

## Reproductive Ecology and Immature Stages of *Copelatus masculinus* RÉGIMBART, 1899 (Coleoptera, Dytiscidae)

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**Abstract** *Copelatus masculinus* RÉGIMBART, 1899 collected from Yonaguni-jima Island in the Ryukyus, were reared in the laboratory and laid at least 16 eggs during a period of 31 days. The developmental period for each stage was as follows: egg, within seven days; first instar larva, two to seven days; second instar larva, five to 15 days; third instar larva, eleven to 19 days; landing to escaping including pupation and emergence, twelve to 27 days. Given that the eggs were few and laying started immediately after mating, we estimated that laying periods of *C. masculinus* are likely to be in the early summer in natural environments. A single third instar larva survived on land for up to 56 days. It is likely that the third instar larvae can survive under detritus for approximately two months when their habitat lacks water in natural environments. The surface of the pupa was covered by many setae, allowing it to float on water. All newly emerged adults escaped from the pupal chamber to the soil.

### Introduction

The dytiscid genus *Copelatus* ERICHSON, 1832 comprises 438 known species worldwide (MILLER & BERGSTEN, 2016). From Japan, 14 *Copelatus* species have been recorded to date (MORI & KITAYAMA, 2002). The Asian species *Copelatus masculinus* RÉGIMBART, 1899 (Fig. 1A) has been recorded from southwestern Japan (islands of Ishigaki-jima, Iriomote-jima, and Yonaguni-jima), China, Philippines, Malaysia, and Indonesia. The Japanese population of the species was recognized as *C. imasakai* MATSUI et KITAYAMA, 2000 (MATSUI & KITAYAMA, 2000), which has recently been treated as a junior synonym of *C. masculinus* by HÁJEK *et al.* (2018). The distribution of the Japanese population is restricted to the southernmost islands of the Ryukyu Islands, i.e., Ishigaki-jima, Iriomote-jima, and Yonaguni-jima (MORI & KITAYAMA, 2002).

The biology of *C. masculinus* remains largely unknown. No information is available, excluding the work by MORI and KITAYAMA (2002) and HÁJEK *et al.* (2018). MORI and KITAYAMA (2002) mentioned that adult individuals of the species were collected in shaded wetlands and paddy field ditches along forest edges in Japan. HÁJEK *et al.* (2018) mentioned that *C. masculinus* were collected in shallow newly emerged pools with clayey bottoms and devoid of vegetation at the borders of forests and *Acacia mangium* plantations in East Kalimantan. Not surprisingly, immature stages of the species have never been reported. Life history of and morphological information on the immature stages will provide essential data for the conservation of aquatic biodiversity, as well as for taxonomic and phylogenetic studies (e.g., OHBA, 2009 a, b; SASAKAWA, 2016; WATANABE *et al.*, 2017). In addition, these data are expected to allow us to investigate the *C. masculinus* biology in the field.

We attempted laboratory rearing of *C. masculinus* with the aim of revealing the basic biology of *C. masculinus*. In this study, we report the results of the rearing, particularly reproductive ecology, and briefly describe its immature stages.

## Materials and Methods

### *Rearing methods*

Adult individuals of *Copelatus masculinus* were collected in Tarumai-shitsugen, Yonaguni-chô, Yonaguni-jima Island, Okinawa Prefecture, Japan on June 17, 2018, by the first author. Three individuals (two males and one female) were captured at the same locality and brought to the rearing room of the Ishikawa Insect Museum (Hakusan-shi, Ishikawa Prefecture) on June 20, 2018. The rearing room was maintained at 26°C with nine hours of light (from 8:15 to 17:15) and 15 hours of darkness (9L : 15D). We placed dead leaves and Java moss (Hypnaceae) in a plastic rearing cup for adults (13 cm in diameter, 6 cm in height) to provide sites for hiding and oviposition. Adequate quantities of frozen chironomid larvae were provided to adults as prey every two to three days. The rearing experiment was terminated on December 5, 2018. Rearing container was checked every one to three days for eggs by washing the Java moss. Eggs that had obviously been delayed in being detected were also counted and the number of days recorded.

When eggs were found, we recorded the date and the developmental stage. Eggs were individually reared in plastic cups ('larval cups'; 8 cm in diameter, 4 cm in height, ca. 5 mm in water depth) containing Java moss and maintained at 26°C. We provided living chironomid larvae as larval feed. We prepared plastic cups ('pupation cups'; the same size as larval cups) with crushed and moistened peat moss (1 cm in depth) as landing soil. We forcibly placed third instar larvae on the peat moss in the pupation cups if they did not eat prey items and instead walked without stopping for up to a day.

The date of hatching, molting, transition to the soil, and escaping of newly emerged adults to the soil surface were recorded in detail for each individual, as well as the condition of individuals in the larval and/or pupation cups. Newly emerged adults were kept individually and fed frozen chironomids in the larval cups.

### *Estimation of duration of immature stages (days)*

To determine the number of days of each developmental stage from egg to emergence, we used histograms. Each larval period can vary, therefore inferring these periods using histograms can provide a more accurate value than using averages.

### *Morphological study*

All specimens for morphological study were reared by the first author: four third instar larvae fixed in 70% ethanol for observations. All specimens were reared as described above. Observations were carried out using a Nikon SMZ stereoscopic microscope. Photographs were taken with a Nikon Digital Sight DS-L2 digital camera attached to the SMZ microscope. Composite images were created using the software Adobe Photoshop CS4 for Mac. The fine structures of a third instar larva were observed using a scanning electron microscope (SEM; JEOL JCM-6000 Neoscope Scanning Electron). The larva was dried using vacuum freeze-drying equipment and given an ultrathin gold coating by high-vacuum evaporation. Morphological terminology follows BALKE (2005). The present description may overlaps that of generic concepts of *Copelatus*. However, the larvae of only a few *Copelatus* species are known and generic limit of larval morphology is still unknown. For a comprehensive study in the future, in this paper we describe the larva in detail as possible.

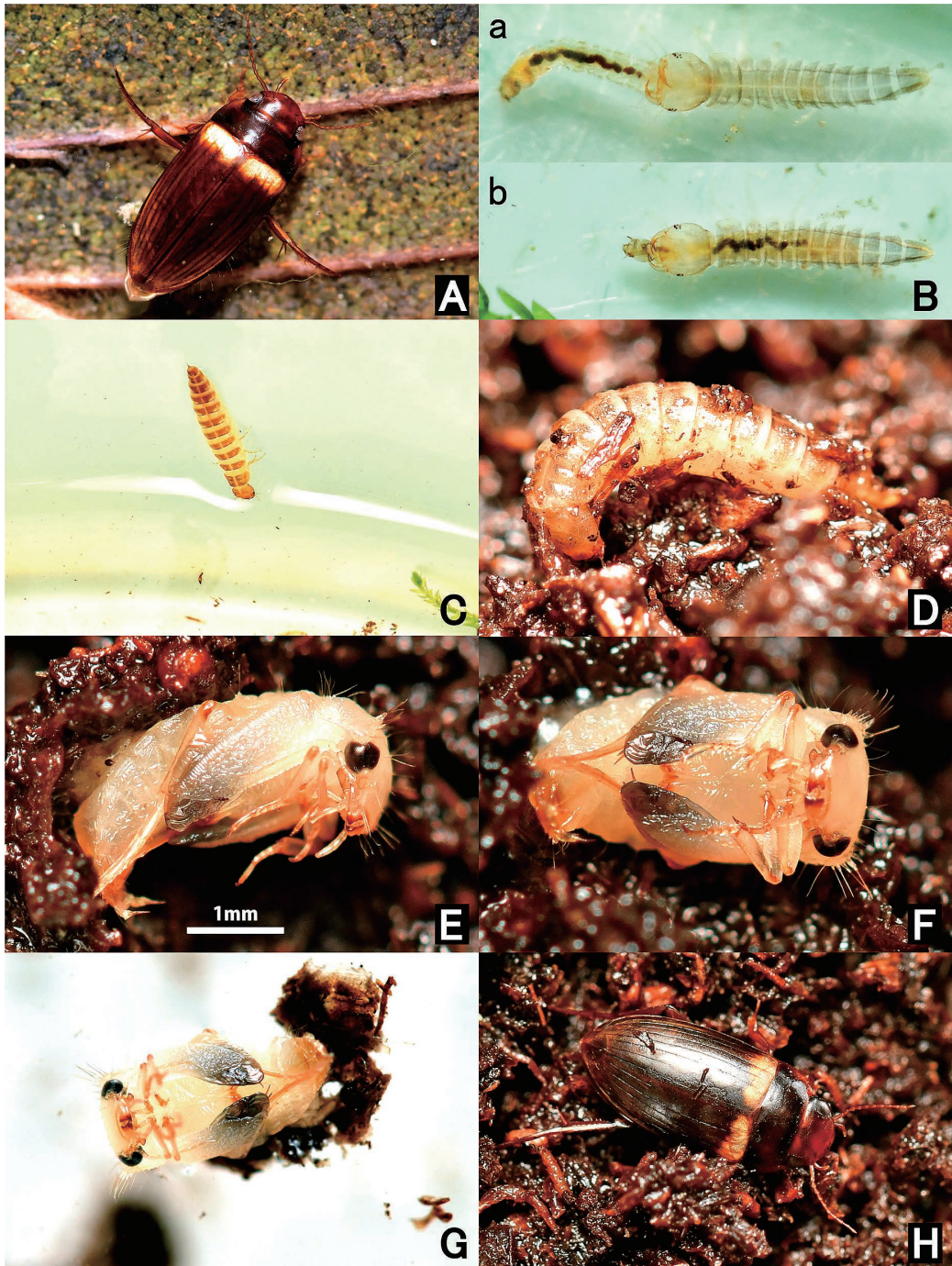


Fig. 1. *Copelatus masculinus*. — A, Adult from Yonaguni-jima Island; B, second instar larva preying on living chironomid larvae (a, after capture; b, swallowing); C, third instar larva climbing the vertical surface of a larval cup; D, third instar larva that remained in the soil for 56 days; E & F, pupa in a pupal chamber; G, pupa floating after pouring water on the soil; H, new adult escaping to the soil surface.

## Results

### *Reproductive ecology*

The first egg was found on June 26, six days after adult rearing. The female continued to lay eggs until July 27, 2018, and in total 16 eggs were found during the 31 days. Eggs were laid singly on Java moss during this period. The eggs were covered with a viscous substance, enabling them to adhere to solid surfaces.

The number of days in each developmental stage is shown in Fig. 5. All 16 eggs were fertile. The egg period was two to seven days (mean 5.3 days,  $n = 16$ ). About 75% of eggs hatched within five to six days (Fig. 5).

The larvae started eating live chironomid larvae from hatching day (Fig. 1B). When larvae encountered a live chironomid larva, the larvae captured the bait using their mandibles, and swallowed them whole (Fig. 1B; Ishikawa Insect Museum, 2019).

The period of each developmental stages was as follows: total larval period, 22 to 33 days (mean 29.2 days,  $sd = 3.8$ ,  $n = 11$ ); first instar larvae, two to seven days (mean 3.1 days,  $sd = 1.2$ ,  $n = 16$ ); second instar larvae, five to 15 days (mean 9.1 days,  $sd = 2.9$ ,  $n = 16$ ); third instar larvae, eleven to 19 days (mean 15.2 days,  $sd = 2.2$ ,  $n = 11$ ). There was several days of individual variation in the developmental period of each larval instar (Fig. 5). Histograms of the developmental stages (Fig. 5) showed the distribution of developmental periods as follows: total larval period, the higher frequency was 28 to 33 days with small differences; first instar larvae, the highest frequency was three to four days; second instar larvae, the higher frequency was six to nine days with small differences; third instar larvae, the higher frequency was 13 to 17 days, with small differences.

After transitioning to land, the larvae dug into the soil and made a pupal chamber. We also observed that pupa floated to the surface when they were immersed in water (Fig. 1G).

Premature transition to the soil of third instar larvae was recorded once. The larva landed on August 27, when we checked on October 22 (56 days later), we discovered the larva in the soil (Fig. 1D). Although in this case, the soil in the rearing container was only slightly moist, the larva was alive, with a swelled body and did not move. The larva started feeding on prey after being returned to a larval cup, and transitioned to the soil again five days later.

The pupal periods were not quantified, because the inside of the pupal chamber could not be observed in all pupation cups. New adults escaped from the pupal chamber after twelve to 27 days (mean 21.6 days,  $n = 10$ ), and moved to soil surface (Fig. 1H). The body was colored, but the abdomen was slightly lighter compared to the parents. Adults began to feed from the day they escaped the pupal chamber.

### *Description of the third instar larva*

Body length ca. 9.0 mm in expanded specimen preserved in 70% ethanol. Body slender, thorax slightly wider than head capsule.

Color. Head yellowish brown. Thorax and abdomen bicolored, with brownish sclerites and much lighter membranous parts.

Head. Head capsule semi-circular, wider than long (Fig. 2C & 3A). Dorsal to lateroventral surfaces of head capsule bearing very densely distributed, fine scale-like microstructures (Fig. 3B); median and lateral part with small patches without the structures. Six stemmata present on anterior corner of head capsule (Fig. 3C), anterior three more closely aggregated than posterior three. Frontal margin of front clypeus gently rounded. Posterolateral corner with small spine-like setae (Fig. 3H). Maxillary stipes elongate (Fig. 3F). Maxilla palpi four-segmented (Fig. 3F). Antenna four-segmented, third an-

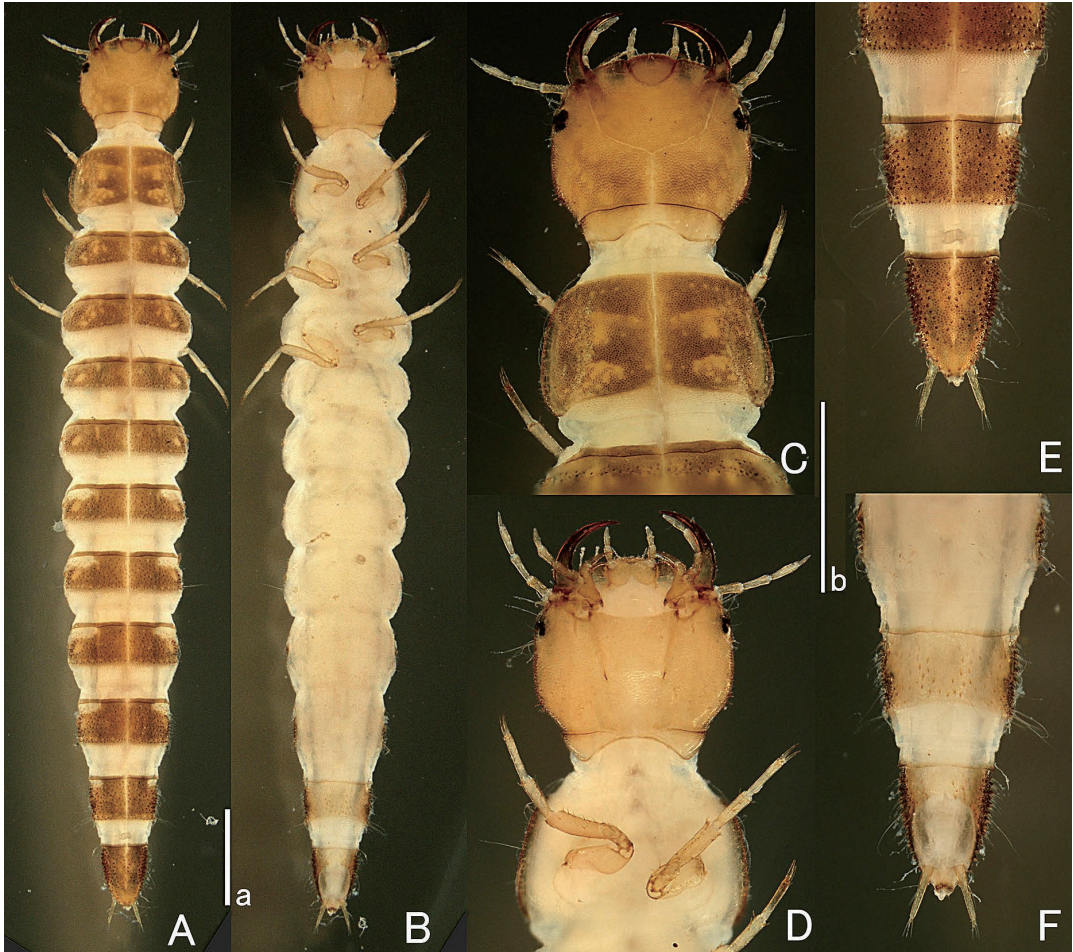


Fig. 2. Third instar larva of *Copelatus masculinus*. — A & B, Habitus; C & D, head and prothorax; E & F, abdominal terminal. — A, C & E, Dorsal view; B, D & F, ventral view. Scale bars: 1.0 mm, left (a) for A & B; right (b) for C–F.

tenal segment with short sensorial appendage (Fig. 3G). Mandible simple, inner margin with fine serrations (Fig. 3F).

**Thorax.** Prothorax narrowly flattened in lateral margin, without short keel and coarse spines on front-lateral area (Fig. 4B). Dorsal surfaces of prothorax with bearing very densely distributed, fine scale-like microstructures; median and lateral part with small patches without the structures, more or less reticulate (Fig. 4A). Meso- and metathorax shorter than prothorax, with sparsely arranged small spines (Fig. 4C). Legs slender, without swimming setae (Fig. 3D).

**Abdomen.** Eight-segmented, bearing small spines on dorsal and lateral surfaces (Fig. 4D–E); all segments without lateral fringe of swimming setae. Eighth segment triangular; urogomphi shorter to subequal relative to length of the last segment (Fig. 4E–H).

**Remarks.** WATANABE *et al.* (2017) described the third instar larva of *C. parallelus* ZIMMERMANN, 1920 based on SEM observations. The SEM photographs of both species suggest that the larva of *C. masculinus* is lacking large spines on postlateral corner of head, lateral keel, and dorsal spines on prothorax. The spines and keels on head and prothorax are useful characters for identification for the

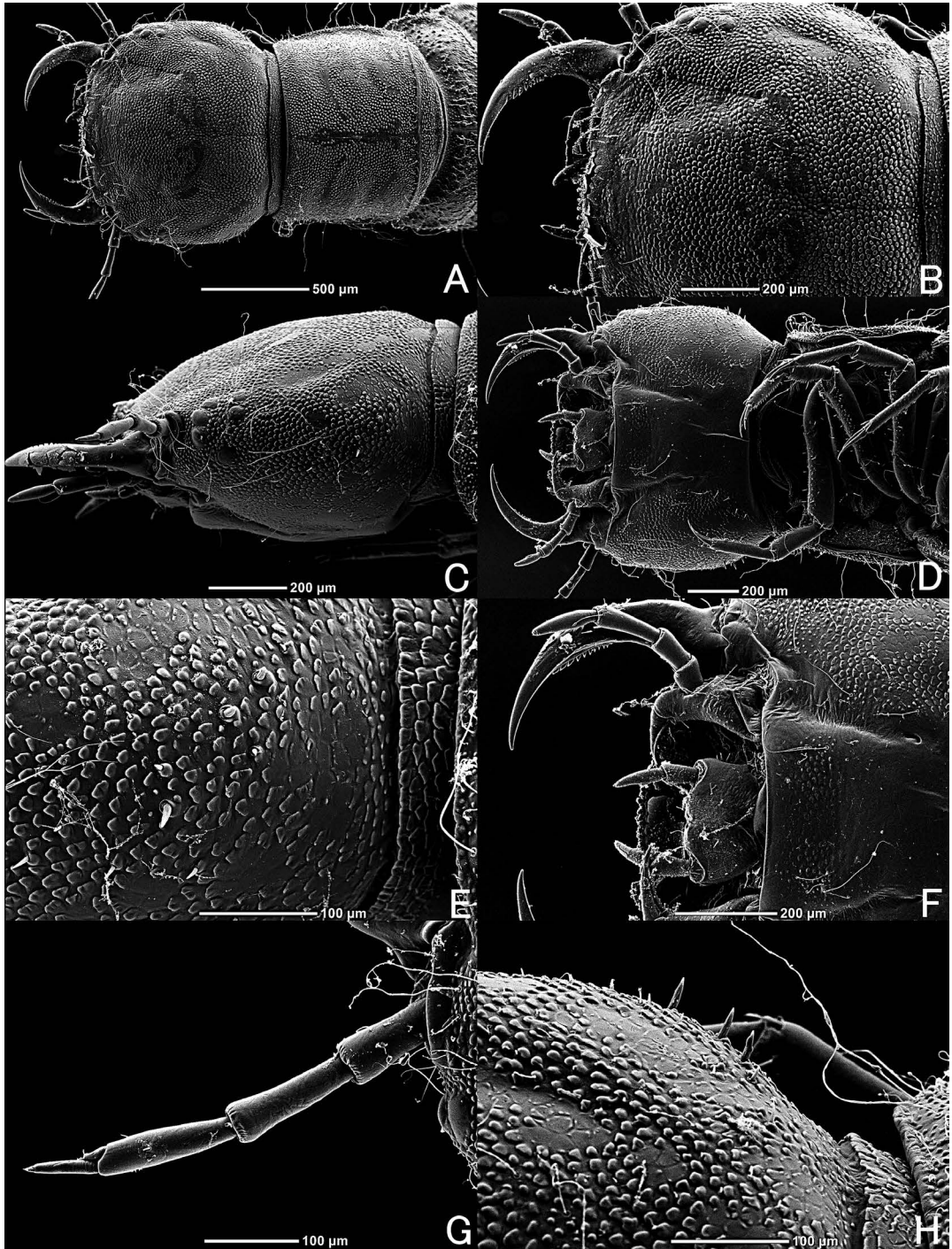


Fig. 3. SEM photographs of a third instar larva of *Copelatus masculinus*. — A & D, Head and prothorax; B, C, E, F & H, head (E & H, spine-like setae on postlateral corner); G, left antenna. — A, B, G & H, Dorsal view; C & E, lateral view; D & F, ventral view. Scale bars: 100 µm for E, G & H; 200 µm for B, C, D & F; 500 µm for A.

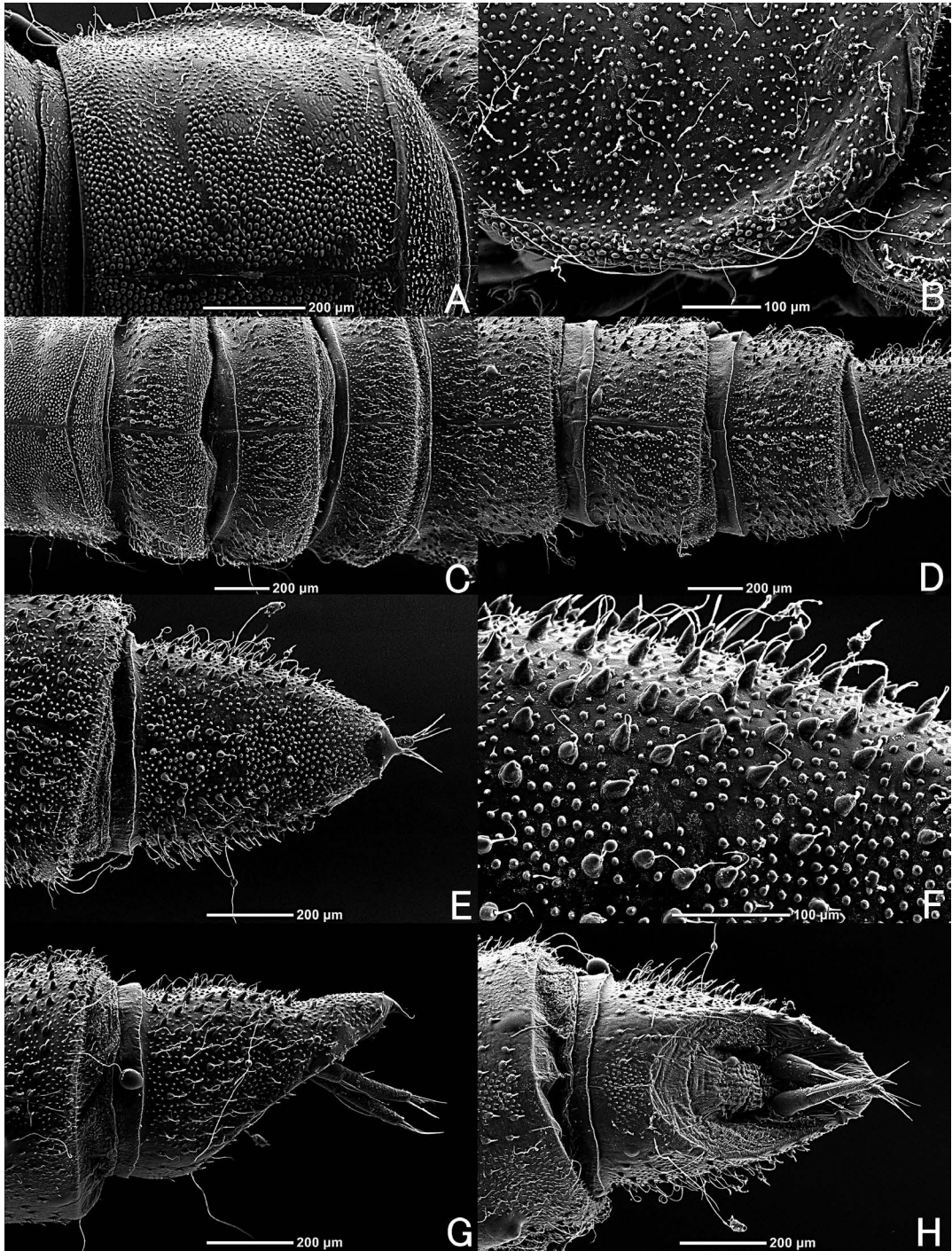


Fig. 4. SEM photographs of a third instar larva of *Copelatus masculinus*. — A & B, Prothorax; C, prothorax to second abdominal segment; D, fifth to eighth abdominal segments; E–H, last (eighth) segment. — A, C–F, Dorsal view; B & G, lateral view; H, ventral view. Scale bars: 100  $\mu\text{m}$  for B & F; 200  $\mu\text{m}$  for A, C–E, G & H.

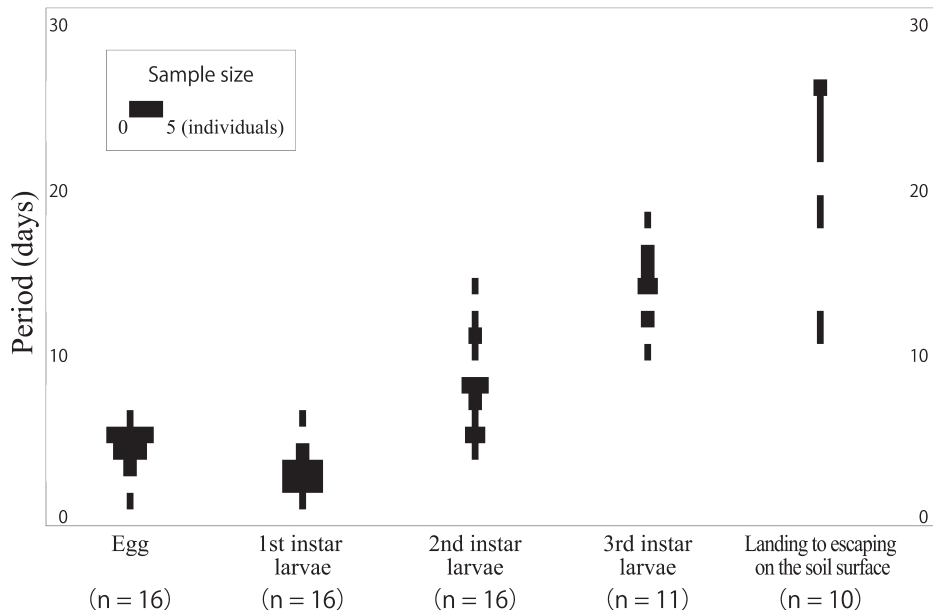


Fig. 5. Histogram of the number of days in each developmental stage, from egg to escaping to the soil surface after emergence.

larvae of Japanese *Copelatus*. Six *Copelatus* species have been recorded in Japan in addition to *C. masculinus*: *C. weymarni* BALFOUR-BROWNE, 1947, *C. oblitus* SHARP, 1882, *C. teranishii* KAMIYA, 1938, *C. kammuriensis* TAMU et TSUKAMOTO, 1955, *C. nakamurai* GUEORGUIEV, 1970 and *C. parallelus* (TAJIMA & YANAGIDA, 2010; HAYASHI, 2015; MITAMURA *et al.*, 2017; WATANABE *et al.*, 2017). Among the larvae, *C. oblitus* is similar to that of *C. masculinus* by the structures on the head and prothorax. Furthermore, both have the same semicircular shape of the head (see MITAMURA *et al.*, 2017, p. 68).

## Discussion

### *Reproductive ecology with special reference to durations of immature stages*

Under laboratory rearing, the single female laid at least 16 eggs during 31 days. In previous research, *Copelatus parallelus* laid 115 eggs during 108 days (WATANABE *et al.*, 2017), therefore the number of laid eggs of *C. masculinus* is apparently few. A possible cause of the difference in the number of eggs laid by *C. masculinus* may be that they were collected during the breeding season. The laying periods of *C. masculinus* may be around the early summer in Japan. The egg laying environment for *C. masculinus* was the same as *C. nakamurai* and *C. parallelus* (TAJIMA & YANAGIDA, 2010; WATANABE *et al.*, 2017).

The egg periods observed in this experiment were from two to seven days. However, not all eggs were found on the ovipositional day; some eggs were collected during development. Based on our observation (Fig. 5), the most reliable estimate of the egg period is approximately six to seven days, with a maximum of approximately seven days. It should be noted that the results of this study are based on a small sample size from one female.

The larval period of *C. masculinus* (mean of 29.2 days at 26°C) is longer than that of *C. nakamu-*



*rai* (mean of 16.6 days at 24–28°C; TAJIMA & YANAGIDA, 2010) and *C. parallelus* (mean of 27.9 days at 26°C; WATANABE *et al.*, 2017). *C. nakamurai* and *C. parallelus* are distributed in temperate climates (Honshu) and their habitats are small water bodies that are unstable and easily become dry riverbeds. However, *C. masculinus* is distributed in the subtropics (the Yaeyama Islands), where rainfall is higher than on Honshu. The Japanese habitats of *C. masculinus* are small wetlands surrounded by forest, where there is relatively frequent flooding. Therefore, larval period differences may be caused by flooding periods and their frequency.

Details of the pre-pupa and pupa periods are unknown, because the inside of the pupal chambers could not be observed in all larval cups. Additionally, some of the adults escaped from the pupal chamber and hid in the soil, and the discovery of individuals may have been delayed.

#### *Remarkable features in the reproductive ecology of Copelatus masculinus*

We confirmed five features of the ecology of *C. masculinus*. First, regarding the predation behavior of larvae, the larvae preyed upon living chironomid larvae, and all larvae swallowed the prey full without first sucking body fluids. The mandibular canal is not present in larvae of the genus *Copelatus* (BALKE, 2005). Therefore, it seems that only small animals could be target prey. It is unconfirmed whether they search for immobile prey by smell cues, similar to *C. parallelus* (WATANABE *et al.*, 2017).

Second, the larvae were resistant to dry periods. Although we observed only one example, it is noteworthy that the larva survived on land for at least 56 days. Thus, it is likely that the larvae of *C. masculinus* can survive under detritus for at least about two months when their habitat lacks water. The period that *C. masculinus* can endure is longer than that reported for *C. parallelus* (the longest was 46 days at 26°C; WATANABE *et al.*, 2017).

Third, when reared in plastic cups, the third instar larvae reaching the timing of landing climbed the vertical surface of the cup, escaped from the water (Fig. 1C), and reached the lid. This behavior is the same as that seen in *C. parallelus* (WATANABE *et al.*, 2017). Since the larvae of *Copelatus* species have similar morphology (e.g., MICHAT & TORRES, 2009; HAYASHI, 2015; MITAMURA *et al.*, 2017), most larvae of this genus may be able to climb walls with flat surfaces like plastic cups.

Fourth, as with *C. parallelus* (WATANABE *et al.*, 2017), the surfaces of the pupae were covered by many setae, allowing it to float on water (Fig. 1E–G). The habitats of *C. masculinus* are unstable wetlands with large water level fluctuations. These pupal surface structures have the advantage that it allows pupae to avoid drowning when the pupal chamber is submerged due to rainfall or water level increases.

Finally, our observation regards new adults revealed that all newly emerged adults escaped to the soil surface from the pupal chamber. This is in contrast to newly emerged adults of *C. parallelus*, which remain in the pupal chamber (WATANABE *et al.*, 2017). It remains unclear whether the difference in behavior of newly emerged adults is interspecific or between species-groups, and therefore further study is required.

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## 要 約

渡部晃平・林 成多：ヤエヤマセスジゲンゴロウ（鞘翅目ゲンゴロウ科）の3齢幼虫および繁殖生態の記載。——本研究では、与那国島産のヤエヤマセスジゲンゴロウ *Copelatus masculinus* RÉGIMBART, 1899 を屋内の実験室で飼育し、繁殖により得られた幼虫（3齢）の形態および繁殖生態について記載した。飼育下において、1雌から31日間で最低16個の産卵が確認された。各成育段階における成育期間は卵（7日以内）、1齢幼虫（2~7日）、2齢幼虫（5~15日）、3齢幼虫（11~19日）、上陸-蛹化-羽化-脱出（12~27日）であった。飼育開始後すぐに産卵したこと、産卵数が同属のコセスジゲンゴロウ *C. parallelus* ZIMMERMANN, 1920 と比べて顕著に少なく繁殖期の途中で採集されたと考えられたことから、自然環境下における繁殖期は初夏であると推測された。幼虫期間は温帯に分布する同属のコセスジゲンゴロウやトダセスジゲンゴロウ *C. nakamurai* GUEORGUIEV, 1970 に比べて長く、湛水頻度が高い亜熱帯地域での生息に適応している可能性が考えられた。一部の3齢幼虫は、陸上で56日間生存した後再び水中で摂食を開始し、その後羽化に至った。3齢幼虫は、少なくとも2ヶ月程度の濁水に耐えられる可能性がある。蛹の表面には毛が生えており、水に浮くのを確認した。羽化した新成虫は自力で蛹室から脱出し、一部の個体は再度土の中に潜った。これは湛水するまで新成虫が蛹室に留まるセスジゲンゴロウと異なるが、この生態の違いが種群あるいは種間差によるものなのかについては、今後の研究が必要である。

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