ISSN 0013-8738, Entomological Review, 2014, Vol. 94, No. 9, pp. 1251–1256. © Pleiades Publishing, Inc., 2014.
Original Russian Text © A.V. Kuprin, V.G. Bezborodov, Dae-Am Yi, A.K. Kotlyar, 2014, published in Zoologicheskii Zhurnal, 2014, Vol. 93, No. 9, pp. 1080–1085.

Developmental Biology and Ecological Peculiarities of the Relict Longhorn Beetle *Callipogon relictus* Semenov, 1899 (Coleoptera, Cerambycidae)

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Received March 20, 2014

Abstract—The developmental biology of the relict longhorn beetle *Callipogon relictus* Semenov, 1899 was studied from egg to adult in the laboratory for the first time. The molting dynamics and morphometric characteristics of all the larval instars were determined. The location of larvae within the tree trunk was investigated by non-destructive modern methods. The reproductive behavior and the process of oviposition are described. Individual development of the species under laboratory conditions, at constant temperature and humidity, took 44 months, suggesting that in the nature it extends over at least four years.

DOI: 10.1134/S0013873814090061

The Neotropical genus *Callipogon* Audinet-Serville, 1832 is represented in the Russian and East Asian faunas by a single species *C. relictus* Semenov, 1899 described from the South Primorskii Territory (Semenov, 1899). The relict longhorn beetle is a typical inhabitant of broadleaved valley forests and is present in the territories of four states: the Russian Federation, the People's Republic of China, the Democratic People's Republic of Korea, and the Republic of Korea (Kuprin and Bezborodov, 2012). Despite its acknowledged status as a protected species (*The Red Book*..., 1983, 2001, 2005, 2008, 2009), the relict longhorn beetle remains poorly studied.

The available literature contains fragmentary data on the biology and ecology of *C. relictus*, including the morphology of preimaginal stages, the host tree species, and the times of adult flight in different parts of its range (Ilyin, 1926; Murayama, 1936; Yakovlev, 1945; Lyubarsky, 1953; Mamaev and Danilevsky, 1975; Cherepanov, 1979; Bong et al., 2007; Lim et al., 2013). Even the chorology of the species in the Russian part of its range was only recently studied (Kuprin and Bezborodov, 2012).

The aim of this work was to study the developmental biology of the preimaginal stages and the ecology of the adults of *C. relictus* under natural and laboratory conditions.

MATERIALS AND METHODS

This work was based on long-term (2008–2012) observations of the relict longhorn beetle in the Russian Far East, which were carried out using the modern biological techniques and routine methods of ecological entomology (Dobrovolsky, 1969; Fasulati, 1971; Tsurikov and Tsurikov, 2001; Kuprin, 2012). The reproductive behavior was studied in adult beetles reared in the laboratory and in those collected in the nature, using video and photo recording.

The ecological and biological study of the relict longhorn beetle and the reproduction experiments were carried out under laboratory conditions and at the field station in the Ussuri Nature Reserve (Far East Branch, Russian Academy of Sciences).

The conditions suitable for successful laboratory rearing of *C. relictus* were developed for the first time. The larvae were reared in 2–3-liter PVC containers densely filled with sawdust of the Japanese elm *Ulmus japonica*. The containers were kept in a MIR-154 incubator (Sanyo, Japan) at a constant temperature of +25°C and humidity of 65–75%. The containers were inspected twice a month, the larvae were weighed, and their total length and the head capsule width were measured.

Larval development in the nature was studied in special model trunk segments which were placed in the typical habitats of the species (Kuprin, 2012). The behavior and position of the larvae inside the trunk were studied using a 318V Digital Inspection Scope (Testo, Taiwan) and a Magfinder II MRI (AILab Co. Ltd) at the Center for the Study of Insect Ecology, Yeongwol, Republic of Korea. Altogether, over 200 preimaginal individuals of *C. relictus* were studied: 136 eggs, 82 larvae, and 22 pupae; 12 adults (10 females and 2 males) were reared under laboratory conditions.

RESULTS AND DISCUSSION

Two main elements can be distinguished in the reproductive behavior complex of adult relict longhorn beetles: mating behavior (including the precopulatory behavior and copulation proper) and oviposition. To attract males, the female releases specific secretion which is produced only after the period of additional feeding.

According to our observations, the female responded to the mate's appearance by assuming the warning posture: it raised itself on the fore legs, opened its mandibles, and made the so-called "intention movement." In turn, the male approached the female and stopped in front of it, touching its head with the antennae or fore tarsi. The precopulatory behavior lasted from 5 to 17 min, during which the receptive female might try to move away, hit the male with the antennae, or make rapid turns and falls. During this period the male tried to grasp the female with its middle and fore tarsi; then it quickly bent its abdomen forwards to establish contact of the terminalia. In the process of copulation the female remained still while the male continued scratching its head. The mates uncoupled about 30 min later.

The female started laying eggs almost immediately (within 3–5 min) after mating. It turned by 180° and extended its ovipositor, using it to find a suitable place for oviposition. Single eggs were glued into bark fissures 15–20 cm apart. Laying of one egg took on average 3–5 min. The freshly laid eggs were milky-white, and gradually turned pink and then black as the embryos developed (Fig. 1).

The bark areas containing the eggs were sprayed with the female's secretion, which was a mechanism regulating the uniform distribution of eggs over the tree trunk. According to our data, one female laid 24–28 eggs, whereas the maximum number of eggs found in the ovaries was 92. In the laboratory, the females sometimes laid unfertilized eggs.

Embryonic development took 20 days under the laboratory conditions. The hatching larva made a relatively large hole at the anterior end of the egg, including the micropylar disc. In the process the larva ate nearly all the gnawed-out parts of the chorion, whereas the rest of the egg envelope either remained in the tunnel or was pushed into the entry part of the gallery, together with the boring dust and excrements.

The larvae of the relict longhorn beetle are saproxylo-mycetophages which develop in the Japanese elm wood infested with white rot. The species can also develop in the Manchurian ash *Fraxinus mandshurica*, the Amur linden *Tilia amurensis*, the Japanese poplar *Populus maximowiczii*, and also in the Mongolian oak *Quercus mongolica*, the Korean birch *Betula costata*, the Manchurian maple *Acer mandshuricum*, the chosenia *Chosenia arbutifolia*, and the Mongolian poplar *Populus suaveolens* (Ilyin, 1926; Lyubarsky, 1953; Kuprin and Bezborodov, 2012).

Immediately after hatching, the I instar larva bored into wood without making any surface tunnels. The position of the larva in the tree trunk is shown in Fig. 2. From the II instar till pupation, the larva gnawed a long tunnel of an irregular brace-like shape, which started with an oval entry hole up to 4 mm in diameter and ended with a pupal cradle 100–120 mm long and 50–80 mm wide and an exit hole 12–38 mm in diameter. The mean total length of the larval tunnel was 110–120 cm.

Under the laboratory conditions, at constant temperature and humidity, development of the relict longhorn beetle took 44 months, the total duration of the larval stage being 42 months. The larvae molted 5 times and passed through 6 instars (table); in the laboratory molting occurred twice a year, usually in March and September. The process of molting took 2 days. The newly molted larvae had pale and soft integuments which hardened and changed their color within a day. The size and body mass of the larvae increased during the first 38 months of development, after which the body mass slightly but abruptly decreased (Fig. 3).

The consecutive molts in the laboratory occurred with intervals of 182–186 days. Thus, at constant temperature, humidity, and trophic regime each instar lasted on average 6 months, with the exception of the VI instar which took 9 months. Pupation started at the minimum body mass of 30 g, whereas the optimum mass of pupating larvae of this species was 32–37 g.

Fig. 1. Changes in the egg color and hatching of the larva of *C. relictus*.

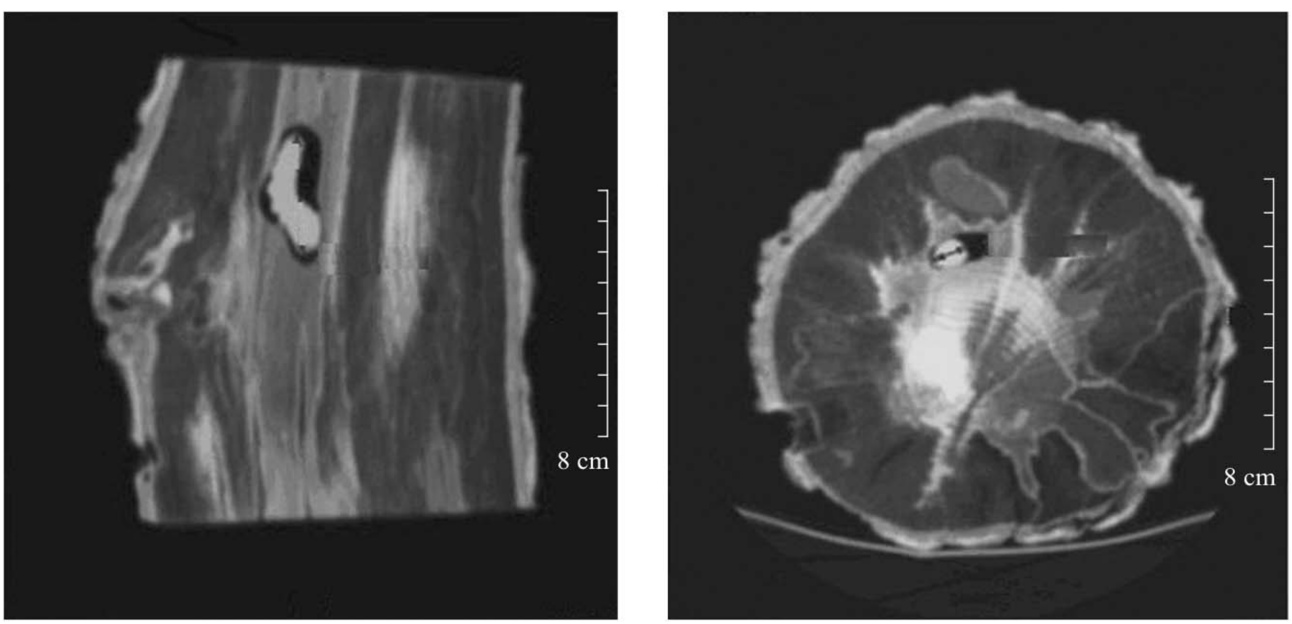

Fig. 2. Position of the larva of *C. relictus* inside the Japanese elm trunk (MRI data).

Measurements of the larvae of *C. relictus*

Instar	Head capsule width, mm		Mandible length, mm		Number of larvae studied
	lim	$\bar{x} \pm SD$	lim	$\bar{x} \pm SD$	
I	1.1–2.3	1.7 ± 0.39	0.3–0.7	0.5 ± 0.16	82
II	2.4–3.4	2.9 ± 0.33	0.7–1.2	0.9 ± 0.19	61
III	3.4–4.2	3.8 ± 0.27	1.2–1.9	1.5 ± 0.24	56
IV	4.1–5.1	4.6 ± 0.33	1.9–2.6	2.3 ± 0.25	42
V	5.0–6.5	5.8 ± 0.47	2.6–3.8	3.2 ± 0.39	36
VI	8.2–8.7	8.4 ± 0.18	3.8–4.9	4.4 ± 0.36	24

Starting from the IV instar, the larvae produced a crackling noise with their mandibles, which "warned" the neighboring individuals about their tunneling direction. Cases of cannibalism were recorded, when older (IV–VI instar) larvae ate conspecific I–III instar larvae.

The movement of the larva in its tunnel was studied by the non-destructive methods. The mechanism of their movement was typical of the xylophagous larvae (Isaev and Girs, 1975). The VI instar larvae fed actively deep inside the tree trunk and gradually moved closer to the surface wood layers. It was experimentally found that they did not require the action of low temperatures before pupation, contrary to what was observed, for example, in larvae of the genus *Monochamus* Dejean, 1821 (Isaev et al., 1988).

Having reached the surface wood layers, the larva made an oval pupal cradle. In standing trees the cradles were positioned horizontally, and their entry tunnels were densely sealed with boring dust. The anterior end of the pupal cradle was separated from the bark by a layer of wood 2–3 cm thick, so that the young adult had to make a short exit tunnel. The length and width of the pupal cradle exceeded those of the larva ready for pupation.

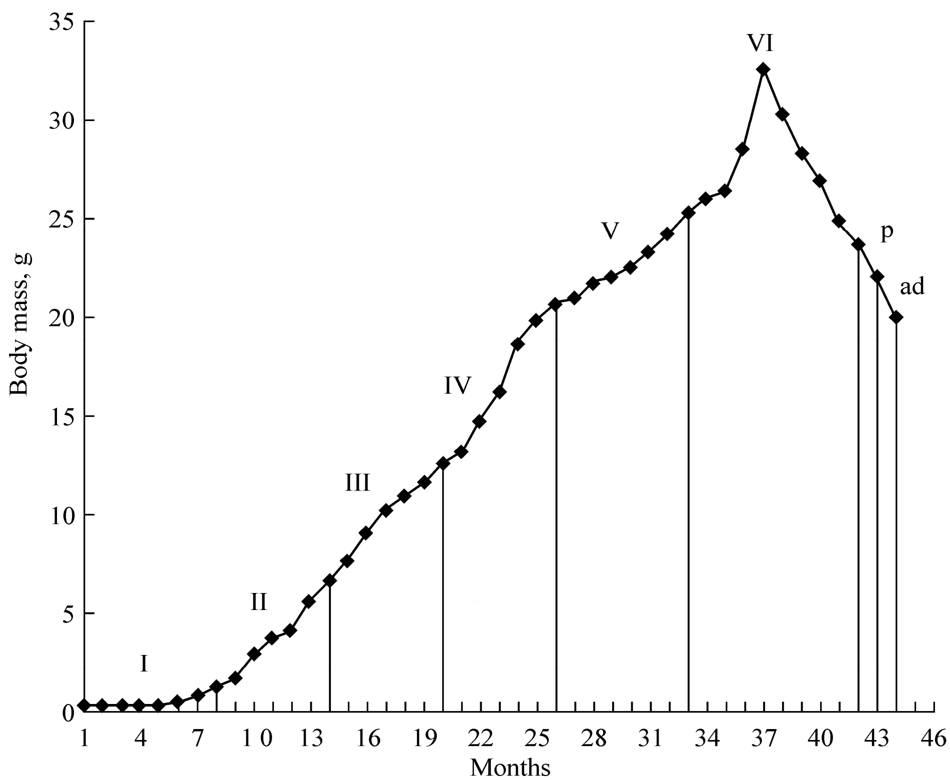

Fig. 3. The body mass dynamics of *C. relictus* during development; I–VI, larval instars; p, pupa; ad, adult.

After the building of the cradle the larva became immobile and started pupating; this stage took 14–16 days. When touched, the pupa made circular movements. Pupal development took 21–25 days, which agrees with the data of Cherepanov (1979). Emergence from the pupal cradle was preceded by a preparatory stage 5 days long. The darkening of the integuments and weight loss were observed already in the pupa. The pupal cuticle was separated 2 days before its shedding. The shedding process took 80 min; the young adult remained in the pupal cradle for 5–6 days, during which its elytra got smoothed out while its integuments hardened and became pigmented. The level of humidity seems to be important for adult development during this period, since the pupae kept in Japanese elm sawdust at low humidity sometimes yielded adults with deformed elytra. On the 5–6th day, the completely developed adult started to make the exit hole.

The adult life duration mostly depends on the weather and trophic conditions, the availability of places for oviposition, and the oviposition rate. According to our data, most adults lived 14–28 days, both in the nature and in the laboratory. The adult life span in Northeast China was estimated at 12–45 days (Yakovlev, 1945, Li et al., 2012). In the nature adult beetles were often observed on sap released by damaged arboreal plants. Feeding occurred in the morning and in the middle of the day; the optimum conditions for feeding were observed at a temperature of +25°C and humidity of 60%. In the laboratory, the beetles actively fed on pureed fruit (apple, peach or pear), diluted honey, and sugar syrup, consuming up to 40 mg of food in about 30 min. Males emerged 5–7 days later than females. According to our data of 2008–2012, the secondary sex ratio varied from 0.3 to 0.5. Females usually died on the 2nd or 3rd day after oviposition. The life span of males was found to be 1–2 days shorter than that of females (Kuprin, 2010).

Thus, it was established that individual development of the relict longhorn beetle took 44 months, including 20 days of embryonic development, 1260–1280 days (42 months) of the larval stage, and 21–25 days of the pupal stage. The adult life span was 14–28 days. In addition, MRI and endoscopy methods allowed us to study the preimaginal stages of *C. relictus* inside the tree trunk in a non-destructive way; this approach is particularly important for studying rare and protected species.

ACKNOWLEDGMENTS

This work was financially supported by the Russian-Korean project “An Ecological and Biological Study of *Callipogon relictus* Sem.”

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