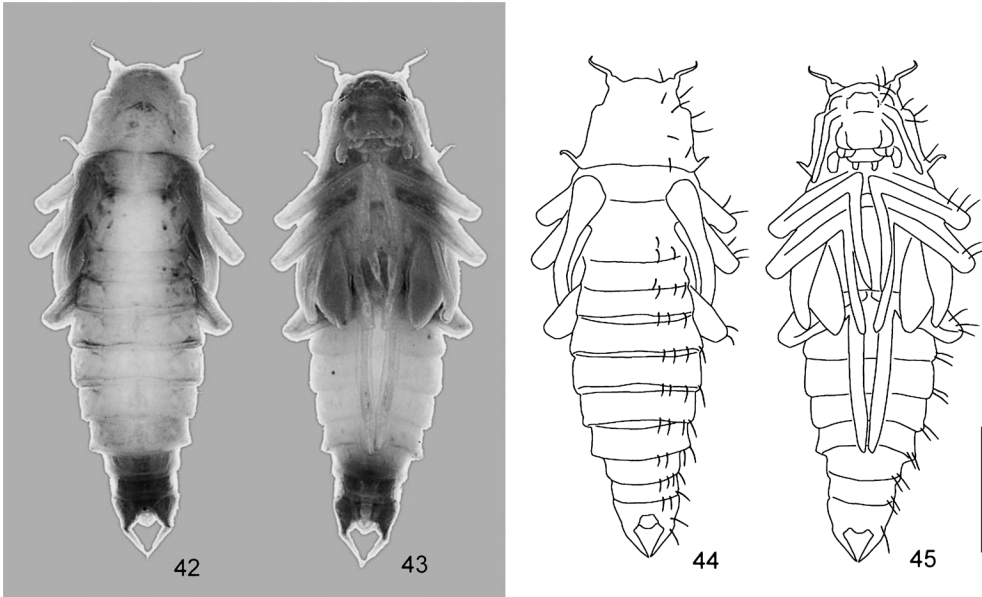




Figs. 36–41. Larva of *G. shirahatai*. — 36, Fore leg; 37, 39, 40 mid leg; 38, 41, hind leg. All legs are left. Scale bars, 0.5 mm in 36–38; 0.1 mm in 39–41. Photographs by right microscope.

but immature larvae (body length ca. 3.0 mm) appear in November. The mature larvae overwintered in the tank and emerged next July. The mature larvae collected from field in May pupated in sands above the water in September. The period of pupal stage is only three days in the room temperature.

These observations show that the estimated life cycle of this species, as follows: breeding season is summer to early autumn; the period of larval stage is more than 12 months; adults appear summer to early autumn and dead until winter; overwintered mature larva are emerged in early summer. The diet of the larvae and adults is decaying woods in running water.



Figs. 42–45. Pupa of *G. shirahatai*. — 41, 42, A specimen preserved in 70% ethanol (Photographs by zoom microscope); 41, 43, dorsal view; 42, 44, ventral view. Scale bar is 1.0 mm.

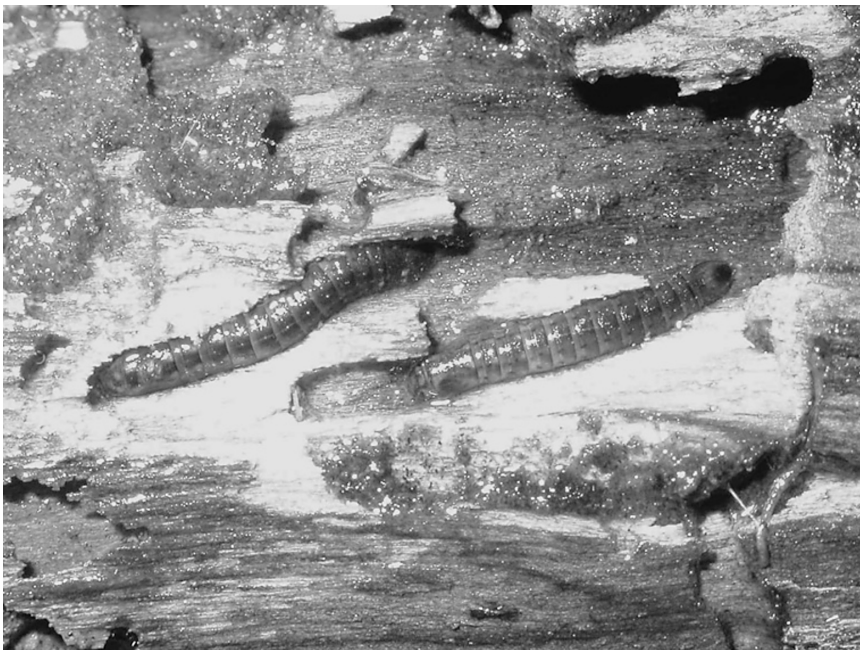


Fig. 46. Living larvae of *G. shirahatai* on decaying wood in Hii-kawa, Izumo, Shimane Pref., Japan.

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### 要 約

林 成多：アヤスジミゾドロムシ（鞘翅目ヒメドロムシ科）の幼虫および蛹の記載と生態的知見。——アヤスジミゾドロムシの幼虫は、飼育およびDNAバーコーディングによって、外見の特徴は判明しているが、その形態は詳しく検討されていなかった。筆者は、野外で採集した老熟幼虫を光学顕微鏡で観察・撮影し、記載を行った。本種の幼虫は体色や腹部腹節末端節の特徴的な形状により、日本産の他のヒメドロムシ科の幼虫とは容易に識別できる。ヒメドロムシ科の幼虫の重要な分類形質としては、本種の前胸の ventral sclerites は3枚で構成され、前基節窩の後方は閉じている (scleriteがある)。中胸の ventral sclerites は3枚で構成されている。体表を詳しく検討すると、鱗状のものも含め、多様な形状の剛毛が確認された。蛹は、飼育によって得られていた標本を記載した。本種の生態について、これまでに得られた情報を総合すると、産卵は夏から秋、幼生期は1年以上、成虫の出現は夏から秋、越冬態は若齢および老齢幼虫であり、越冬した老齢幼虫は初夏に蛹化・羽化することが推定される。蛹化場所は水面より高い場所の地中であることが確認されている。また、成虫・幼虫共に流水を食べている。

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