

Morphology and biology of *Malachius prolongatus* (Coleoptera: Melyridae: Malachiinae)

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Abstract. Oviposition behaviour, early instars and adult sexual dimorphism of *Malachius (Malachius) prolongatus* Motschulsky, 1866 (Coleoptera: Melyridae: Malachiinae) are described. Larval morphogenesis and the timing of developmental events are compiled and compared with larval development of three malachiine species studied before. Larvae of *Malachius prolongatus* at the time of egg hatch exhibit the most advanced morphogenesis currently known in malachiine beetles, especially in the development of labrum, antennae, mandibular condyles, maxillary and labial palps and prolegs. However, similar to previously studied genera, they also bear thoracic egg bursters.

Key words. Coleoptera, Cleroidea, Malachiinae, Melyridae, morphology, ontogeny, hatching, first instar larva, foetomorphic larva, embryonic molt

Introduction

Three species of malachiine beetles (family Melyridae), i.e., *Laius asahinai* Nakane, 1955, *Intybia niponicus* (Lewis, 1895) and *Nepachys japonicus* (Kiesenwetter, 1874) have been found to go through ‘foetometamorphosis’ during their life cycles. The first instar larva after hatching from the egg is inactive, non-feeding and with vestigial head appendages and legs (so-called foetomorph), and completes larval morphogenesis only after hatching from the egg (ASANO & KOJIMA 2013; ASANO 2013, 2014). Foetometamorphosis was first observed in cantharid beetles by VERHOEFF (1917) and was interpreted as an abnormal metamorphosis, similar to hypermetamorphosis in Endopterygota (VERHOEFF 1917, JANBEN 1963). Later, EVERS (1960) found that the hatchlings of the malachiine species *Axinotarsus (Axinotarsus) pulicarius* (Fabricius, 1777) are inactive and non-feeding, but he did not describe them in detail. ASANO & KOJIMA (2013) investigated the ontogeny of *L. asahinai*, described early instars and revealed that hatchlings (= first instars) and second instar larvae were in the foetomorphic state. This study was the first description of foetomorphic early instars in the malachiine melyrids. Foetomorphosis was subsequently discovered in malachiine genera *Laius* Guérin-Méneville, 1838 and *Intybia* Pascoe, 1866 as well (ASANO 2013, 2014).

Malachiine genera in which foetomorphosis was found are classified in two subtribes: Apalochrina (*Laius* Guérin-Méneville, 1838 and *Intybia* Pascoe, 1866) and Attalina (*Intybia* Pascoe, 1866). Nothing is known for the five remaining subtribes recognized today (Ebaei-na, Colotina, Illopina, Malachiina and Troglolina). Based on the fact that complex kinds of metamorphosis are usually shared between closely related taxa (e.g., in case of hypermetamorphosis; CROWSON 1981), it can be assumed that foetomorphosis may be more widespread in Malachiinae.

Recently, I collected many adults of *Malachius* (*Malachius*) *prolongatus* Motschulsky, 1866 (classified in the subtribe Malachiina). *Malachius prolongatus* is a common species distributed in the Japanese islands, except for the Nansei Islands (YOSHITOMI & HAYASHI 2011). However, little biological information is known so far (HAYASHI & TAKENAKA 1959, HAYASHI 1974), and the early instars have never been observed. Therefore, I reared adults of *M. prolongatus*, observed their oviposition and hatching in captivity, closely examined the larval morphogenesis of early instars, and the timing of developmental events.

In this paper, the foetomorphic first instar and second instar larvae of *M. prolongatus* are described. Additionally, I describe the sexual dimorphism of adults, and provide a differential diagnosis and additional biological information about the species.

Material and methods

Adult specimens (5 males and 13 females) of *Malachius prolongatus* were collected from the inflorescence and leaves of *Leucanthemum paludosum* (Poiret) Bonnet et Barratte (Asteraceae), *Viburnum plicatum* Thunberg var. *tomentosum* (Thunberg ex Murray) (Adoxaceae), *Rumex acetosa* L. (Polygonaceae), *Rosa multiflora* Thunberg, *Spiraea cantoniensis* Loureiro and *Stephanandra incisa* (Thunberg) (all Rosaceae) at various locations in Shizuoka prefecture, in the central region of Honshû island, Japan, from 21st April through 8th May 2016. Females were transferred one each to plastic containers with the dead branches and bark of *Pueraria lobata* Ohwi (Fabaceae), as well as dead branches of *Miscanthus* sp. (Poaceae) for egg-laying sites, and inflorescences of *Leucanthemum paludosum* as a source of food. Containers were kept at room temperature. In total, 125 eggs were found on the bark pieces and branches in the containers from 7th through 20th May 2016. On 16–28th May 2016, a total of 89 eggs had hatched, the remaining eggs died from drying up or mold. The first larval molt occurred on 18th–30th May 2016. For further observations, 10 specimens each of the first and second instar larva were enclosed alive in glycerin. Three adult males and 3 adult females were fixed in 70% alcohol and immersed in 10% KOH solution for about 24 hours at room temperature. Specimens were examined under compound microscopes (Olympus BX41 and CHS), and drawn with the aid of attached drawing tubes.

In larval description, the following abbreviations are used:

BL – body length, from the anterior margin of the frons to apex of the urogomphi;	an – antenna;	lp – labial palp;
BW – maximum body width;	cr – chorion;	md – mandible;
HL – head capsule length;	ct – cuticle;	meb – eggburster of mesonotum;
HW – maximum head capsule width;	eb – egg burster;	mp – maxillary palp;
UL – urogomphi length;	L1 – first instar larva;	peb – egg-burster of pronotum;
	hp – hypopharinx;	pl – proleg;
	lb – labrum;	st – larval stemma.

In adult description, the terminology of male genitalia follows YOSHITOMI (2014), and the following abbreviations are used:

BL – length of body, from anterior margin of frons to elytral apices;	EW – maximum width of elytra;
HL – maximum length of head capsule;	HTL – length of hind tibia;
HW – maximum width of head capsule, including eyes;	gl – the aggregation of glands
PL – maximum length of pronotum;	gp – gonoporal piece;
PW – maximum width of pronotum;	is – intarnal sac;
EL – maximum length of elytra;	sc – sclerite;
	tf – tuft of hairs.

The specimens examined are deposited in the collections of Makoto Asano, Shizuoka, Japan.

Results

Malachius (Malachius) prolongatus Motschulsky, 1866

(Figs 1–30)

Malachius prolongatus Motschulsky, 1866: 167 (original description)

Malachius (Malachius) prolongatus: EVERS (1985): 23 (establishment of the subgenus *Malachius* and key to species)

Additional description of adult (Figs 1–9, 27). **Male.** Measurements in mm ($n = 2$). BL: 6.7 (6.5–6.8); HL: 1.2 (1.1–1.2); HW: 1.4 (1.3–1.4); PL: 1.4 (1.3–1.5); PW: 1.6 (1.5–1.6); EL: 4.1 (4.0–4.1); EW: 2.1 (1.8–2.5); HTL: 2.0.

Head capsule rather wide (HW/HL 1.1); apical margin of frons prominent, with dense short setae and glandularium (Fig. 6: gl); gula with a tuft of hairs (Figs 7, 9: tf).

Abdomen rather soft; tergite VIII subtrapezoid, long and rather slender (Fig. 3); VIII sternite in caudal margin bifid, each apex hook-shaped (Fig. 2). Endophallus elongate and almost straight. Internal sac (Fig. 4: is) with numerous minute spines densely all over the inner surface and with a gonoporal piece (Fig. 4: gp) but without ligra. Gonoporal piece with a long and thin sclerite (Fig. 4: sc). Spicular fork thin, anterior half cover-up endophallus and posterior part bifid (Fig. 5).

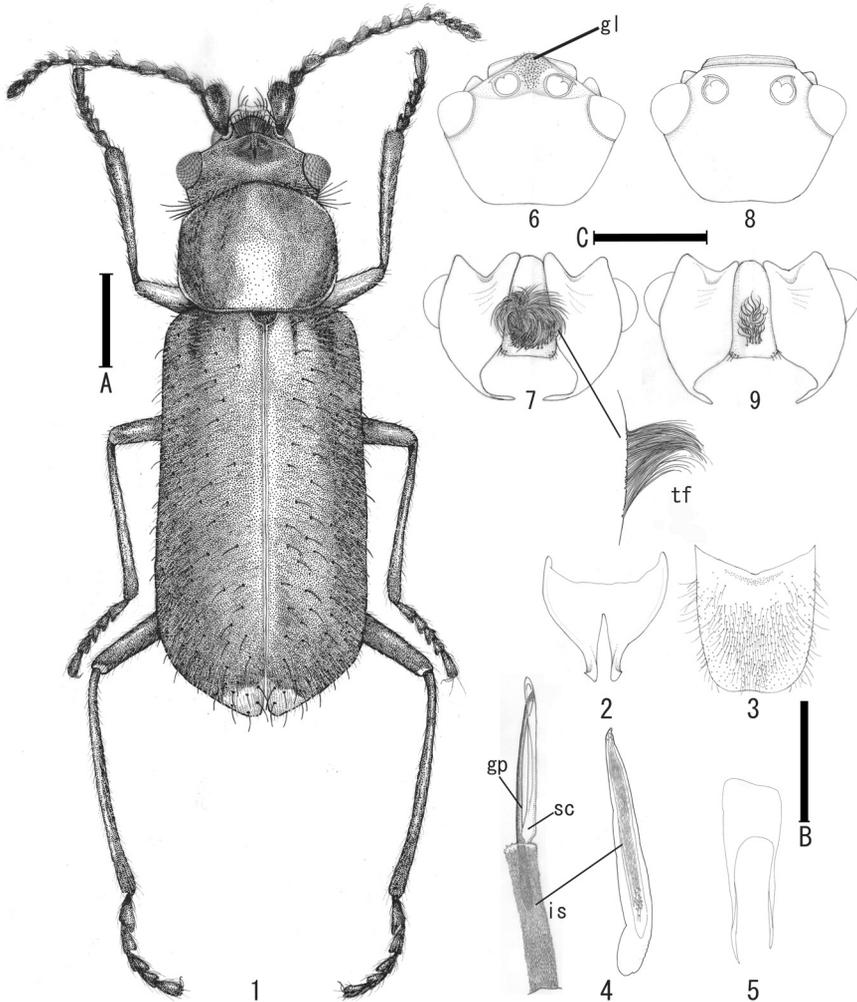
Female. Measurements in mm ($n = 2$). BL: 6.6 (6.4–6.8); HL: 1.0 (0.9–1.0); HW: 1.4; PL: 1.1 (0.9–1.2); PW: 1.6; EL: 3.9 (3.6–4.2); EW: 1.9 (1.8–2.0); HTL: 1.7.

Head capsule rather wide (HW/HL 1.4), frons simple (Fig. 8), gula with a small tuft of hairs (Fig. 9).

Description of egg (Fig. 24). Length: 1.0–1.1 mm ($n = 2$). Light orange in color, chorion unpigmented and transparent. Slender, soft and smooth. Embryos are visible through very thin chorion (Fig. 24).

Description of first instar (larva at the egg hatch) (Figs 10–13, 17–23, 25). Measurements in mm ($n = 1$). BL: 1.07; BW: 0.31; HL: 0.13; HW: 0.20; UL: 0.06.

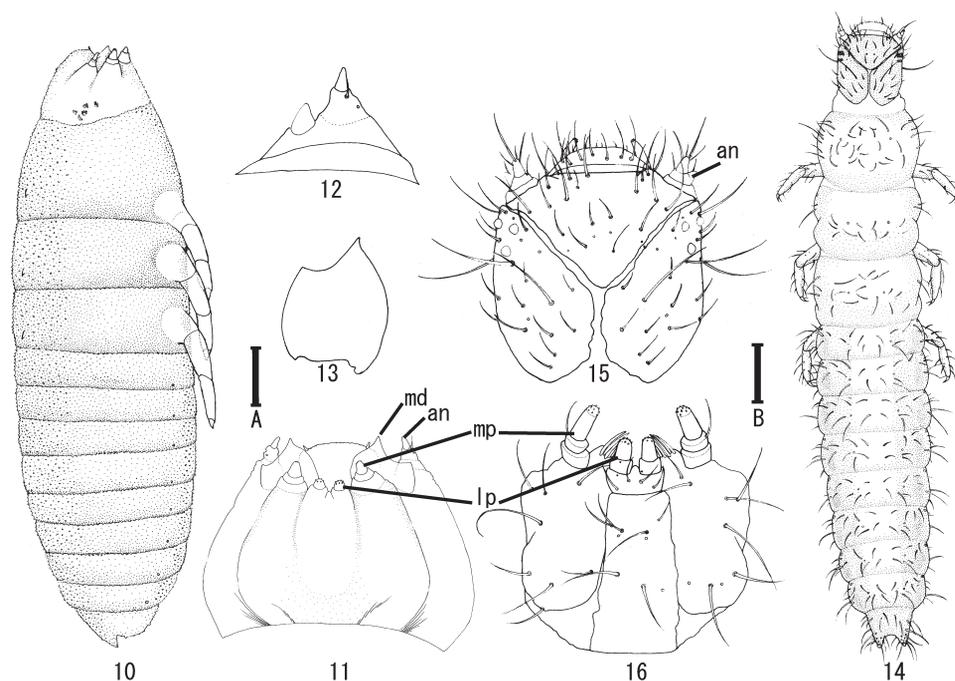
Body unpigmented and translucent, very soft, rugose, oblong oval in form, setae absent except on antennae and labrum. Yolk stocked in midgut, chorion still covering abdomen (Figs 10–13, 17, 18). Head capsule subtrapezoidal; setae, pore, frontal arm and epicranial stem indistinct. Labrum distinguishable, with 2 setae. Five stemmata obscurely visible, 3 arranged in transverse row anteriorly and 2 posteriorly (Fig. 10, 17). Antennae (Fig. 11: an) obscurely 3-segmented; segment I short, segment II with a conical sensorium, seta and pore, segment III slender and long (Fig. 12). Mandibles (Fig. 11: md) protruding, with two pointed



Figs 1–9. Illustrations of *Malachius (Malachius) prolongatus* Motschulsky, 1866. male (1–5), sexual dimorphisms in adult (6–9). 1 – habitus; 2 – 8th sternite in ventral view; 3 – 8th tergite in dorsal view; 4 – endophallus in lateral view with extrusive gonoporal piece; 5 – spicular fork; 6 – male head in dorsal view with a tuft in lateral view; 7 – ditto, in ventral view; 8 – female head in dorsal view; 9 – ditto, in ventral view. Scales = 1.0 mm: A (1), B (2–5), C (6–9). Abbreviations: gl – the aggregation of glands; gp – gonoporal piece; is – intarnal sac; sc – sclerite; tf – tuft of hairs.

parts, divided from head capsule and not overlapping each other, condyle formed obscurely (Fig. 13, 21). Maxillary palpi (Fig. 11: mp) obscurely 3-segmented. Labial palpi (Fig. 11: lp) 1-segmented. Prementum, maxillae, and postmentum undivided and fused with each other.

Pronotum about 1.1× as long as broad, about 1.4× as broad as head, obtusely angulate. Pro- to metanota each with egg-bursters (Fig. 22, 30), setae and markings absent (Fig. 10).



Figs 10–16. Early instars of *Malachius (Malachius) prolongatus* Motschulsky, 1866. 1st instar (10–13), 2nd instar (14–16). 10 – habitus in lateral view; 11 – head in ventral view; 12 – antenna in ventral view; 13 – right mandible in ventral view; 14 – habitus in lateral view; 15 – head in dorsal view; 16 – mouth parts in ventral view. Scale A = 0.1 mm: (10), 0.04 mm: (11), 0.015 mm: (12–13), Scale B = 0.1 mm: (14), 0.02 mm: (15), 0.01 mm: (16). Abbreviations: an – antenna; lp – labial palp; md – mandible; mp – maxillary palp.

Prolegs conical, femora, tibiae and claws segmented (Fig. 10, 19).

Abdomen 9-segmented, widest at segment I and then narrowing posteriorly. Urogomphi short, pointed apically, setae absent (Fig. 23).

Description of second instar (larva after the first molt) (Figs 14–16). Measurements in mm ($n = 1$). BL: 1.19; BW: 0.2; HL: 0.14; HW: 0.12; UL: 0.18.

Body unpigmented and translucent, soft, rather rugose, oblong. Yolk presented immediately after molt, then consumed. Head capsule subquadrate, about as long as broad and flattened above, frontal arm and epicranial stem distinct; with 17 or 18 pairs of setae and 4 pairs of pores on epicranial plates, with 8–10 pairs of setae and 1 or 2 pairs of pores on frons (Fig. 15). Five visible stemmata, arranged as in first instar. Antennae (Fig. 15: an) visible 3-segmented; segment II with conical sensorium and 2 long setae, segment III with a long and 3 short setae. Labrum about $4.0\times$ as broad as long, with 6 long setae in middle and 8 short setae on labro-epipharyngeal margin. Maxillary palpi (Fig. 16: mp) 3-segmented, obscurely visible, segment II with seta and segment III with sensilla. Labial palpi (Fig. 16: lp) 2-segmented, obscurely visible, segment II with pore and segment III with sensilla. Prementum subtrapezoidal, with 2 pairs of setae and a pair of pores. Maxillary stipes and postmentum

Table 1. Larval morphogenesis of *Malachius* (*s. str.*) *prolongatus*.

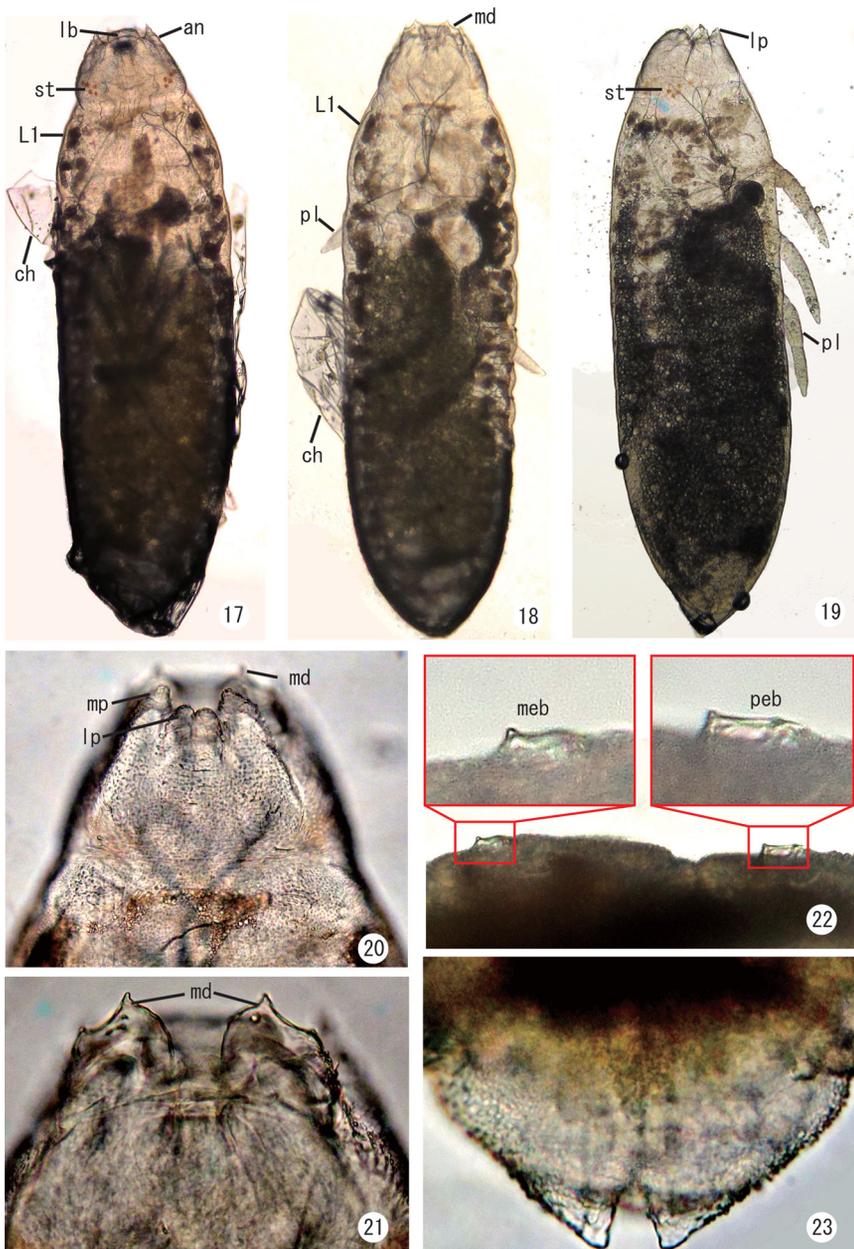
	First instar	Second instar	Mature larva (after HAYASHI & TAKE- NAKA 1957)
Body length (mm)	1.07	1.19	10.00 (maximum values)
Larval cuticle	unsclerotized and unpigmented	unsclerotized and unpigmented	strongly pigmented, densely covered by setae
Head capsule	unsclerotized and unpig- mented	slightly sclerotized and pigmented	strongly sclerotized and pigmented
Cephalic suture	indistinct	distinct	distinct
Cephalic setae	absent	21–22 pairs	46–52 pairs
Labrum	slightly divided	divided	divided
Arrangement of stemmata	3–2	3–2	3–1
Mandible	unsclerotized and unpig- mented, pointed apically, short, protheca absent	completely formed, sclerotized apically, rather short	strongly sclerotized, long
Condyle	formed (small protrusion)	formed	formed
Labium	slightly divided	divided	divided
Pronotum	unsclerotized and unpig- mented	unsclerotized and unpig- mented	strongly pigmented
Setae of pronotum	absent	14–21 pairs	densely covered by setae
Marking of thorax	absent	slightly pigmented	strongly pigmented
Egg burster	present (on thorax)	absent	absent
Yolk	remains	present immediately after molt, then consumed	absent
Urogomphi	defined (short), setae absent	completely formed (short), slightly pigmented, with several setae	long, with 23 pair of se- tae, strongly sclerotized and pigmented
Proleg	completely segmented, setae absent	completely segmented, sparsely covered by setae	completely segmented, densely covered by setae

distinctly visible, the former bearing 7 pairs of setae and the latter with 5 setae and 3 pores. Cardo invaginated behind stipes (Fig. 16).

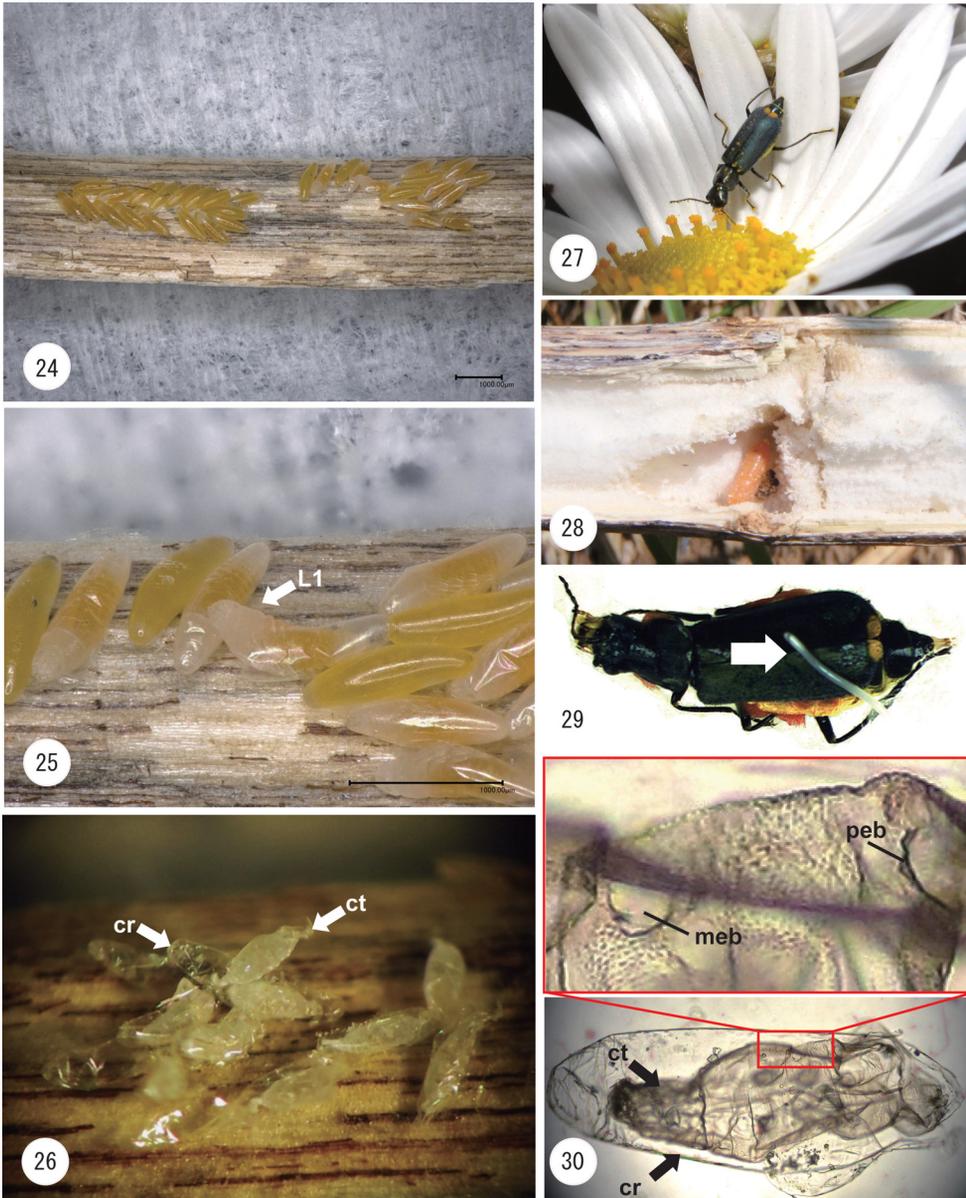
Pronotum about 1.3× as long as broad, about 1.4× as broad as head, dorsum unpigmented, with pair of glandularium, 14–21 pairs of setae. Meso- and metanota subequal in width, each with a pair of glandularium in middle; former with 7–8 pairs of setae; latter with 9–11 pairs of setae. Prolegs long; femur with 6 setae, tibiotarsi with 5 setae, claw with 1 short seta (Fig. 14).

Abdomen 9-segmented, slightly narrowed anteriorly and posteriorly, abdominal tergites I–VIII each usually with 12 pairs of setae and pair of glandularium dorsally. Urogomphi subparallel, apices suddenly recurved, with 11 pairs of setae and 5 pairs of pores (Fig. 14).

Seasonal abundance. In temperate regions of Japan, many males and females were found in the middle of April, however males were hardly found since around May 2, and females could not be found on May 20 in the lowland area of Shizuoka city. Mature larvae and pupae were found in early April in the field. Eggs were laid from early until middle of May.



Figs 17–23. Photographs of first instar of *Malachius (Malachius) prolongatus* Motschulsky, 1866. 17 – habitus in ventral view; 18 – ditto in ventral view; 19 – ditto in lateral view; 20 – maxillary and labial palpi; 21 – mandibles; 22 – egg bursters; 23 – urogomphi. Abbreviations: an – antenna; cr – chorion; L1 – first instar larva; lb – labrum; lp – labial palp; md – mandible; meb – eggburster of mesonotum; mp – maxillary palp; peb – egg-burster of pronotum; pl – proleg; st – larval stemma.



Figs 24–30. Photographs of *Malachius (Malachius) prolongatus* Motschulsky, 1866. 24 – eggs; 25 – hatching; 26 – first molting; 27 – adult; 28 – pupa; 29 – adult parasitized by nematode (indicated by an arrow); 30 – chorion and cuticles first molted. Abbreviations: cr – chorion; ct – cuticle; L1 – first instar larva; meb – eggburster of mesonotum; peb – egg-burster of pronotum.

Table 2. Number of eggs and the dating of developmental events of *Malachius (Malachius) prolongatus* (asterisk showed in the earliest date).

Collection date of adults	3-May-2016	3-May-2016	8-May-2016
Number of eggs	38	40	22
1st oviposition	7-May-2016	9-May-2016	unobserved
Dorsal closure	15-May-2016*	18-May-2016*	unobserved
Egg hatch	16-May-2016*	18-May-2016*	22-May-2016*
1st molt	18-May-2016*	20-May-2016*	25-May-2016*
Number of eggs of 2nd oviposition	25		
2nd oviposition	20-May-2016		
1st larval molt of 2nd oviposition	28-May-2016*		

Biology and larval morphogenesis (Figs 17–30, Tables 1–2). *Malachius prolongatus* inhabits bushes and thickets near inland water areas. Eggs are laid under bark of dead branches of *Pueraria lobata* and leaf sheaths of dead *Miscanthus* sp. in captivity (Fig. 24). At each egg site, 1–40 eggs were laid. Oviposition was observed twice per female. Second oviposition began 11 days after the first one (Table 2). Eggs were not covered by anything. Embryos grew larger in the chorion; eggs were filled to bursting with embryos immediately before egg burst.

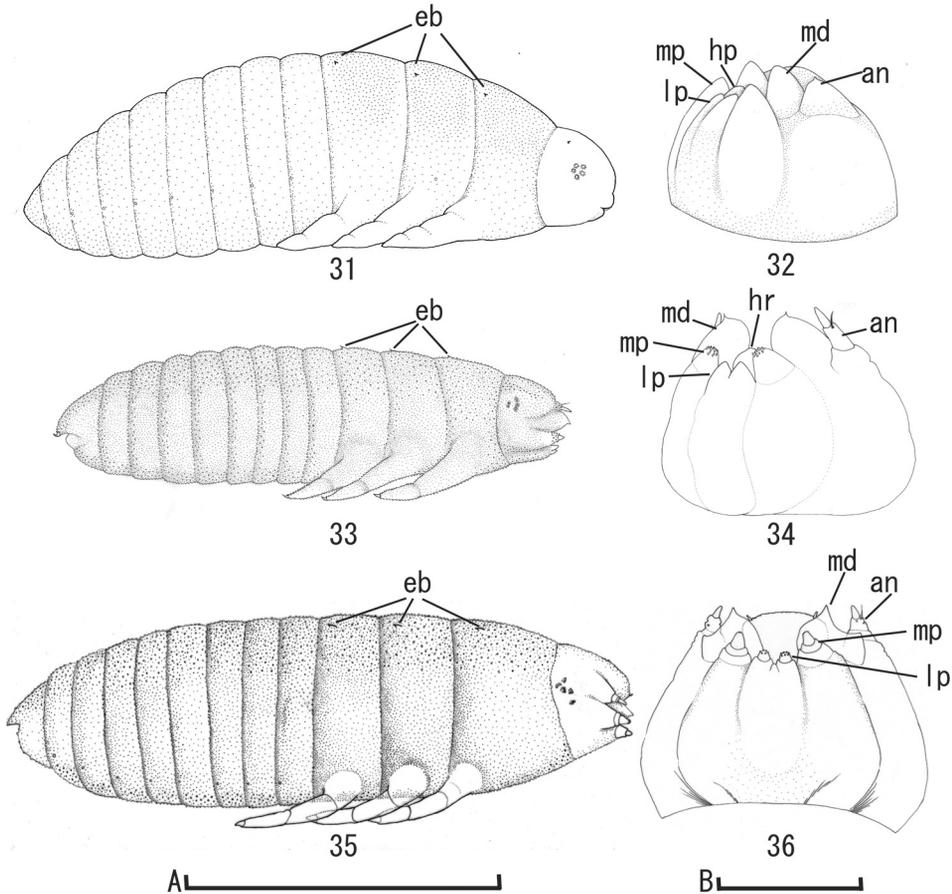
Egg burst and egg hatch begins 9 or 10 days after oviposition (Table 2). The eggs of the same clusters did not all begin to burst at once. Chorion bursts around the larval head to thorax and the larvae partly wriggle out, but remain largely hidden in the chorion for 2 days (Fig. 25). They were inactive and non-feeding; however, they consumed water and wriggled the upper half of the body to expose themselves to the sun. Some body parts remained in the foetomorphic state, the whole body was translucent and flabby, cephalic suture and setae were unformed, mandibles were short and not overlapping each other, condyles were vestigial, they had an extra stemma than normal, urogomphi were short and vestigial and prolegs were short and conical (Figs 17–23) (Table 1).

First molt generally began two days after hatching ($n = 89$) (Fig. 26). Egg bursters (peb, meb) are present in the cuticle of the first instar (Fig. 30: peb, meb). After the first molt, larvae were immediately active, walked on dead branches and consumed the nymphs of aphids which were provided. The yolk which is still present immediately after the first molt, was completely digested in second instar. Larval morphs drastically changed by the first molt. Cephalic and pronotal setae formed; antennae, maxillary and labial palpi were distinctly segmented; egg bursters were absent; mandibles, condyle and urogomphi were completely formed. The head capsule, the tip of mandibles and urogomphi became slightly pigmented (Table 1). The molted cuticle of the first instar larva remains adhered inside of the empty chorion (Fig. 26).

Second molt began about 5 days after the first molt. After the second molt, larvae were also very active. They stayed on the dead branches and consumed small arthropods. Mature larvae probably overwinter, pupate in early spring within dead *Miscanthus* stems, and adults emerge in the spring in the lowland area of Shizuoka city. (Fig. 28). Number of larval instars is however still not known. The morphology of mature (= likely last instar) larvae was described by HAYASHI & TAKENAKA (1959) (Table 1).

The adults are aggressive and prey on many small insects, as well as on each other. While in captivity, they also consume the small insects provided and the pollen of the above plants (Fig. 27). They prey on nymphs of aphids very well, but were in distress to prey on the fast-moving, jumping plant lice and thrips. They were often parasitized by nematodes (Fig. 29).

Mating was observed at the end of April. For this species, the male and female contact each other with their mouthparts (TAGO 2004). This is possibly a precopulatory behavior. For the related species *Malachius bipustulatus* (Linnaeus, 1758), it is known that the female contacts the frons of the male (excitator) with her labial palpus. It is suggested that a secretion from the excitator is used for precopulatory behavior (MATTHES 1962). There is a possibility that the female of *M. prolongatus* also contacts the male glandularium on the frons (Fig. 6) by the labial palpus or a tuft of hairs on the gula (Figs 7, 9).



Figs 31–36. The hatchlings of malachiine. habitus (31, 33, 35), head in ventrolateral view (32, 34), head in ventral view (36). 31 – *Intybia niponicus* (Lewis, 1895); 32 – ditto; 33 – *Nepachys japonicus* (Kiesenwetter, 1874); 34 – ditto; 35 – *Malachius (Malachius) prolongatus* Motschulsky, 1866; 36 – ditto. Figs 31 & 32 from Asano (2013), 33 & 34 from Asano (2014). Scale A = 0.5 mm: (31, 33, 35), Scale B = 0.1 mm (32, 34, 36). Abbreviations: an – antenna; eb – egg burster; lp – labial palp; hp – hypopharinx; md – mandible; mp – maxillary palp.

Table 3. Comparison of first instar larval structure of malachiine species.

Subtribe	Apalochrina		Attalina	Malachiina
Species	<i>Laius asahinai</i>	<i>Intybia niponicus</i>	<i>Nepacys japonicus</i>	<i>Malachius prolongatus</i>
Reference	ASANO & KOJIMA 2013	ASANO 2013	ASANO 2014	this paper
Body length (mm)	0.84	1.16	0.75	1.07
Larval cuticle	unsclerotized and unpigmented	unsclerotized and unpigmented	unsclerotized and unpigmented	unsclerotized and unpigmented
Head capsule	unsclerotized and unpigmented	unsclerotized and unpigmented	unsclerotized and unpigmented	unsclerotized and unpigmented
Cephalic setae	5pairs	5 pairs	absent	absent
Labrum	undivided	undivided	undivided	slightly divided*
Stemma	3—2	3—2	3—2	3—2
Antenna	unsegmented, sensorium absent	unsegmented, sensorium absent	2- segmented, sensorium absent	3-segmented, with sensorium*
Mandible	unsclerotized and unpigmented, protheca absent	unsclerotized and unpigmented, protheca absent	unsclerotized and unpigmented, pointed apically, protheca absent	unsclerotized and unpigmented, short, pointed apically, protheca absent
Condyle	invisible	invisible	invisible	slightly divided*
Hypopharynx	visible	visible	visible	invisible*
Maxillary palps	unsegmented	unsegmented	1-segmented	3-segmented*
Labial palps	unsegmented	unsegmented	unsegmented	2-segmented*
Pronotum	unsclerotized and unpigmented	unsclerotized and unpigmented	unsclerotized and unpigmented	unsclerotized and unpigmented
Egg burster	present (on thorax)	present (on thorax)	present (on thorax)	present (on thorax)
York	remains	remains	remains	remains
Urogomphi	undeveloped	undeveloped	defined (short), setae absent	defined (short), setae absent*
Proleg	unsegmented	unsegmented	claws segmented	completely segmented, setae absent*
Spiracles	visible	visible	visible	visible

It is conceivable that females oviposit at least twice from early through late May. They require about 11 days from first to the second oviposition (Table 2).

Remarks. HAYASHI (1974) described an interesting case of the oviposition of *M. prolongatus* on a dipteran larva, but it was probably accidental. The closely related species *Axinotarsus pulicarius* (Fabricius, 1775) lays 10–30 eggs per oviposition per female (EVERS 1960).

Discussion

The first instar larva of *Malachius prolongatus* (Figs 35, 36) has a single foetomorphic larval stage, similar to *Nepachys japonicus* (subtribe Attalina; ASANO 2014). In contrast, the two species of the subtribe Apalochrina with known larval morphology, i.e. *Laius asahinai* and *Intybia niponicus* have two foetomorphic instars (ASANO & KOJIMA 2013, ASANO 2013).

In comparison among the structures of first instar larvae of the above four species (Table 3), we find that the degree of completion of the larval morphogenesis at egg hatch differs between species. The larval morphogenesis at egg hatch of *M. prolongatus* is more advanced than in the remaining three genera studied (see Figs 31, 32 for *Intybia niponicus*, and Figs 33, 34 for *N. japonicus* (the most advanced morphogenesis is marked by an asterisk in Table 3). Two species of the subtribe Apalochrina, *I. niponicus* and *L. asahinai* hatched with the most immature structures, which are unsegmented antenna, maxillary and labial palps, undeveloped urogomphi and unsegmented prolegs in principle (Figs 31, 32). *Nepachys japonicus* hatched with a more advanced morph than the above two species of Apalochrina, however the morphogenesis of the labrum, antenna, maxillary palps, and prolegs are more retreated than *M. prolongatus* (Figs 33, 34). Nevertheless, the first instar larvae of all the above species have egg bursters of some kind on their thorax.

In the order Coleoptera, the larval structure is usually completed in the egg (CROWSON 1981), and the first instar larva hatches with structures allowing it to survive on its own. The egg bursters are usually presents on the cuticle of the first instar larvae. However, the timing of their occurrence seems to be earlier than in the studied malachiine species, in which the first instar larval structures are not completed upon egg hatch. This also suggests that egg bursters are formed in different developmental stages in different species.

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