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Larval description with notes on biology of *Holotrichia fissa* (Coleoptera: Scarabaeidae: Melolonthinae) from central India

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Abstract - Though economically important, the field of description of larval morphology and taxonomy of Scarabs is restricted to a very few publications specifically from India. This study was planned to study and document the morphology of developmental stages of one of the most commonly occurring species of scarab, *Holotrichia fissa* (Coleoptera: Scarabaeidae: Melolonthinae) from central India. Here we describe in detail the morphological characteristics of egg, first, second and third instar larva and pupa along with notes on biology of the species. The details of third instar larva are described focussing on the structure of Cranium, Antenna, Labrum, Clypeus, Epipharynx, Mandibles, Maxilla, Thorax, Abdomen, and Raster pattern. This study adds to the knowledge of morphology of the developmental stages the species.

Keywords - White grubs, India, larval description, Melolonthinae, scarabs

Introduction - Beetles belonging to the family Scarabaeidae, order Coleoptera of Class Insecta are commonly known as ‘Scarabs’ or ‘Scarab beetles.’ Larval stages of scarabs are famous as ‘White Grubs’. This group is known to have around 41,818 species all over the world (Schoolmeesters, 2024). White grubs are with a ‘C’ shaped body, well developed mandibles, antennae and legs and have cribriform spiracles. Grubs of most of the species of scarabs under subfamilies Melolonthinae, Rutelinae, Cetoninae and Dynastinae are economically significant as they are known to feed on roots of a variety of crops (Ritcher, 1966). Though economically important, the field of morphology and taxonomy of larval Scarabaeidae is restricted to a very few publications. As most of the taxonomic publications are descriptions of the adult forms, one needs to wait for an adult to emerge for correct identification of the species. Forbes (1891) while studying the life histories of insects, pointed out the need for identifying species in the grub/larval stage. This requires a significant period of time as the study of larval forms requires rearing, proper feeding and maintaining the necessary conditions for larval growth.

Hayes (1929) and Ritcher (1966) gave the first monographs on ecology and morphology of larval Scarabaeidae. Adults emerge after the pre-monsoon showers at the end of May – June, hence they are likewise known as June beetles. Larval stages of scarabs belonging to subfamilies Cetoniinae, Dynastinae, Memonthinae and Rutelinae are polyphagous and feed on roots of Maize, Pearlmillet, Groundnut, Moongbean, Ginger, Potato, Sugarcane, Coffee, Millet, Grape, seed potato, cedar nursery, Coconut

nursery, Arecanut, paddy, bamboo, chilli, okra, brinjal, butter fruit, alfalfa, onion etc (AINPWG, 2024). While larvae of Aphodiinae and Scarabaeinae are coprophagous in nature and feed on mammalian dung (AINPWG, 2024).

Life cycle of most of the scarabs completes in 1 – 3 years. Due to such long period of time, studying the taxonomy of immature stages is restricted. Almost a century ago, Hayes (1929) concerned about lack of study on the larval taxonomy of scarabs. Owing to Indian scarabaeidae not much has been done apart from some notable works by Veeresh (1980) and Ritcher (1966). Recently Sreedevi & Tyagi (2014) gave diagnostics in larvae of white grubs of India. Indian larval Scarabaeidae studies are mostly focused on biology rather than description of larval morphology.

Though economically important, larval taxonomy and nidification of scarabs are poorly documented from Indian subcontinent. Hence, this study was planned to document natural history and morphological characters of developmental stages of some commonly occurring scarabs of central India. This study is focussed on *Holotrichia fissa* belonging to the subfamily Melolonthinae. Morphology of developmental stages of the species are described herewith.

Methodology - The study area, 'Bor wildlife sanctuary' is situated along the Southern boundary of Nagpur district and Northern boundary of Wardha district of Maharashtra, and extends over an area of 61.10 sq km excluding the reservoir at 20° 57' N and 78° 37' E altitude. Bor wildlife sanctuary is covered with southern mixed dry deciduous forest and supports a dense population of Tiger and Panther which is comparable to Kanha and Pench Tiger Reserves in central India in terms of animals/km^2.

Adults of *H. fissa* were collected from the study area with the help of light traps and were brought to the laboratory. These were further reared under laboratory conditions with optimum temperature and humidity. Rearing pots were made of transparent glass with a diameter of 15 cm. Single pair of adults were released per pot. The adults were fed with fresh, tender neem leaves. The pots were checked every day for egg laying. Eggs were collected and transferred to small plastic cups with a diameter of 5 cm and length 7 cm with moist soil. Single egg was kept per cup. Larvae were fed with tender groundnut roots. Developing stages were reared in individual plastic cups as the larvae are cannibalistic. The cups were covered with cloth mesh to prevent escape. As the second and third instar larvae are voracious feeders, adequate food supply was ensured.

For morphometry, larvae were dipped in near boiling water for about 3 minutes and preserved in 70 % alcohol or glycerol to prevent them from shrinking. Five to ten specimens of each instar grubs and pupae were taken for taxonomic studies, photographed and documented. *Leica Stereozoom Microscope* was used for morphometry and photography. Studied material is submitted in the entomology section of Centre for Sericulture and Biological pest management and Research Laboratory (CSBR), RTM Nagpur University, India for future reference.

Results and Discussion –

Ecology and Distribution - Adults of the subfamily Melolonthinae feed extensively on foliage of host plants whilst larvae commonly known as 'white grubs' are root feeders of plants. Our species of interest, *Holotrichia fissa* is a pest of various cash crops such as, Groundnut, Sugarcane, Coffee, Cotton, Pomogranate, Ber, Guava, Rose etc (AINPWG, 2024). Various Indian authors earlier studied the biology and life cycle of economically important *Holotrichia fissa* and other species of the genus *Holotrichia.* Lolage & Patil (1984) studied the biology of *Holotrichia fissa* from Konkan region in western India. Shrilakshmi (2015) studied biology of *Holotrichia fissa* from Dharwad region which is a part of southern India. Amongst other species of genus *Holotrichia,* Veeresh (1977) gave the biology of *H. serrata* from laboratory conditions of Bengaluru and Anitha (1997) gave the biology and morphometry of *H. reynaudi* from Andhra Pradesh which forms a prt of southern India. From Northern India, Brar & Sandhu (1982) gave the biology for *H. consanguinea* from Punjab. Considering central India, Dadmal et al. (2013a) gave the field biology of *H. serrata* from Vidarbha. However, the detailed morphological description of the larval instars is still lacking.

Collection of adults, mating and egg laying – Adults emerged after the first monsoon showers in large numbers and were found gathering on ground near the light source. Adults emerged in dark usually from 7 PM to 8.30 PM. On field, mating continued for 5 – 6 minutes. After mating females tried to bury themselves into the soil. In some locations, adult females were observed to bury themselves into cement or clay if soil is not available in their immediate vicinity after mating. The adult emergence continued for about 25 days. The freshly emerged adult pairs were collected and brought into the laboratory. These were kept in cages filled with soil and host plant. Adults continued mating inside the cages. The mating period was observed to be 5 – 6 minutes. Mated females were found to bury themselves into the soil for egg laying. Majority of the eggs were laid singly in moist soil in earthen cells up to 10 cm below soil. Some of the eggs were laid in openly in soil without earthen cell. For a group of 30 females observed, a single female laid 2 to 25 number of eggs. Egg laying continued for 7 – 9 days per female. Field collected females laid more eggs as compared to lab reared females.

A. Description of larval morphology

1. Eggs: Eggs are white, oval when freshly laid. After 3 to 4 days they turn spherical. Fully grown egg is 2.94±0.06 mm in length and 2.24±0.21 mm in breadth. After nearly 4 days the egg membrane becomes transparent on one side and the developing embryo can be seen breathing from outside. Other half of the egg remain white (Fig1. C-D).

2. First instar: Head capsule measures about 1.98±0.03 mm in length and 1.82±0.02 mm in width. Antenna is 1.26±0.03 mm in length. Right mandible is slightly larger than left mandible. Right mandible is trilobed while left mandible is bilobed (Fig1. E-F). According to previous studies in western India, (Konkan region), *H. fissa* First instar developmental period was recorded to be 19.7 days (Lolage & Patil, 1986) and from Dharwad 20.48 days (Shrilakshmi, 2015). For *H. renaudi* it is 15.5 days (Anitha, 2006) and for *H. serrata* it is recorded to be 30.2 days (Veeresh, 1977).

3. Second instar: Second instar larva is whitish in coloration and is much slender than the third instar larva.

4. Third instar larva: Fully grown 3rd instar larva is whitish in coloration and is 31.41±0.55 mm in length. Detailed morphological characterization of the Third instar larva of *H fissa* can be given as follows (Fig 2-3).

Cranium The cranium is about 6.21±0.02 mm in length and 5.64±0.01 mm wide. The epicranial suture and frontal suture are well marked (Fig2. A-B).

Labrum The Apical projection is irregular in shape. It has a series of 4 transversely place setae (mid two long and lateral two moderate in length). Along to the lateral margin are 2 setae on each side. A pair of setae near the lateral margin. In front of the median transverse complete ridge there is a series of 4 long setae. Behind the median transverse ridge are a series of 4 medium length setae.

Clypeus Clypeus is isosceletally trapezoidal in shape. Anteroclypeus is slightly smaller than post-clypeus. On the junction of Anteroclypeus and Postclypeus is a series of 4 long setae (two on lateral margins and two placed in middle).

Epipharynx Situated under the labrum and clypeus. The boundry towards pharynx is marked by strongly curved crepis. The plegmatia (Pig) are well developed, consist of 16 to the right and 13 to the left, transverse plegmata which gradually diminish in length anteriorly and posteriorly. The acanthopariae (Actp) carry 16 to the right and 13 to the left. Chetopariae (Chp) are large, with strong straight setae nearest the pedium (ped) and gradually weaker and slender setae towards the mid and shorter towards the plegmata. Along the inside of Zygum is a single curved series of 7 dark, large punctures. The helli (Hi) are strong, curved, dark, arranged in 2 arched concentric series with 10 in the front series and 5 each in the inner group. Pedium (Ped) is longitudinal and slender (Fig2. C).

Mandibles A pair of strong, black mandibles is present. Scissorial part has a sensorial pit on its dorsal side. The molar area of the right mandible is trilobed with the lobes increasing in size towards the base. The left mandible molar area is bilobed. A well-developed brush of long bristles is found at the base of both right and left mandibles. In the dorsomolar region of the right mandible are 7 setae arranged in single vertical series. In the dorsomolar part of the left mandible an oblique series of 2 - 3 setae are present. On the ventral lateral carinae of each mandible is a longitudinal series of 8 – 9 long setae (Fig2. E).

Maxilla A pair of maxilla is present on head with other mouth parts. For each maxilla, Galea and lacinia are free distally and fused proximally. Maxillary palps 4 segmented. 12 stridulatory teeth are present in a row on stipe dorsally (Fig2. D,F).

Antenna Antenna 4 segmented with 2.65±0.06 mm length. First antennal segment is about ⅓ size of 2nd (Fig. 3A).

Thorax Three pairs of 4 segmented legs are present on thoracic region. Forelegs shorter and hindlegs longer. Mid legs with intermediate length in between foreleg and hindleg. Legs are covered with small to large thick black bristles. Single pair of thoracic spiracle

present on prothoracic segment. Thoracic spiracle is oval with 0.71±0.01 mm length and 0.61±0.01 mm width (Fig. 3B,D).

Abdomen 8 pairs of abdominal spiracles on each abdominal segment. First abdominal spiracle is oval, and smaller than the thoracic spiracle with 0.61±0.01 mm length and 0.51±0.01 mm width. Size of abdominal spiracles decrease consecutively towards last abdominal segment. Last abdominal spiracle being the smallest with 0.40±0.01 mm in diameter (Fig. 3C).

Raster Raster is present ventrally on the 10th abdominal segment. Raster comprise of a longitudinal row of 19 pali on right and 17 pali on left of the septula. Septula is a bare, long, slender region placed centrally on the ventral side of the 10th abdominal segment (Fig. 4E). Anal slit Y shaped (Fig. 3F).

Third Instar larva of *Holotrichia serrata* was described earlier by Veeresh (1977) from South India and Dadmal et al. (2013a) from Vidarbha. Description of third instar larva by these authors was compared with the characters of third instar larva of *H. fissa.* It is found that larva of *H. serrata* differs significantly from *H. fissa* and can be identified in larval stage as well with the help of the given description. Most significant differences differentiating the two species can be compiled as size of cranium, number of antennal segments 5 in *H. serrata* and 4 in *H. fissa.* Structure and position of setae on labrum, position of setae on clypeus, structure of Epipharynx and Mandibles, structure of Maxilla and Raster pattern.

5. Pupa: Freshly formed pupa is white in colour which is further turned into ochraceous yellow. It is about 22.06±0.27 mm in length and 9.08±0.13 mm wide (Fig. 3G-H).

B. Notes on biology (Fig. 4)

1. Egg - Eggs were found in soil balls in the rearing pot. Single egg was laid per ball. Incubation period ranged from 5 to 9 days with an average of 6.88±1.31 days. From Konkan region *H fissa* incubation period was recorded to be 7.5 days (Lolage & Patil, 1986) and from Dharwad it is found to be 9.1 days (Shrilakshmi, 2015). For *H renaudi* it is 11.5 days (Anitha, 2006) and for *H serrata* it is recorded to be 10 to 12 days (Veeresh, 1977).

Incubation period and host plants: An interesting phenomenon was observed during incubation. Some of the eggs were kept in rearing pots supplemented with groundnut seedlings to enquire sufficient food for the newly hatched larva, while, half the eggs were simply kept in soil, single egg per pot. In case of pots with groundnut seedlings incubation period of eggs was found to be 5 to 7 days with an average of 5.85±0.78 days. In case of pots without groundnut seedlings, incubation period was delayed and found to be 7 to 9 days with an average of 7.92±0.80 days. The experiment was repeated thrice after each egg laying keeping half the number of eggs (n=30) in pots with groundnut seedlings and half the number of eggs in soil without any seedling. For 70% of the cases in pots with groundnut seedlings, incubation period of eggs was found to

be 5 to 7 days while incubation period of eggs without any seedling ranged from 7 to 9 days. This data suggest that the presence of groundnut seedling may minimize the incubation period of *H fissa* eggs.

2. First instar - Newly hatched first instar larva is transparent and whitish in appearance. It lasted for 11.35±2.54 days with a range of 8 days to 15 days. First instar larva is 10.16±0.38 mm in length. Transparent appearance of the larva turned to milky white coloration within 12 hours of hatching and head capsule began turning light brown.

3. Second instar - Second instar larva took 15.31±1.95 days for development. It is an active feeder of the roots of host plant. Second instar larva is whitish in coloration and is much slender than the third instar larva.

From Konkan region *H fissa* second instar developmental period was recorded to be 26 days (Lolage & Patil, 1986) and from Dharwad 26.82 days (Shrilakshmi, 2015). For *H renaudi* it is 17.5 days (Anitha, 2006) and for *H serrata* it is recorded to be 35.8 days (Veeresh, 1977).

4. Third instar - Third instar larva lasted for 52.58±1.81 days taking the longest duration in developmental cycle with minimum duration of 50 days and maximum of 55 days. From Konkan region *H fissa* third instar larval duration was recorded to be 30.1 days (Lolage & Patil, 1986) and from Dharwad 33 days (Shrilakshmi, 2015). The developmental period of third instar larva is longest in this study. For *H. renaudi* third instar larva lasted for 34 days (Anitha, 2006) while for *H. serrata* it is the longest 102.9 days (Veeresh, 1977).

Third instar larvae are found to be cannibalistic in nature. They show active locomotion and tend to escape the rearing pot. During this period, larva feeds voraciously on roots of host plants. Larvae starved during larval stages were found to develop into malformed pupae and do not emerge as adults. All the 10 starved larval stages undergo pupation but were malformed and never attained adulthood. For biology study 26 specimens of each developmental stage were observed and biology was described.

Third instar larva creates a pupal chamber around itself as it develops into Pupa. Pupal duration varied from 8 to 10 days with an average of 9.15±0.78 days. During this study the pupal period is recorded to be the shortest. Pupal period for *H. fissa* given by Lolage & Patil (1986) was 14.95 days and by Shreelakshmi (2015) was 15.7 days. Pupal duration for other species of genus *Holotrichia* such as *H. renaudi* is about 17 days (Anitha, 2006) and for *H. serrata* is about 11.8 days (Veeresh, 1977).

5. Adults - Longevity for adults is recorded to be 9.54±1.14 days.

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Fig 1. *Holotrichia fissa* rearing, eggs. First instar larva: A- rearing pots, B – uneaten roots and eaten roots of groundnut by the larva, C – Egg (magnified view), D – Eggs in the soil, E- First Instar larva, F – Head of First instar larva

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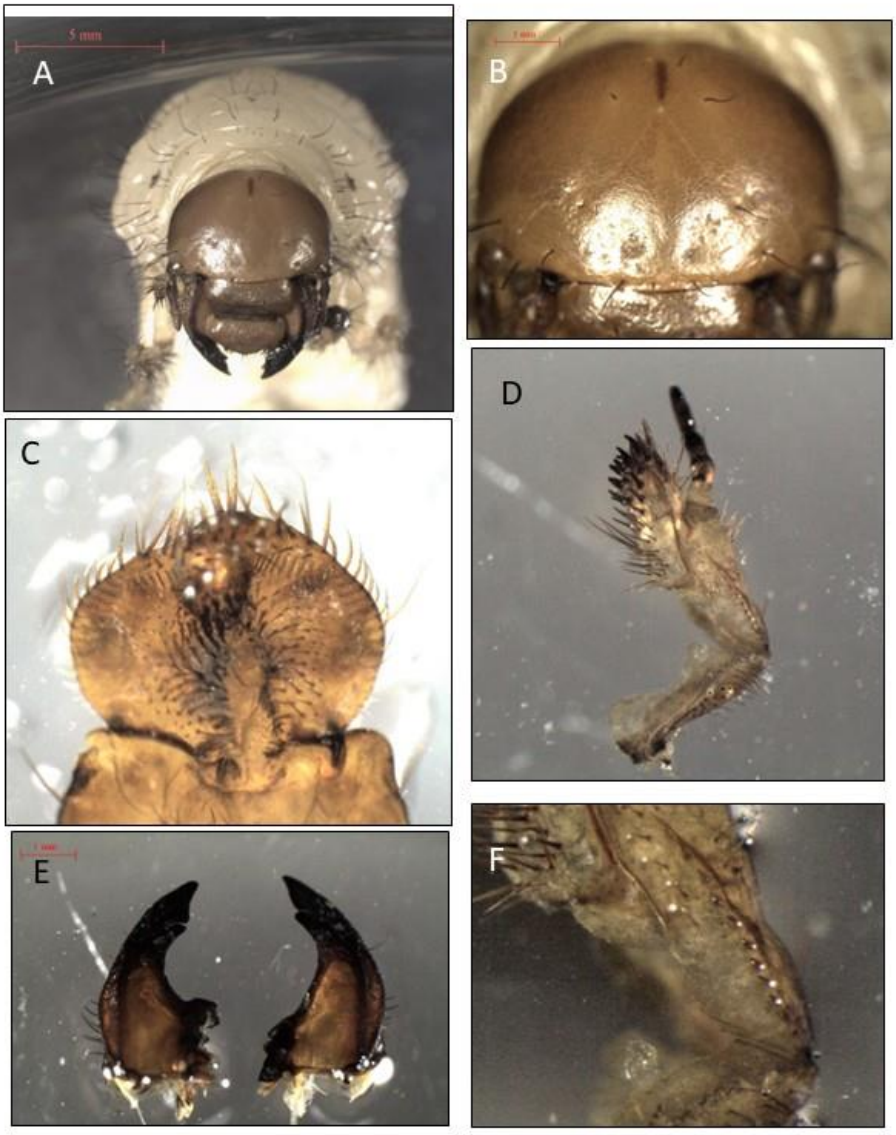

Fig 2: Morphological characters of Third Instar larva of *Holotrichia fissa* A- Head, B – Cranium, C – Epipharynx, D – Maxilla, E – Mandibles, F – Maxillary teeth

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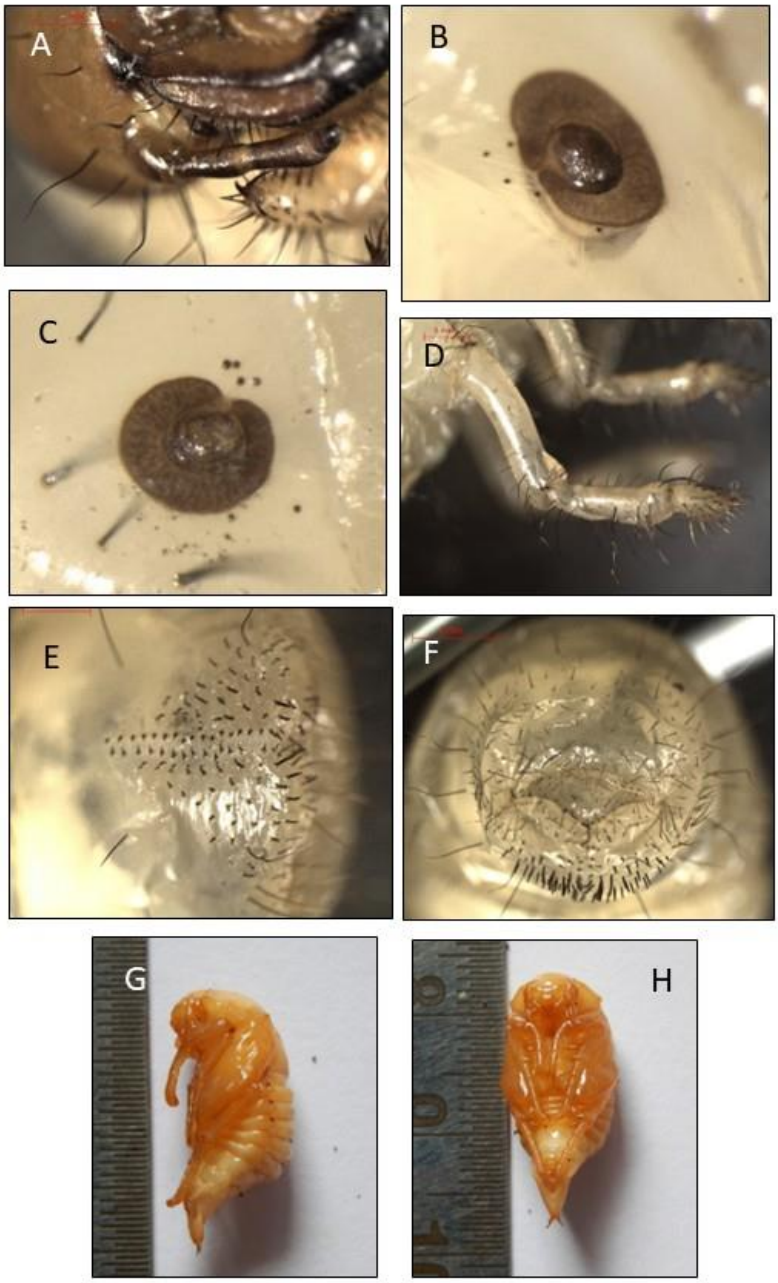

Fig 3: Morphological characters of prepupa and pupa of *Holotrichia fissa* A- Antenna, B – Thoracic spiracle, C – Abdominal spiracle, D – Hind leg, E – Raster, F – Anal slit, G- Male pupa, H – Pupa ventral view

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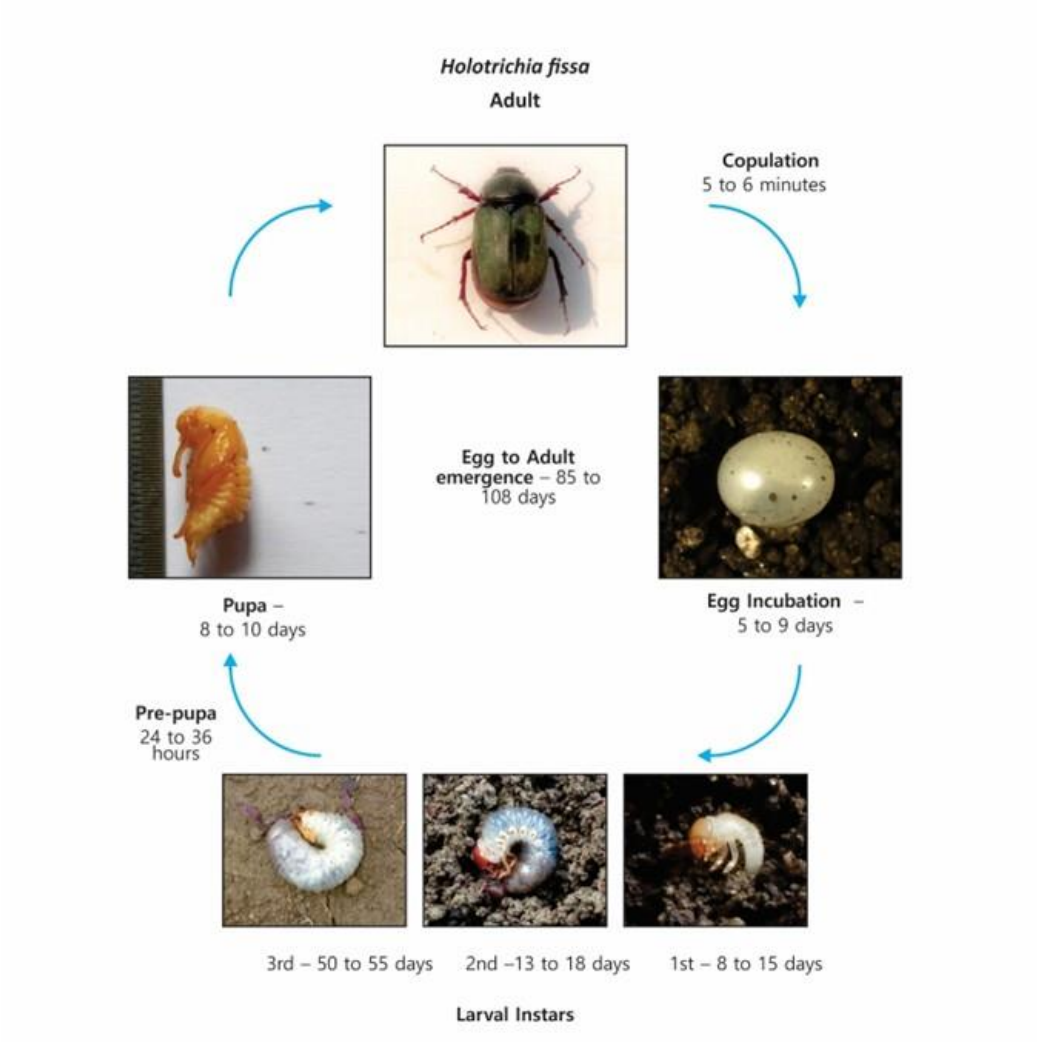

Fig 4. Biology of *Holotrichia fissa*

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