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# Description of the instar III larva of *Agabus (Acatodes) browni* Kamiya, 1934 (Coleoptera: Dytiscidae, Agabinae)

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## Abstract

The instar III larva of *Agabus (Acatodes) browni* Kamiya, 1934 (Coleoptera: Dytiscidae: Agabinae) is described for the first time with emphasis on chaetotaxy. The larva is characterized by a yellow-brown frontoclypeus with a central anterior diamond-shaped dark brown macula, the serrated inner ventral margin of the distal half of the mandible, and the abdominal segment VIII with both short and three pairs of long hair-like setae. The larva is compared to those of *A. conspicuus* Sharp, 1873, and *A. japonicus* Sharp, 1873 from Honshu and western Japan. A key to identify the instar III larvae of these species is provided.

Key words: Coleoptera, Dytiscidae, Agabinae, Agabus browni, immature stages, mature larva

### Introduction

A total of 177 species in the genus *Agabus* Leach, 1817 (Coleoptera: Dytiscidae: Agabinae) are known worldwide (Nilsson & Hájek 2025), including seven species from Japan (Nakajima *et al.* 2020; Watanabe & Yoshitomi 2022). This paper focusses on the larval morphology of three Japanese species—*Agabus (Acatodes) browni* Kamiya, 1934, *Agabus (A.) conspicuus* Sharp, 1873, and *A. (A.) japonicus* Sharp, 1873. Currently only the larvae of the latter two species are known (instar III only; Fukuda *et al.* 1959; Mitamura *et al.* 2017). All three species are often found and reproduce in the same locality in Honshu and western Japan (Saijo 2001; Watanabe & Hisamatsu 2016). Hence, field surveys of the larval stages are difficult without accurate larval identification. The aim of this paper is to describe the mature larva of *A. browni* and to provide an identification key to the instar III larvae of the three species.

# Materials and methods

The larvae were fixed in 70% ethanol and subsequently mounted on slides with 70% ethanol or HS-slides (Shirayama *et al.* 1993) with Euparal. The specimens were observed under an optical microscope (Nikon ECLIPSE E400) at 400-fold magnification and sketched using a Nikon Y-IDT drawing tube. After scanning the sketch, line drawings were prepared using an iPad Pro 11-inch (4th generation). Photographs of the living larvae were made using a Nikon D500 digital camera equipped with a Nikon AF-S Micro NIKKOR 60 mm f/2.8G ED lens. Measurements were performed using a stereomicroscope (Leica M205C, Planapo  $1.0\times$ ) with a transmitted light base (Leica TL3000 Ergo), a camera (Leica DFC420), and software (LAS version 3.3.1). The fine structures of the specimens were observed using a scanning electron microscope (SEM; JEOL JCM-6000 Neoscope Scanning Electron). These larvae

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were freeze-dried and coated with ultrathin gold layers by high-vacuum evaporation. The examined larvae have been deposited in the larval collections of the Ishikawa Insect Museum (IIM), Ishikawa, Japan, and the Hoshizaki Green Foundation (HOWP), Shimane, Japan. The observation methods used in the study followed Watanabe & Hayashi (2024). The measurements and notation of primary setae, and pores used in this study follow Alarie & Michat (2020) and Alarie *et al.* (2023).

# The instar III larva of Agabus browni Kamiya, 1934

(Figs 1, 5-28)

**Source of material:** The specimens studied were collected in association with adults at the following localities: One instar III (IIM), Imain, Munakata-shi, Fukuoka Prefecture, Japan, 28 III 2005, Y. Kamite leg.; two instar III (IIM), Osashimachokusumi, Ena-shi, Gifu Prefecture, Japan, 4 III 2023, Y. Kamite & N. Kamite leg.; two instar III (IIM, HOWP), Namikata, Namikata-cho, Imabari-shi, Ehime Prefecture, Japan, 28 III 2023, L. Takechi & A. Shirakata leg.; two instar III (IIM, HOWP), idem, 11 IV 2023, L. Takechi leg. At these sites were found either *A. browni* alone or accompanied by *A. japonicus* Sharp, 1873, which can be distinguished by their head length and width differences.

**Diagnosis:** The larvae of *A. browni* can be distinguished from the other two by the following combination of characters: body large, head length approximately 2.0 mm; head width approximately 1.7 mm (*A. japonicus*: small, head length approximately 1.6 mm, head width approximately 1.3 mm); abdominal segment VIII with short setae and long hair-like setae (*A. japonicus* has only short setae (Fukuda *et al.* 1959; Hayashi 2015)); yellow-brown frontoclypeus with one diamond-shaped dark brown macula centrally (Fig. 1, 5) (*A. conspicuus* has many dark brown maculae, and a centrally anterior cross-shaped macula (Fig. 2; Hayashi 2015; Mitamura *et al.* 2017)).

**Color (Fig. 1):** Head capsule yellow-brown, with diamond-shaped dark brown macula centrally on frontoclypeus; w-shaped dark brown macula centrally on parietal region; coronal line dark brown; dark brown line laterally from stemmata to temporal spines; neck with T-shaped dark brown macula on each lateral side; stemmata dark brown; antennae light yellow-brown; mandible dark orange; maxilla light yellow-brown; labium light yellow-brown. Body yellow-brown; anterior and posterior regions of pronotum and posterior region of mesonotum, metanotum, and abdominal segments I–VII dark brown; legs yellow-brown; urogomphus yellow-brown.

**Body (Fig. 1):** Subcylindrical, narrowing towards abdominal apex. Total length 13–18 mm, but this length is highly variable between individuals after molting and mature ones. Other measurements and body shape ratios are presented in Table 1.

**Head (Figs 5–12, 16–28):** Cephalic capsule (Figs 5, 16) flattened, sub-rounded, longer than broad, maximum width at level of posterior stemmata, constricted at level of occipital region, occipital suture absent, coronal suture elongate, occipital foramen broadly emarginate ventrally; frontoclypeus subtriangular, rounded outwards, anterior margin with approximately 60 variably sized spatulate lamellae clypeales (Figs 18–19). Antenna (Figs 8, 20) slender, shorter than HL and HW; A4 shortest; A1, A2, and A3 subequal in length; A3' pore-like. Mandible (Figs 6–7, 21–28) prominent, broad basally, 2.4–2.7 times longer than wide, apex acute, with groove on inner margin (Fig. 27), distal half serrated on inner ventral margin (Figs 27–28); proximal half of inner ventral margin bearing many brush-like hairs (Figs 25–28), with many spinulae behind these hairs (Figs 21–22). Maxilla (Figs 9–10) with cardo subovate; stipes short, broad, dorsally with many hairs from base to palpifer and many minute spinulae around setae MX5–MX6; galea well developed, subconical, dorsally with many hairs and several long setae at base; palpifer short, palpomere-like, with dorsodistal spinulae and short hairs; palpus three-segmented, MP2 longest, MP1 and MP3 subequal in length. Labium (Figs 11, 12) with prementum sub-rectangular, broader than long, with minute spinulae on dorsobasal surface; palpus two-segmented, LP1 longer than LP2.

Thorax (Figs 1, 13, 14): TI and TA with strong spinulae along ventral margin.

Abdomen (Figs 1, 15): All sclerites with anterotransverse carina, covered with minute spinulae; segments I–VII transverse; segment VIII subcylindrical, extending dorsally into a short siphon; segments I–V sclerotized dorsally, membranous ventrally; segment VI completely sclerotized except for a ventral narrow area; segments VII–VIII completely sclerotized; spiracles present on segments I–VII; tergites I–VI and anterior region of tergite VII with sagittal line, tergite VIII without sagittal line. Urogomphus two-segmented, longer than LAS, with segment 2 very short.



**FIGURES 1–4.** *Agabus* spp. larvae and reproductive habitats. 1, Instar III larva of *Agabus browni* Kamiya, 1934; 2, instar III larva of *A. conspicuus* Sharp, 1873; 3, reproductive habitat in Gifu Prefecture; 4, reproductive habitat in Ehime Prefecture.



**FIGURES 5–12.** Instar III larva of *Agabus browni*. 5, Cephalic capsule, dorsal aspect; 6, mandible, dorsal aspect; 7, same, ventral aspect; 8, antenna, ventral (left) and lateral (right) aspect; 9, maxilla, ventral aspect; 10, same, dorsal aspect; 11, labium, dorsal aspect; 12, same, ventral aspect. \*, Additional sensilla.



FIGURES 13–15. Instar III larva of *Agabus browni*. 13, metathoracic leg, anterior aspect; 14, same, posterior aspect; 15, abdominal segment VIII and urogomphus, dorsal aspect.

**Chaetotaxy:** Similar to that of the generalized *Agabus* larvae (Alarie & Michat 2020). Cephalic capsule with minute spinulae on dorsobasal surface (Figs 16–18). Parietale with 3–6 temporal spines on lateral surface (Fig. 5). Antenna without secondary setae. Mandible with several secondary setae, one long seta behind pore MNa (Figs 7, 26), other setae minute, inserted near outer margin from behind pore MNc to near apex (Figs 6, 21–24, 26). Setae MX5–MX6 stout, strongly curved posteriorly (Fig. 10); stipes with two secondary minute setae ventrally, one posteromesal to pore MXb, and another behind seta MX2 (Fig. 9). Legs without natatory setae. Posterior margins of abdominal tergites I–VIII with two pairs of long setae dorsally (Figs 1, 15), tergites VI, VII, and VIII with two, three, and one ventral pairs, respectively. Numbers and positions of secondary setae on legs are listed in Table 2.

**Ecology:** Agabus browni are generally found and reproduces in ponds (Figs 3–4). Instar III larvae were collected in March and April, which suggests that *A. browni* overwinter at larval stage or reproduce in winter in Japan.

#### Key to instar III larvae of Agabus species from Honshu and western Japan

1.	Body small; head length approximately 1.6 mm; head width approximately 1.3 mm. Abdominal segment VIII with only short
	setae Agabus japonicus Sharp, 1873
-	Body large; head length approximately 2.0 mm; head width approximately 1.7 mm. Abdominal segment VIII with short and
	long hair-like setae

2.	Frontoclypeus with several small dark brown maculae and a large cross-shaped macula centrally
	Agabus conspicuus Sharp, 1873
-	Frontoclypeus almost entirely yellow-brown, with a diamond-shaped dark brown macula centrally
	Agabus browni Kamiya, 1934



18 \_\_\_\_\_200 μm High-vac. SEI PC-std. 15 kV x 150 2025/01/03 008625



FIGURES 16–20. Scanning electron micrographs of *Agabus browni*, instar III: 16, cephalic capsule, dorsal aspect; 17, right adnasalia, dorsal aspect; 18–19, anterior margin of frontoclypeus, dorsal aspect; 20, antennomeres III–IV, ventral aspect.



**FIGURES 21–28.** Scanning electron micrographs of *Agabus browni*, instar III: mandibles. 21–26, right mandible. 21, 23, 24, dorsal aspect; 22, outer aspect; 25, 26, ventral aspect; 27, 28, left mandible, inner aspect.

Measure	Range	Measure	Range	Measure	Range
HL (mm)	2.0-2.15	MNL/MNW	2.42-2.69	L3/HW	3.42-3.48
HW (mm)	1.65-1.7	MNL/HL	0.40-0.43	L3 (CO/FE)	1.05-1.09
FRL (mm)	0.85-0.9	A/MP	1.42-1.47	L3 (TI/FE)	0.63-0.66
OCW (mm)	1.05-1.1	GA/MP1	0.23-0.36	L3 (TA/FE)	0.63-0.68
HL/HW	1.19-1.28	PPF/MP1	0.34-0.40	L3 (CL/TA)	0.48-0.52
HW/OCW	1.49–1.59	MP2/MP1	1.06-1.18	LAS (mm)	1.55-1.65
COL/HL	0.57 - 0.58	MP3/MP2	0.88-0.94	LAS/HW	0.94-1.01
FRL/HL	0.42-0.43	MP/LP	1.09-1.15	U (mm)	2.7–2.9
A/HW	0.53-0.59	LP2/LP1	0.76-0.84	U/LAS	1.69–1.83
A3/A1	0.82-0.93	L3 (mm)	5.65-5.8	U/HW	1.61-1.77
A3/A2	0.97 - 1.04	L3/L1	1.47-1.52		
A3/A4	1.81-2.19	L3/L2	1.22-1.26		

TABLE 1. Measurements and ratios for the instar III of Agabus browni Kamiya, 1934. Three specimens were examined.

**TABLE 2.** Number and position of secondary setae on the legs of instar III of *Agabus browni* Kamiya, 1934. Numbers between slash marks refer to pro-, meso- and metathoracic legs, respectively. A = anterior, AD = anterodorsal, AV = anteroventral, Di = distal, PD = posterodorsal, PV = posteroventral, Total = total number of additional and secondary setae on segment. Three specimens were examined.

Segment	Position	Range	
Coxa	PD	3-4/4-5/4-5	
	А	3-4/1-5/3-7	
	Total	6-8/6-9/7-12	
Trochanter	Di	0/0/0-1	
Femur	AD	2/2-4/2-4	
	AV	0-2/1-2/2-4	
	PV	2-4/5-6/5-6	
	Total	4-8/8-11/9-14	
Tibia	AV	0/1/2	
Tarsus	AV	0/0/3-4	

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