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Descriptions of immature stages of six aphidophagous Coccinellini (Coleoptera: Coccinellidae) species from China

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Abstract

The morphological diversity of ladybird beetle larvae can provide supplementary evidence for taxonomic identification. However, studies on the larval morphology of Coccinellidae are very limited. In this study, the immature stages of six species from the tribe Coccinellini are examined, including *Phrynocaria unicolor* (Fabricius, 1792) and *Microcaria pupillata* (Swartz, 1808), which are described for the first time, as well as re-examination of *Cheilomenes sexmaculata* (Fabricius, 1781), *Harmonia dimidiata* (Fabricius, 1781), *Harmonia sedecimnotata* (Fabricius, 1801) and *Coelophora biplagiata* (Swartz, 1808). The illustrations, and brief diagnoses for identification of the fourth instar larvae and pupae of examined species are provided. Larval morphology of other *Harmonia* Mulsant, *Cheilomenes* Dejean, *Phrynocaria* Timberlake and *Coelophora* Mulsant species are also compared.

Key words: larva, pupa, morphology, Coccinellini, Coccinelloidea

Introduction

Ladybird beetles (Coleoptera: Coccinellidae) show a significant feeding preference for various sternorrhynchan groups, including aphids, scales, thrips, mealybugs, coccids, whiteflies, psyllids, as well as mites and beetles (Hodek & Honěk 1996; Araujo-Siqueira 2006; Ślipiński 2007; Hodek *et al.* 2012; Zazycki *et al.* 2015; Escalona *et al.* 2017; Boopathi *et al.* 2019). In particular members of the tribe Coccinellini mainly prey on aphids but also feed on a variety of insects such as psyllids, leafhoppers, planthoppers, early-stage Lepidoptera, which are considered harmful insects because they feed on many cultivated plants (Hodek *et al.* 2012; Bouvet *et al.* 2019).

The beneficial status of these ladybird beetles has a rich historical background, which is widely acknowledged by both the public and researchers involved in biological control programs (Hodek 1973; Hodek *et al.* 2012; Bouvet *et al.* 2019). Within agricultural ecosystems, they are also recognized for their effectiveness as biocontrol agents. In terms of utilizing natural enemies for pest management purposes, native natural enemies such as *Harmonia axyridis* (Pallas, 1773) in the north and *Cheilomenes sexmaculata* (Fabricius, 1781) in the south have widespread distribution across China and are commonly employed to regulate aphid populations. Moreover, common aphidophagous species such as *C. sexmaculata*, *Harmonia dimidiata* (Fabricius, 1781), *H. sedecimnotata* (Fabricius, 1801), *Phrynocaria unicolor* (Fabricius, 1792), *Microcaria pupillata* (Swartz, 1808) and *Coelophara biplagiata* (Swartz, 1808) exhibit potential for pest control and play a crucial role in maintaining the biological balance of South China.

The traditional identification method of Coccinellidae primarily relies on the genital characteristics of male adult beetles. Some ladybird beetles, particularly in *C. sexmaculata* and *H. axyridis*, exhibit variable elytral color patterns; however, their larval stage morphology remains stable. Currently, there are approximately 6900 recorded

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species of Coccinellidae worldwide, with around 150 species described or recorded in their larval stages, accounting for 2.17 % of the total species diversity within this family (Gage 1920; Sasaji 1968; Park & Yoon 1993; Ślipiński 2007; Casari & Teixeira 2015; Celli *et al.* 2021). Detailed descriptions exist for larvae belonging to 17 tribes of Coccinellidae. Among these tribes, Coccinellini larvae have been most extensively documented with a total of 63 species recorded representing 42 % of all documented larvae. The tribe Epilachnini has descriptions available for 22 species while Chilocorini and Scymnini have descriptions available for 17 and 12 species respectively; other tribes have fewer than 10 described species.

Several studies have provided descriptions and discussions on the immature stages of Coccinellini. Stehr (1991) provided an identification key for the larvae of North American tribes, highlighting the distinguishing characteristics of Coccinellini larvae such as variable body colorations and reduced third antennomere. Gordon & Vandenberg (1993) described the North American species of *Cycloneda* Crotch, 1871, comparing it with *Olla* Casey, 1899 and *Hippodamia* Dejean, 1836. Ślipiński (2007) provided a key to the larvae of Australian genera including *Menochilus* Chevrolata, 1836, *Coelophora* Mulsant, 1850, *Harmonia* Mulsant, 1850 and *Phrynocaria* Timberlake, 1943. Additionally, Gordon & Vandenberg (1991) and Rees *et al*. (1994) presented identification keys for recognizing common species based on characteristics observed during their immature stages. Furthermore, Sasaji (1968) presented descriptions and keys of Coccinellidae larvae from Japan and Ryukyus encompassing 10 species within the tribe Coccinellini. Celli *et al*. (2021) provided descriptions and keys for 4th instar larvae and pupae of major predatory ladybird beetles found in southern Brazil. More recently, Poorani (2023) presented an illustrated guide to the tribe Coccinellini of the Indian Subcontinent comprising 119 species within 30 genera, including 31 species with illustrations depicting their immature stages. However, only brief descriptions of immature stages are available for 18 species.

Detailed studies on the immature stages of Coccinellidae in Chinese fauna, especially the tribe Coccinellini, are lacking. At present, immature stages of 46 species have been described from China, encompassing 10 tribes (Tian *et al.* 1982; Qi & Tian 1984; Qi & Zhang 1988; Zhang & Qi 1991; Wang 1992; He 1993; Zeng 2000, 2001; Zeng & Pang 2001). He (1993) presented a key for identifying larvae of 14 species of Coccinellini without detailed descriptions. Zeng (2000, 2001) and Zeng & Pang (2001) provided descriptions for larvae of 13 species within the tribe Epilachnini. Tian *et al*. (1982) presented descriptions of the larvae for 12 species belonging to six coccinellid tribes from China. Qi & Tian (1984) described the larvae of 10 species within six tribes from China. Yu (2010) provided photographs of immature stages of 46 species, including 38 Coccinellini species from China which visually demonstrated the diverse color patterns and processes of Coccinellini larvae; however, a detailed description was lacking.

Therefore, in order to improve the understanding of Coccinellini larvae in South China, we provide detailed descriptions, illustrations and important characters to identify immature stages of six common species. The immature stages of *P. unicolor* and *M. pupillata* are described in detail for the first time.

Material and methods

All examined materials are preserved in the collection of the Department of Entomology, South China Agricultural University (SCAU), Guangzhou, China. Larvae of *C. sexmaculata*, *H. sedecimnotata*, *H. dimidiata*, *P. unicolor*, *M. pupillata* and *C. biplagiata* were collected from Guangdong Province in China. These larvae were reared in 500 mL plastic dishes under controlled conditions of 25 ± 1°C, 70 % ± 10 % relative humidity (R.H.) and 12:12 h light: dark (L:D) photoperiod. Aphids were supplied daily to maintain the population stock. We reared the specimens to adulthood (Fig. 1), and adults were identified based on the examination of their male genitalia.

For morphological studies, the larvae were preserved in 75 % ethanol. The images of larvae were prepared using a digital camera (Canon 5D with 65 mm lens; WeMacro) and adjusted using Helicon Remote and Helicon Focus 7 softwares. For further study, heads and tibiotarsi were dissected, cleared in a 10% NaOH solution, boiled for several minutes, washed in water, dissected into different parts and placed in neutral balsam (Mounting Medium for Microscopy, Shanghai Macklin Biochemical Co., Ltd.) on slides. Images were captured with digital camera (Axiocam 506 Color) attached to a dissecting microscope (ZEISS Imager M2) using ZEN 2.3 software. Photographs were edited using Adobe Photoshop CC 2018.

The terminology used for the larval descriptions follows Ślipiński (2007) and Celli *et al*. (2021).

Results

Cheilomenes sexmaculata (Fabricius, 1781)

(Figs 1A–C, 2–3)

Larval descriptions or illustrations of *Cheilomenes sexmaculata*: Ślipiński 2007: 170, Yu 2010: 48, Poorani 2023: 72.

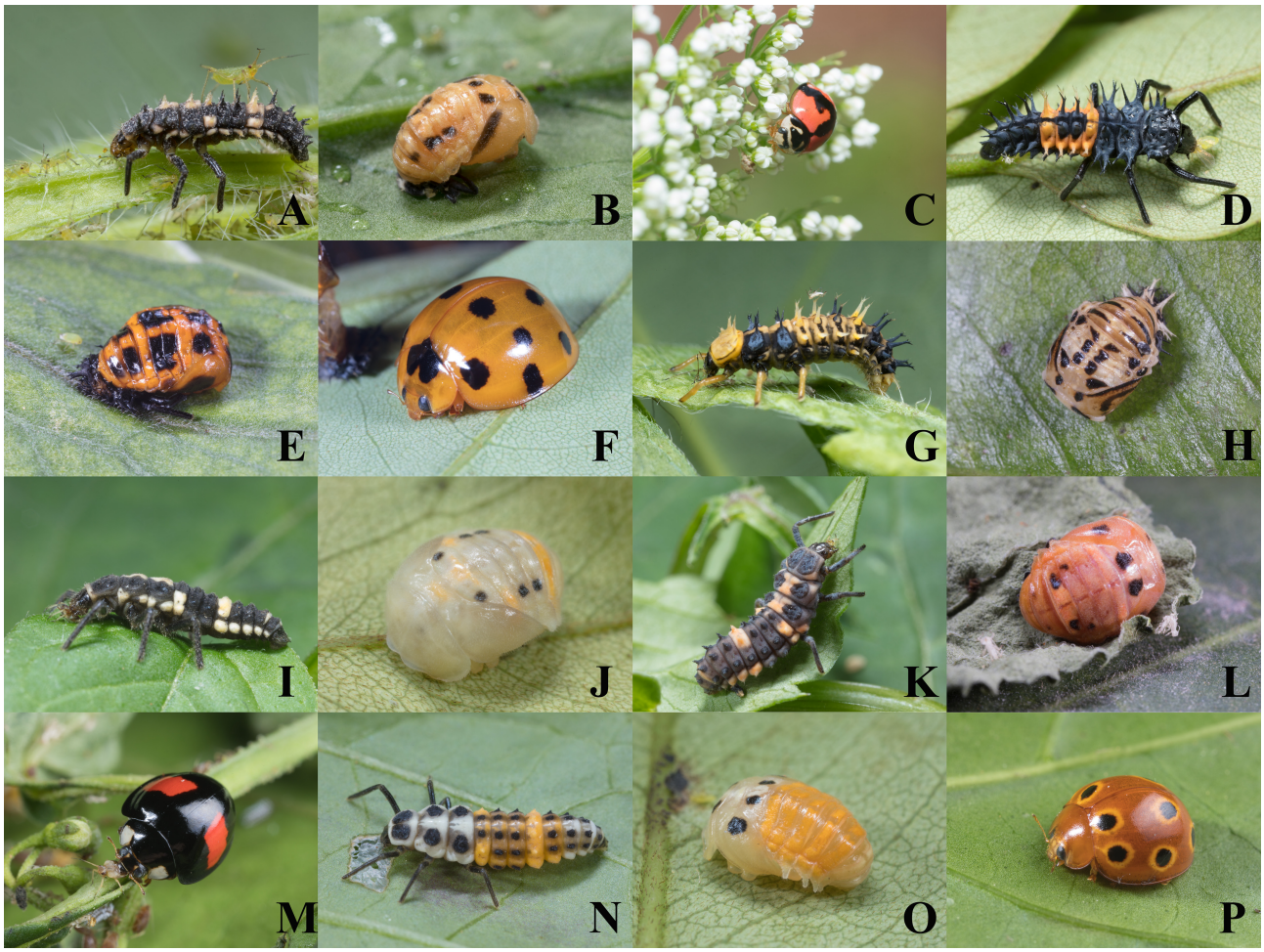

FIGURE 1. Life stages of six studied species of the tribe Coccinellini. **A–C.** *Cheilomenes sexmaculata*, larva (A), pupa (B) and adult (C). **D–F.** *Harmonia dimidiata,* larva (D), pupa (E) and adult (F). **G–H.** *Harmonia sedecimnotata*, larva (G) and pupa (H). **I–J.** *Phrynocaria unicolor*, larva (I) and pupa (J). **K–M.** *Coelophora biplagiata*, larva (K) pupa (L) and adult (M). **N–P.** *Microcaria pupillata*, larva (N)**,** pupa (O) and adult (P).

Fourth instar larva (Figs 2A–B, 2E, 3). Length 6.7–7.3 mm; width at metathorax 2.3–2.4 mm. Body elongate, cylindrical and tapered, with black senti and yellow spots (Figs 2A–B). Head: black, nearly round. Epicranial stem absent; frontal arms inverted omega-shaped (Fig. 3A), do not reach antennae; they appear to be separated at bases. Three hemispherical stemmata dark, arranged in a triangle, near at the base of antenna. Antenna with 3 antennomeres; antennomere 1 wider than and as short as antennomere 2; antennomere 3 with preapical setae and sensilla (Fig. 3B). Labrum with setae (Fig. 3C). Mandible well-sclerotized with two apical teeth (Fig. 3D). Maxilla with mala brush-shaped, with two long setae and a pair of papillae at the apex in ventral side (Fig. 3F). Maxillary palp with two setae in palpomere 2, and apex with sensilla (Fig. 3H). Labium with spare and thin setae, labial palpi with two palpomeres, bearing setae and small stout sensilla at apex (Figs 3G, 3I).

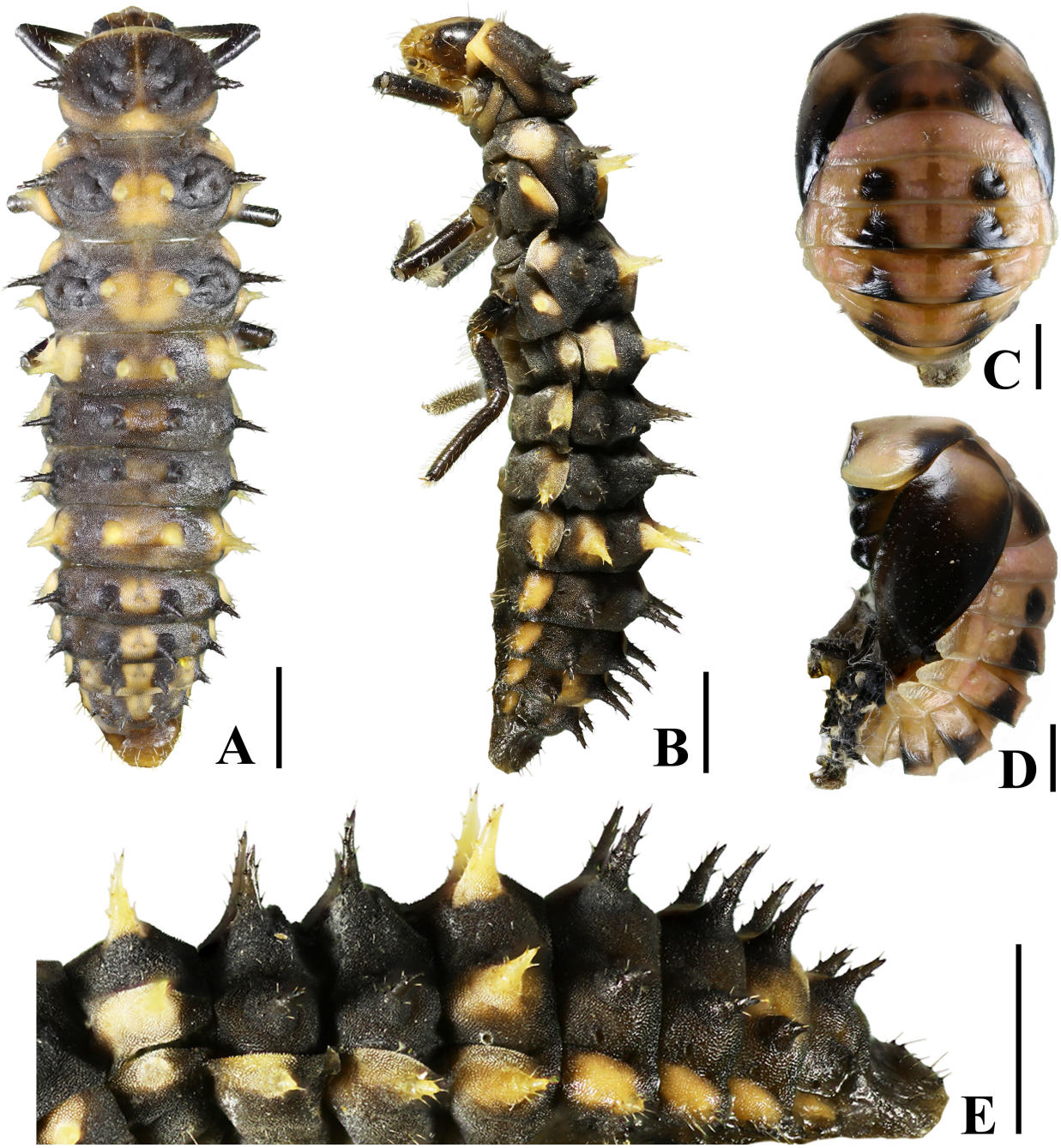

FIGURE 2. The larva and pupa of *Cheilomenes sexmaculata*. **A–B.** fourth instar larva, in dorsal (A) and lateral (B) view. **C–D.** pupa, in dorsal (C) and lateral (D) view. **E.** nine segments of abdomen of fourth instar larva, lateral view. Scale bars: 1 mm.

Thorax. Pronotum black, with two semi-oval dorsal plates, separated by a narrow band and embrace yellow stripes in lateral margins (Figs 2A–B). Each lateral and posterior margins of plates with a black sentus; nearly 28 setae in external margin; central margin of plates without sentus, only with short setae (Figs 2A–B). Mesonotum and metanotum plates black, with yellow spots in central surface (Figs 2A–B). Lateral margins of plates with black sentus, and a yellow sentus in central surface of plates (Figs 2A–B). Lateral margins of mesonotum each with a yellow parascolus bearing 1 to 3 branches, without seta (Figs 2A–B). A pair of circular spiracles with lamellae at the opening in anterolateral part of mesonotum (Fig. 2B). Metanotum laterally each with a yellow parascolus bearing 1 to 3 branches, and three short setae (Figs 2A–B). Legs black, long, with setae; ventral part with a bristle brush near the tarsal claw, and row of external setae in the tibia; tarsal claw with a subquadrate basal tooth and dense setae around the tooth (Fig. 3E).

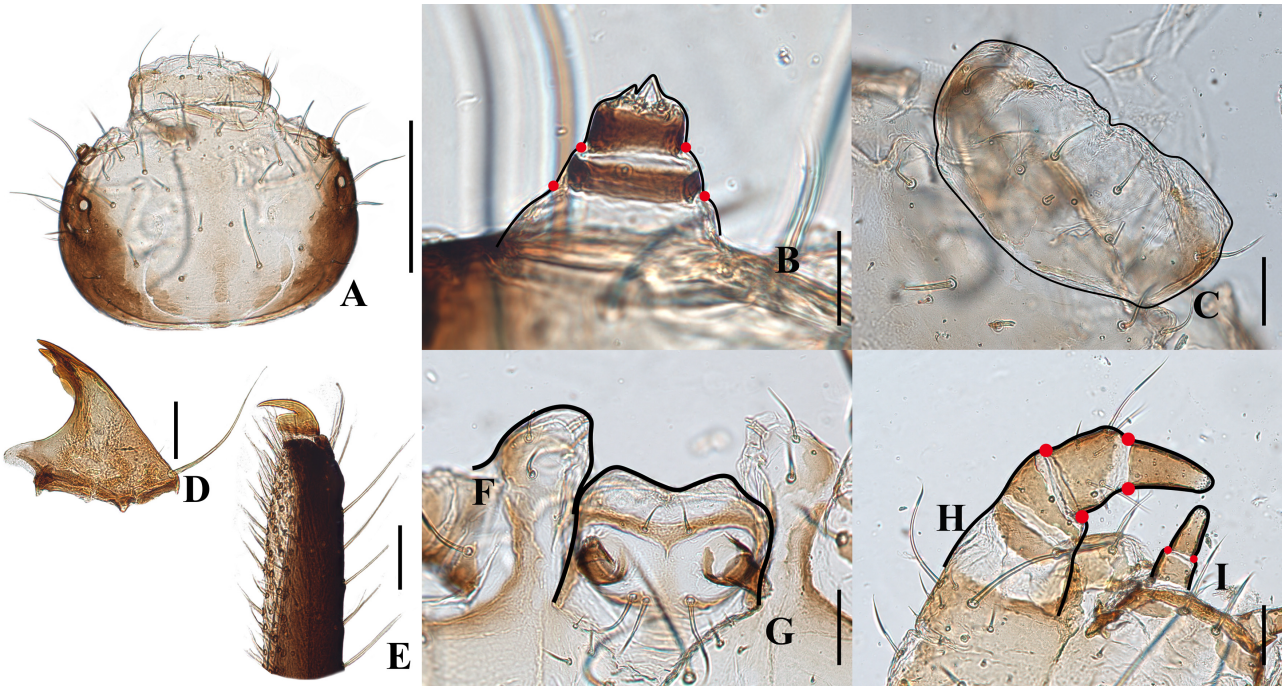

FIGURE 3. Main characters of the fourth instar larva of *Cheilomenes sexmaculata*. **A.** head, dorsal view. **B.** antenna. **C.** labrum. **D.** mandible. **E.** tibiotarsus. **F.** maxilla. **G.** labium. **H.** maxillary palp. **I.** labial palp. Scale bars: 500 μm in A; 100 μm in C–I; 50 μm in B. Black lines: outlines of structures; red dots: boundaries of segmentation of maxillary and labial palpi.

Abdomen with nine segments (Fig. 2E). Segment S1 to S8 with a pair of anterolateral spiracles similar to those of the mesothorax (Fig. 2E). Segment S1 dark brown to black, and tan semi-circular spots in the basal dorsal projections, dorsolateral and lateral parts yellow, with three pairs of yellow senti in dorsal, dorsolateral and lateral parts (Figs 2A–B, 2E). Segment S2 and S3 dark brown, brown in central surface, lateral parts yellow, with two pairs of black senti in dorsal and dorsolateral plates, and a pair of yellow senti in lateral areas (Figs 2A–B, 2E). The color and the location of projection of S4 are similar to those of S1 (Figs 2A–B, 2E). Segment S5 and S6 dark brown, yellow in central surface and lateral parts, with two pairs of black senti in dorsal and dorsolateral plates, and a pair of yellow parascoli in lateral areas (Figs 2A–B, 2E). Segment S7 and S8 dark brown, yellow in central surface and between dorsal and dorsolateral plates, lateral parts yellow, with a pair of black senti in dorsal plates, a pair of black parascoli in dorsolateral plates, and a pair of yellow strumae in lateral areas. Segment S9 dark brown with nearly 20 setae (Figs 2A–B, 2E).

Pupa (Figs 2C–D). Length 3.9–4.0 mm; width 2.0–2.9 mm. Body oval, convex, with extremely short setae. Thorax: pronotum yellow, posterior regions black; mesonotum pink, black border in lateral regions with black stripes connected with meso- and metanotum on each side; metanotum pink, with two large and two small black spots. Abdomen: seven visible segments. S1 pink. Segment S2–S6 pink, with a pair of round and black spots on each side.

Notes. Ślipiński (2007) provided concise description of the larval stage of this species under the name *Menochilus sexpunctatus*. Subsequently, Yu (2010) presented illustrations of immature stages for this species. Poorani (2023) contributed comprehensive illustrations of this species accompanied by brief descriptions of its immature stages.

***Coelophora biplagiata* (Swartz, 1808)**

(Figs 1K–M, 4–5)

Larval descriptions or illustrations of *Coelophora biplagiata*: Kamiya 1965: 85, Sasaji 1968: 121.

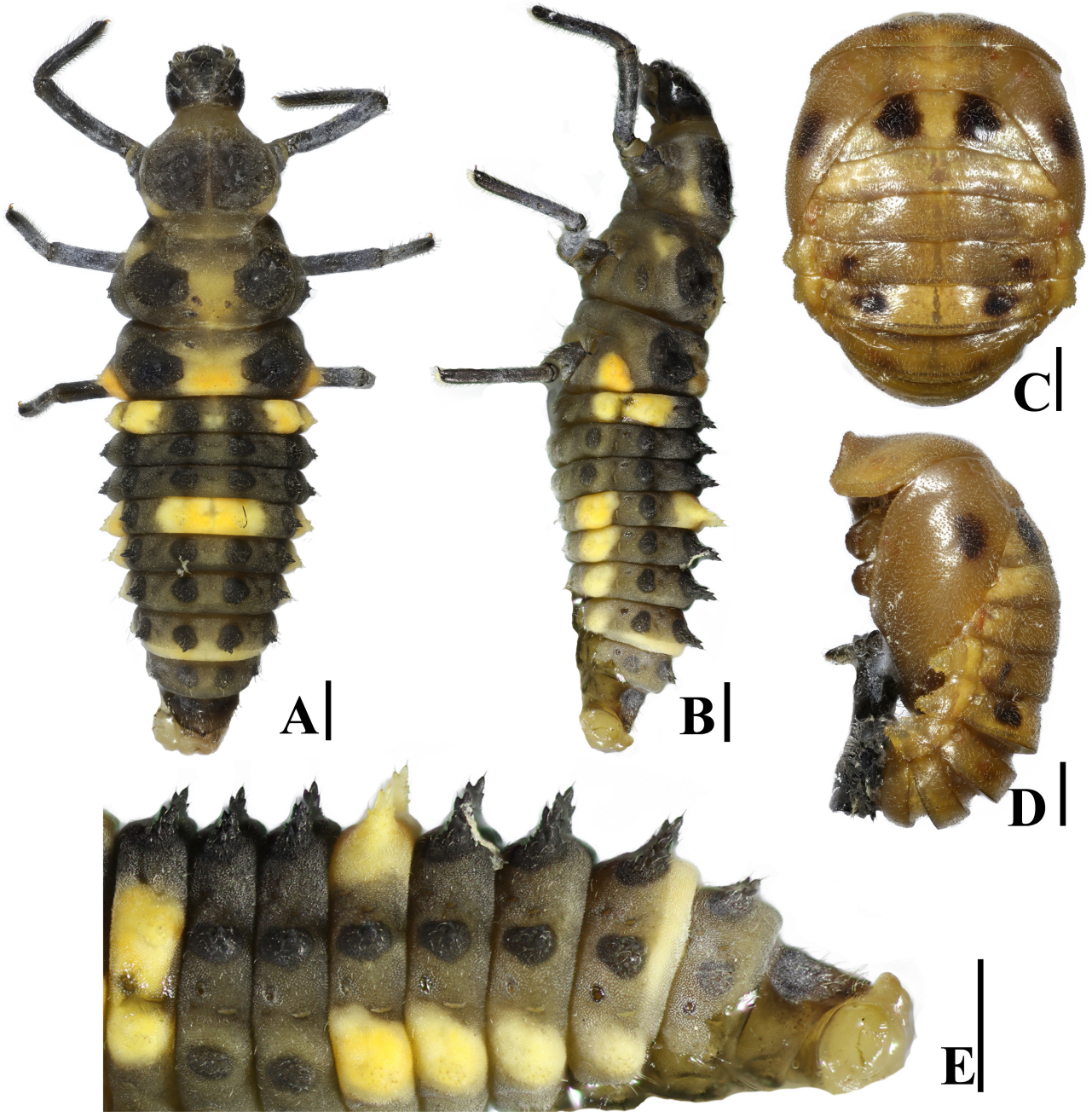

FIGURE 4. The larva and pupa of *Coelophora biplagiata*. **A–B.** fourth instar larva, in dorsal (A) and lateral (B) view. **C–D.** pupa, in dorsal (C) and lateral (D) view. **E.** nine segments of abdomen of fourth instar larva, lateral view. Scale bars: 1 mm.

Fourth instar larva (Figs 4A–B, 4E, 5). Length 12.0 mm; width 3.9 mm. Body elongate, cylindrical and tapered, with parascoli and chalazae, black and yellow color (Figs 4A–B). Head: black, tapered. Epicranial stem absent; frontal arms inverted omega-shaped, do not reach antenna (Fig. 5A). Three hemispherical stemmata dark, arranged in a triangle, near the base of antenna. Antenna with 3 antennomeres; antennomere 3 with preapical setae and sensilla (Fig. 5B). Labrum with setae and brown curves on each side, posterior margin V-shaped (Fig. 5C). Mandible with two apical teeth (Fig. 5D). Maxilla with mala trapezoidal, rounded at apex, with spare long curved setae and outer margin with thin and short setae in ventral side (Fig. 5F). Maxillary palp with three palpomeres (Fig. 5H). Labium with setae in ventral side, posterior margin with numerous short setae, labial palpi with three palpomeres (Figs 5G, 5I).

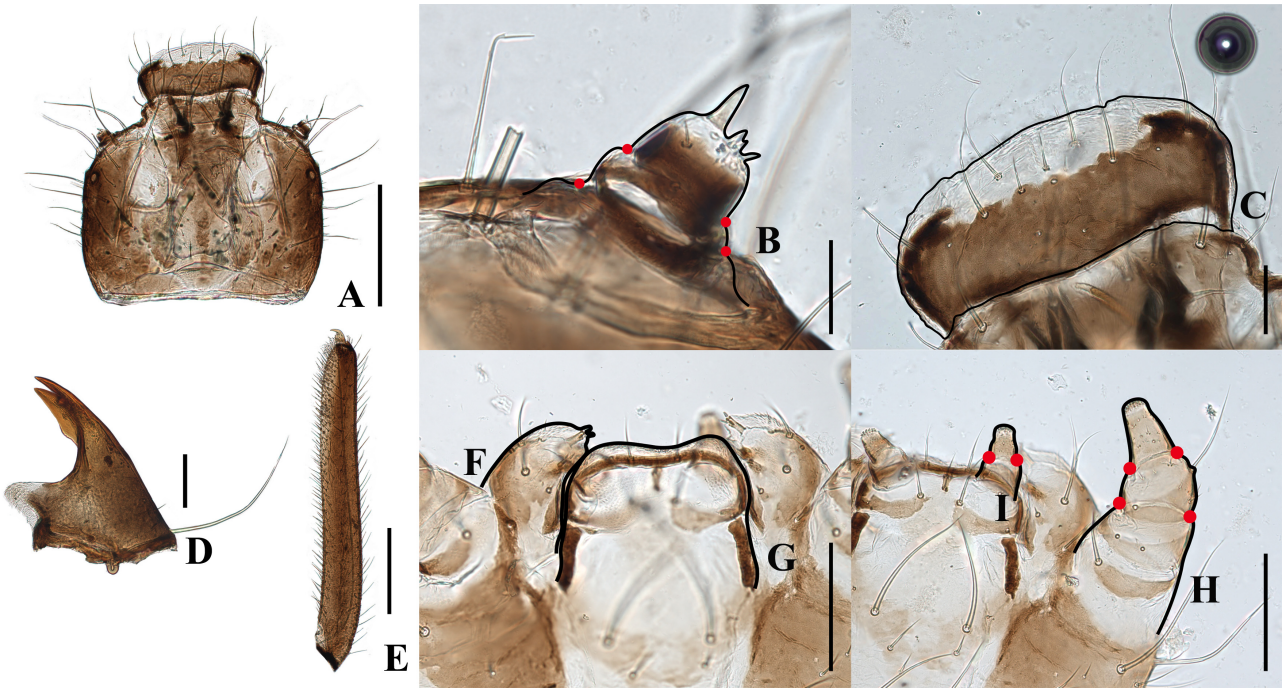

FIGURE 5. Main characters of the fourth instar larva of *Coelophora biplagiata*. **A.** head, dorsal view. **B.** antenna. **C.** labrum. **D.** mandible. **E.** tibiotarsus. **F.** maxilla. **G.** labium. **H.** maxillary palp. **I.** labial palp. Scale bars: 500 μm in A, E; 100 μm in C, D, F–I; 50 μm in B. Black lines: outlines of structures; red dots: boundaries of segmentation of maxillary and labial palpi.

Thorax. Pronotum with two black dorsal plates, semi-oval and sclerotized, separated by a narrow band and posterior marginal areas without projection, only with dense short setae (Figs 4A–B). Mesonotum yellow and black, with dense short setae; black plates with semi-circular outer margins and trapezoid inner margins; lateral margins with black strumae (Figs 4A–B). Metanotum yellow and black, with dense short setae; black plates with semi-circular outer margins and trapezoid inner margins; laterally with yellow strumae (Figs 4A–B). Legs black, with setae; ventral part with a seta brush near the tarsal claw, and row of external setae in the tibia; tarsal claw with a square small basal tooth and dense setae around the tooth (Fig. 5E).

Abdomen with nine segments (Fig. 4E). Segment S1 to S8 with a pair of anterolateral spiracles similar to those of the mesothorax (Fig. 4E). Segment S1 yellow in dorsolateral and lateral areas and dark brown elsewhere, with a yellowish white spot in central part; dorsal plates with black parascoli; dorsolateral and lateral areas with yellow parascoli (Figs 4A–B, 4E). Segment S2 and S3 dark brown, with three pairs of black parascoli in dorsal, dorsolateral and lateral areas (Figs 4A–B, 4E). Segment S4 yellow, and black in dorsolateral regions; dorsal and lateral areas with two pair of yellow parascoli; dorsolateral plates with a pair of black parascoli. Segment S5 and S6 black and yellow; dorsal and dorsolateral plates with two pairs of black parascoli; laterally with a pair of yellow strumae (Figs 4A–B, 4E). Segment S7 chequered with black and yellow, with a yellow stripe in posterior margin; dorsal and dorsolateral plates with two pairs of black parascoli; lateral areas with a pair of black strumae (Figs 4A–B, 4E). Segment S8 black, with yellow strumae in dorsal, dorsolateral and lateral areas (Figs 4A–B, 4E). Segment S9 black with nearly 20 setae (Figs 4A–B, 4E).

Pupa (Figs 4C–D). Length 6.2 mm; width 4.8 mm. Yellowish brown with black spots. Body oval, convex and truncate in the anterior region. Pronotum with two black spots in anterior part. Mesonotum with a pair of spots in lateral margins. Metanotum with a pair of black spots connected to mesonotum. Abdomen with seven visible segments. Segment S1 and S2 yellowish brown without black spot. Segment S3 and S4 with a pair of black spots. Segment S5 to S7 yellowish brown without black spot.

Notes. Yu (2010) provided illustrations of the larval and pupal stages of this species under the name *Lemnia biplagiata* (Swartz). Additionally, Kamiya (1965) outlined the general characteristics of various Coccinellidae tribes including *Lemnia biplagiata* (Swartz) as an example, though no specific description was provided.

***Harmonia dimidiata* (Fabricius, 1781)**
(Figs 1D–F, 6–7)

Larval descriptions or illustrations of *Harmonia dimidiata*: Sasaji 1977: 2, Singh & Phaloura 1990: 89, Phaloura & Singh 1991, Poorani 2023: 126.

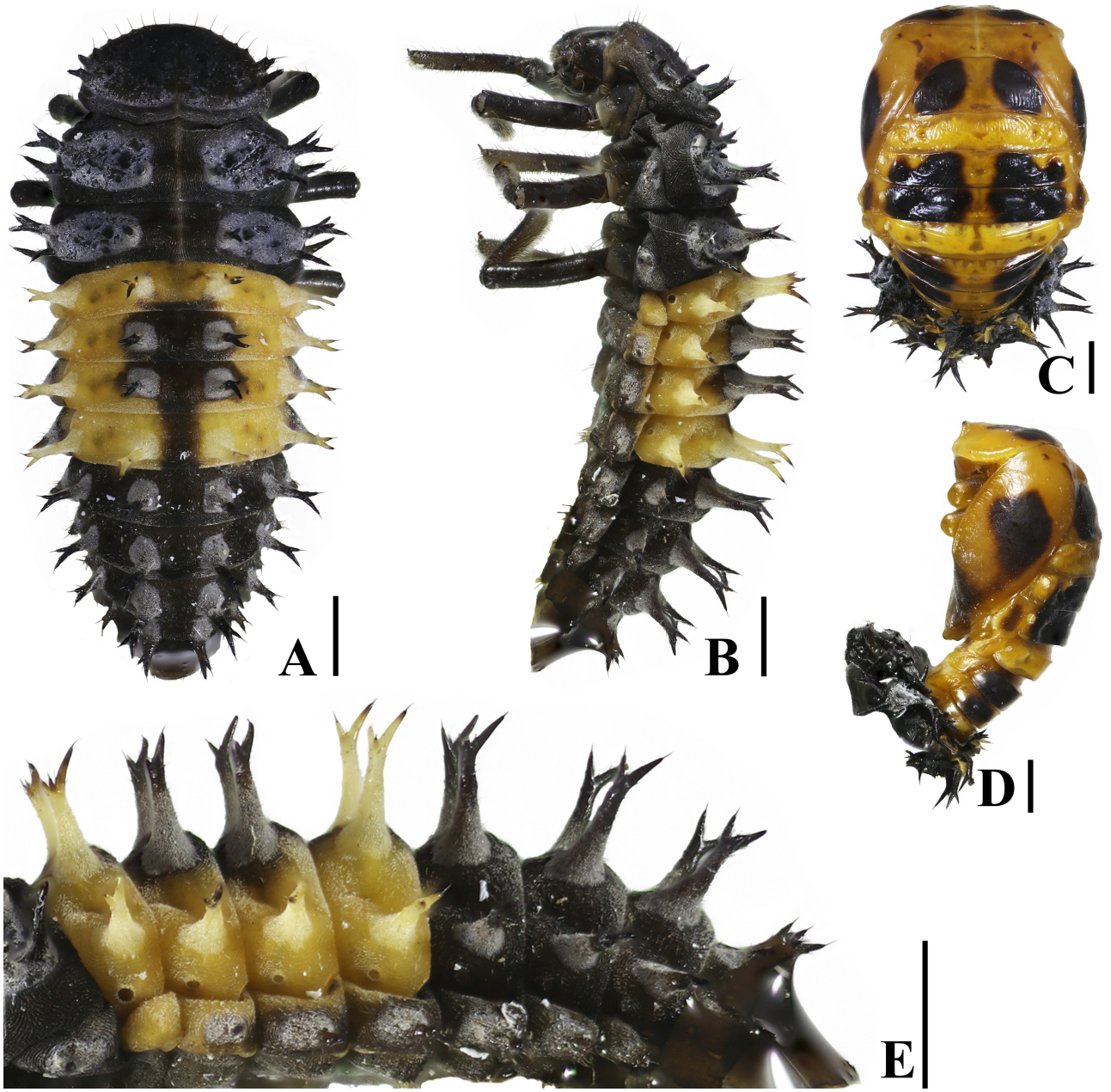

FIGURE 6. The larva and pupa of *Harmonia dimidiata*. **A–B.** fourth instar larva, in dorsal (A) and lateral (B) view. **C–D.** pupa, in dorsal (C) and lateral (D) view. **E.** nine segments of abdomen of fourth instar larva, lateral view. Scale bars: 1 mm.

Fourth instar larva (Figs 6A–B, 6E, 7). Length 6.1–6.8 mm; width 3.0–3.3 mm. Body elongate, cylindrical and tapered, with parascoli and chalazae, black and yellow color (Figs 6A–B). Head: black, tapered. Epicranial stem absent; frontal arms inverted omega-shaped (Fig. 7A), do not reach antennae. Three hemispherical stemmata dark, arranged in a triangle, near at the base of antenna (Fig. 7A). Antenna with 3 antennomeres; antennomere 3 with preapical setae and sensilla (Fig. 7B). Labrum nearly square, bended at two sides, with setae (Fig. 7C). Mandible with two apical teeth (Fig. 7D). Maxilla with mala trapezoidal, rounded at apex, with two long curved setae and outer margin with thin and short setae in ventral side (Fig. 7F). Maxillary palp with three palpomeres (Fig. 7H).

Labium with spare setae, posterior margin with numerous short setae, labial palpi with three palpomeres (Figs 7G, 7I).

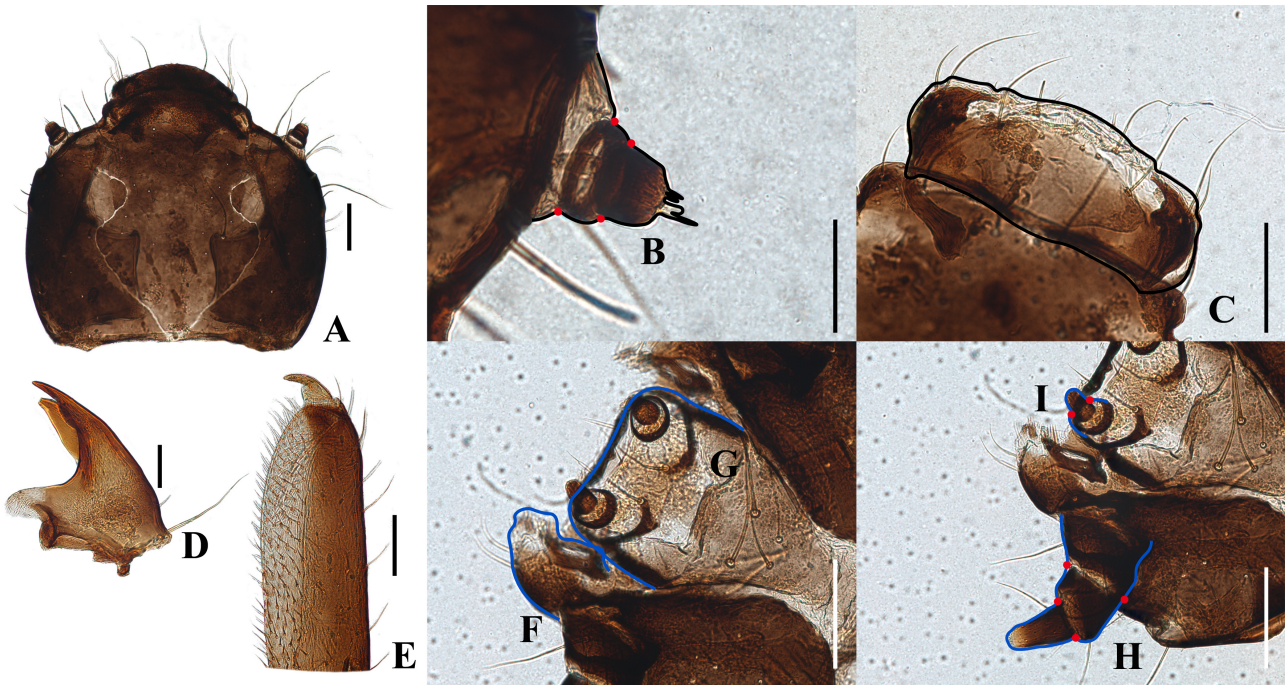

FIGURE 7. Main characters of the fourth instar larva of *Harmonia dimidiata*. **A.** head, dorsal view. **B.** antenna. **C.** labrum. **D.** mandible. **E.** tibiotarsus. **F.** maxilla. **G.** labium. **H.** maxillary palp. **I.** labial palp. Scale bars: 200 μm in A, B, E, F–I; 100 μm in B, D. Black and blue lines: outlines of structures; red dots: boundaries of segmentation of maxillary and labial palpi.

Thorax. Pronotum with two black, semi-oval dorsal plates separated by a narrow band; each lateral margin of plates with four black parascoli; about twelve long chalazae and spare short setae in anterior margin of plates; central surface and posterior margin of plates without projection (Figs 6A–B). Meso- and metanotum black, and their plates pale (Figs 6A–B). Lateral margins of plates each with a long black and a short parascoli, and a black parascoli bearing three branches in each central surface of plates (Figs 6A–B). Lateral margins of mesonotum each with a black sentus, without seta (Figs 6A–B). A pair of circular spiracles with lamellae at the opening in anterolateral part of mesonotum. Metanotum laterally each with a black sentus (Figs 6A–B). Legs black, long, with setae; ventral part with a bristle brush near the tarsal claw, and row of external setae in the tibia; tarsal claw with a square small basal tooth and dense setae around the tooth (Fig. 7E).

Abdomen with nine segments (Fig. 6E). Segment S1 to S8 with a pair of anterolateral spiracles similar to those of the mesothorax (Fig. 6E). Segment S1 yellow, with black stripe in central surface closed S2; dorsal plates with a pair of yellow parascoli, black at the apex, bearing three branches; dorsolateral plates and lateral areas with two pairs of yellow parascoli bearing two branches. Segment S2 yellow, black in central surface, and pale in basal black projections; dorsal plates and lateral areas with two pairs of black parascoli bearing two branches; dorsolateral plates with a pair of yellow parascoli black at the apex (Figs 6A–B, 6E). The color and the location of projection of S3 are similar to those of S2, only different in the surface color of lateral areas (Figs 6A–B, 6E). Segment S4 yellow in surface of dorsal and dorsolateral plates, and black in surface of lateral areas, with a black band in central part; dorsally and dorsolaterally with two pairs of yellow parascoli bearing two branches; lateral area with black parascoli (Figs 6A–B, 6E). Segment S5 and S6 black and pale in basal black projections, with two pairs of black parascoli in dorsal and dorsolateral plates, and a pair of short black parascoli in lateral areas (Figs 6A–B, 6E). Segment S7 and S8 black, and pale in basal black projections, with two pairs of black parascoli in dorsal and dorsolateral plates, a pair of black strumae in lateral areas (Figs 6A–B, 6E). Segment S9 black with nearly 20 setae (Figs 6A–B, 6E).

Pupa (Figs 6C–D). Length 5.2–5.9 mm; width 3.3–3.6 mm. Body oval, convex and truncate in the anterior region, brown with black spots. Thorax: pronotum and metanotum with a pair of black spots. Mesonotum with a pair of black spots in lateral margins. Abdomen: seven visible segments. Segment S1 with two black spots; S3 and S4 with a black stripe on each side; S5 and S6 are similar to S3 and S4; S2, S5, S7 brown, without black spot.

Notes. The larval characters of this species were described by Sasaji (1977). Singh & Phaloura (1990) presented a field key to the larvae of four species within the genus *Harmonia*, including *H. dimidiata* and pointed out that *H. dimidiata* can be distinguished from other *Harmonia* species by the projections of S5 in dorsal and dorsolateral areas. Additionally, it can be distinguished from *H. axyridis* by the coloration of S5 (Celli *et al.* 2021). Subsequently, Phaloura & Singh (1991) provided a description of the larval chaetotaxy for this species. Yu (2010) included an illustration of the larva for this species; however, no further descriptions regarding its larva were given. Poorani (2023) presented comprehensive illustrations of this species along with brief descriptions of its immature stages.

Harmonia sedecimnotata (Fabricius, 1801)

(Figs 1G–H, 8–9)

Larval descriptions or illustrations of *Harmonia sedecimnotata*: Singh & Phaloura 1990: 90, Phaloura & Singh 1991, Poorani 2023: 146.

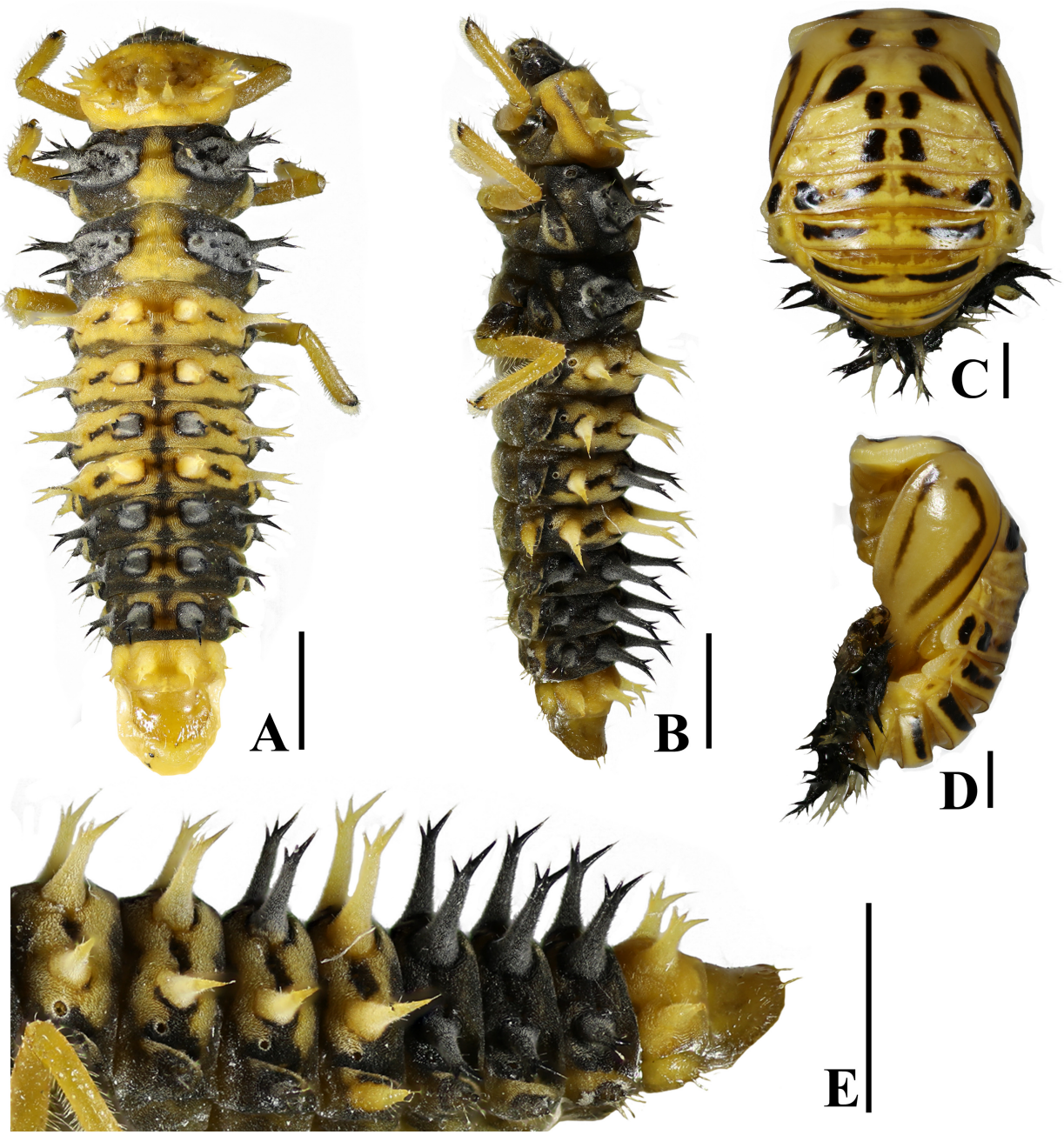

FIGURE 8. The larva and pupa of *Harmonia sedecimnotata*. **A–B.** fourth instar larva, in dorsal (A) and lateral (B) view. **C–D.** pupa, in dorsal (C) and lateral (D) view. **E.** nine segments of abdomen of fourth instar larva, lateral view. Scale bars: 1 mm.

Fourth instar larva (Figs 8A–B, 8E, 9). Length 6.9–7.5 mm; width 2.4–2.5 mm. Body elongate, cylindrical and tapered, with parascoli and chalazae, black and yellow color (Figs 8A–B). Head: black, tapered. Epicranial stem absent; frontal arms inverted omega-shaped, do not reach antennae (Fig. 9A). Three hemispherical stemmata dark, arranged in a triangle, near the base of antenna. Antenna with 3 antennomeres; antennomere 3 with preapical setae and sensilla (Fig. 9B). Labrum with setae, posterior margin V-shaped, with brown curves on each side (Fig. 9C). Mandible with two apical teeth (Fig. 9D). Maxilla with mala trapezoidal, rounded at apex, with long curved setae and outer margin with thin and short setae in ventral side (Fig. 9F). Maxillary palp with three palpomeres (Fig. 9H). Labium with sestae, posterior margin with numerous short setae, labial palpi with three palpomeres in ventral side (Figs 9G, 9I).

Thorax. Pronotum yellow with two dorsal plates, semi-oval and sclerotized, boundary indistinct; lateral margins of plates with four yellow parascoli bearing two branches; posterior margin of plates with four yellow smaller parascoli; about eight charazae in the anterior area; central surface with spare short setae (Figs 8A–B). Mesonotum and metanotum black separated by yellow band and yellow circular ring in the root of lateral projections; anterolateral margin of plates each with a black parascoli bearing three to four branches; posterolaterally each with a black sentus (Figs 8A–B). Lateral margin of mesonotum each with a black sentus, without setae (Figs 8A–B). A pair of circular spiracles with lamellae at the opening in anterolateral part of mesonotum (Fig. 8B). Metanotum laterally each with a black sentus (Figs 8A–B). Legs yellow, long, with setae; ventral part with a seta brush near the tarsal claw, and row of external setae in the tibia; tarsal claw with a square small basal tooth and dense setae around the tooth (Fig. 9E).

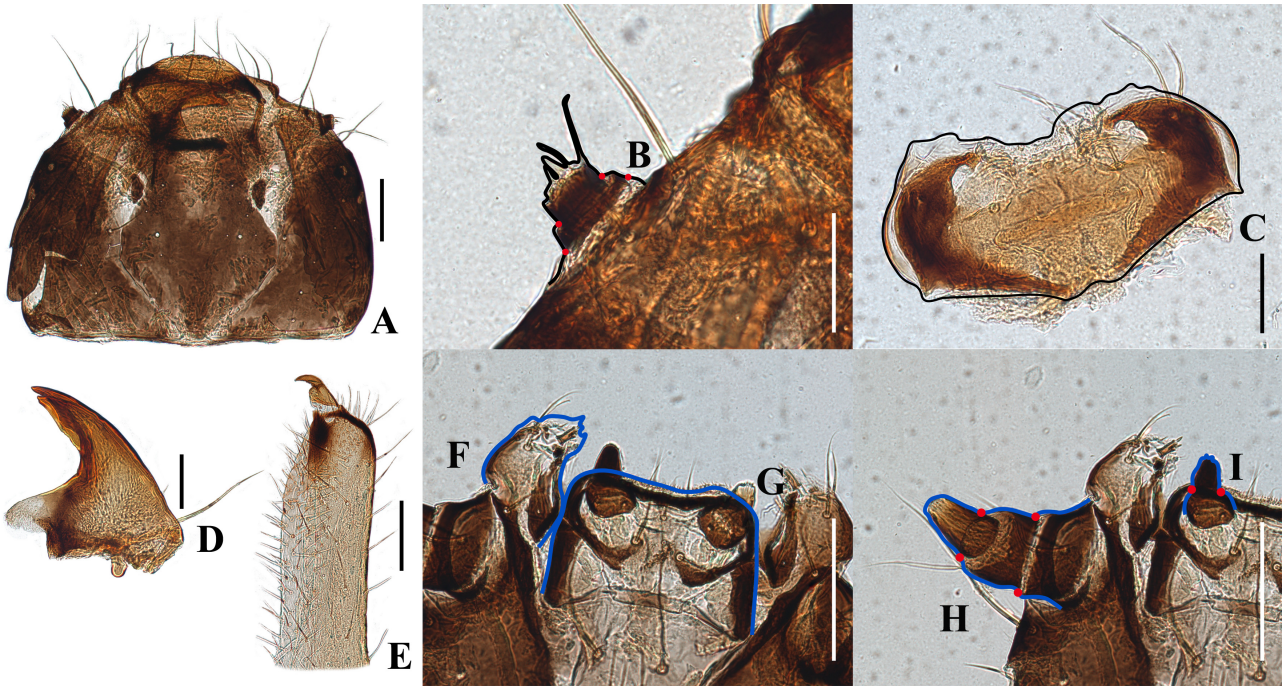

FIGURE 9. Main characters of the fourth instar larva of *Harmonia sedecimnotata*. **A.** head, dorsal view. **B.** antenna. **C.** labrum. **D.** mandible. **E.** tibiotarsus. **F.** maxilla. **G.** labium. **H.** maxillary palp. **I.** labial palp. Scale bars: 200 μm in A, B, D–I; 100 μm in B, D. Black and blue lines: outlines of structures; red dots: boundaries of segmentation of maxillary and labial palpi.

Abdomen with nine segments (Fig. 8E). Segment S1 to S8 with a pair of anterolateral spiracles similar to those of the mesothorax (Fig. 8E). Segment S1 yellow, black in lateral areas, a black oblique stripe between dorsal and dorsolateral plates, and a black spot closed to S2; dorsal and dorsolateral plates with two pairs of yellow parascoli, developed two branches; lateral areas with a pair of yellow parascoli (Figs 8A–B, 8E). Segment S2 yellow, black in lateral areas, a black oblique stripe between dorsal and dorsolateral plates, and black irregular stripe separates two sides; dorsal and dorsolateral plates with two pairs of yellow parascoli bearing three branches; lateral projections are black parascoli (Figs 8A–B, 8E). The surface color and location of projection of S3 are similar to those of S2, differing only in the dorsal projections (Figs 8A–B, 8E). Segment S4 is similar to S1 (Figs 8A–B, 8E). Segment S5 to S7 black, with yellow base of dorsal projections and lateral areas; dorsal and dorsolateral plates with black

parascoli, developed two branches; laterally with smaller black parascoli, only S7 with strumae (Figs 8A–B, 8E). Segment S8 yellow, with yellow parascoli in dorsal and dorsolateral plates and yellow strumae in lateral areas (Figs 8A–B, 8E). Segment S9 yellow with nearly 20 setae (Figs 8A–B, 8E).

Pupa (Figs 8C–D). Length 4.9–5.1 mm; width 3.0–3.5 mm. Yellowish brown with black spots and stripes. Body oval, convex and truncate in the anterior region. Pronotum with two black spots at both sides. Mesonotum with a pair of black spots in central region, and a brown arc on each side in lateral area. Metanotum with two black oblique stripes at both sides. Abdomen with seven visible segments. Segment S1 and S2 with four black spots in central surface. Segment S3 with six black spots: two oblique stripes in central region, four irregular spots arrange in parallel in lateral area; S4 to S6 with one black arc on each side; S7 without black spot.

Notes. Singh & Phaloura (1990) presented a field key to the larvae of four species within the genus *Harmonia*, including *H. sedecimnotata*. Authors mentioned that *H. sedecimnotata* can be distinguished from other *Harmonia* species by the color of S4 in lateral areas. Subsequently, Phaloura & Singh (1991) provided a description of the larval chaetotaxy for this species. Poorani (2023) presented comprehensive illustrations of this species along with brief descriptions of its immature stages.

Microcaria pupillata (Swartz, 1808)

(Figs 1N–P, 10–11)

Fourth instar larva (Figs 10A–B, 10E, 11). Length 11.2 mm; width at metathorax 3.6 mm. Body elongate, cylindrical and tapered, with strumae and chalazae, black and yellow color (Figs 10A–B). Head: black, tapered. Epicranial stem absent; frontal arms inverted omega-shaped (Fig. 11A), do not reach antennae; they appear to be separated at bases. Three hemispherical stemmata dark, arranged in a triangle, near base of antenna. Antenna with 3 antennomeres; antennomere 3 with preapical setae and sensilla (Fig. 11B). Labrum with setae, posterior margin V-shaped, with brown curves on each side (Fig. 11C). Mandible with two apical teeth (Fig. 11D). Maxilla with mala trapezoidal, rounded at apex, with spare long curved setae and outer margin with thin and short setae in ventral side (Fig. 11F). Maxillary palp with three palpomeres, bearing a seta at palpomere 2 (Fig. 11H). Labium with sparse setae, posterior margin with numerous short setae, labial palp composed with three palpomeres (Figs 11G, 11I).

Thorax. Pronotum yellowish white with two semi-oval and sclerotized black dorsal plates, separated by a narrow band; lateral, anterior and posterior marginal areas without projections, only with dense short setae (Figs 10A–B). Mesonotum yellowish white, with dense short setae; black plates with semi-circular outer margins and trapezoid inner margins; lateral margins with yellowish white strumae (Figs 10A–B). A pair of circular spiracles with lamellae at the opening in anterolateral part of mesonotum. Metanotum yellowish white, with dense short setae; black plates with semi-circular outer margins and trapezoid inner margins; laterally with yellowish white strumae. Legs black, long, with sparse setae; ventral part with a seta brush near the tarsal claw, and row of external setae in the tibia; tarsal claw with a square small basal tooth and dense setae around the tooth (Fig. 11E).

Abdomen. Nine segments (Fig. 10E). Segment S1 to S8 with a pair of anterolateral spiracles similar to those on mesothorax (Fig. 10E). Segment S1 yellowish white and black; dorsal plates with a pair of black strumae; dorsolateral plates with a pair of yellow strumae; lateral areas with a pair of yellow strumae; each struma with three short chalazae and two short setae (Figs 10A–B, 10E). Segment S2 and S3 yellowish white and black; dorsal and dorsolateral plates with two pairs of black strumae; lateral areas with a pair of strumae; each struma with three long chalazae and approximately five short chalazae (Figs 10A–B, 10E). Segment S4 yellowish white, with two pairs of yellow strumae in dorsal and dorsolateral plates; laterally with a pair of yellow strumae; each struma with three long chalazae and approximately three short chalazae (Figs 10A–B, 10E). Segment S5 and S6 yellowish white with black dorsal and dorsolateral strumae; lateral regions with a pair of yellow strumae; each struma with approximately eight short chalazae (Figs 10A–B, 10E). Segment S7 yellowish white with black dorsal and dorsolateral strumae, bearing yellow stripe in posterior margin; laterally with yellow strumae (Figs 10A–B, 10E). Segment S8 dark brown, with yellow stripe in central surface; dorsal and dorsolateral plates with two pair of black strumae; lateral areas with yellow strumae (Figs 10A–B, 10E). Segment S9 dark brown, with nearly 20 setae (Figs 10A–B, 10E).

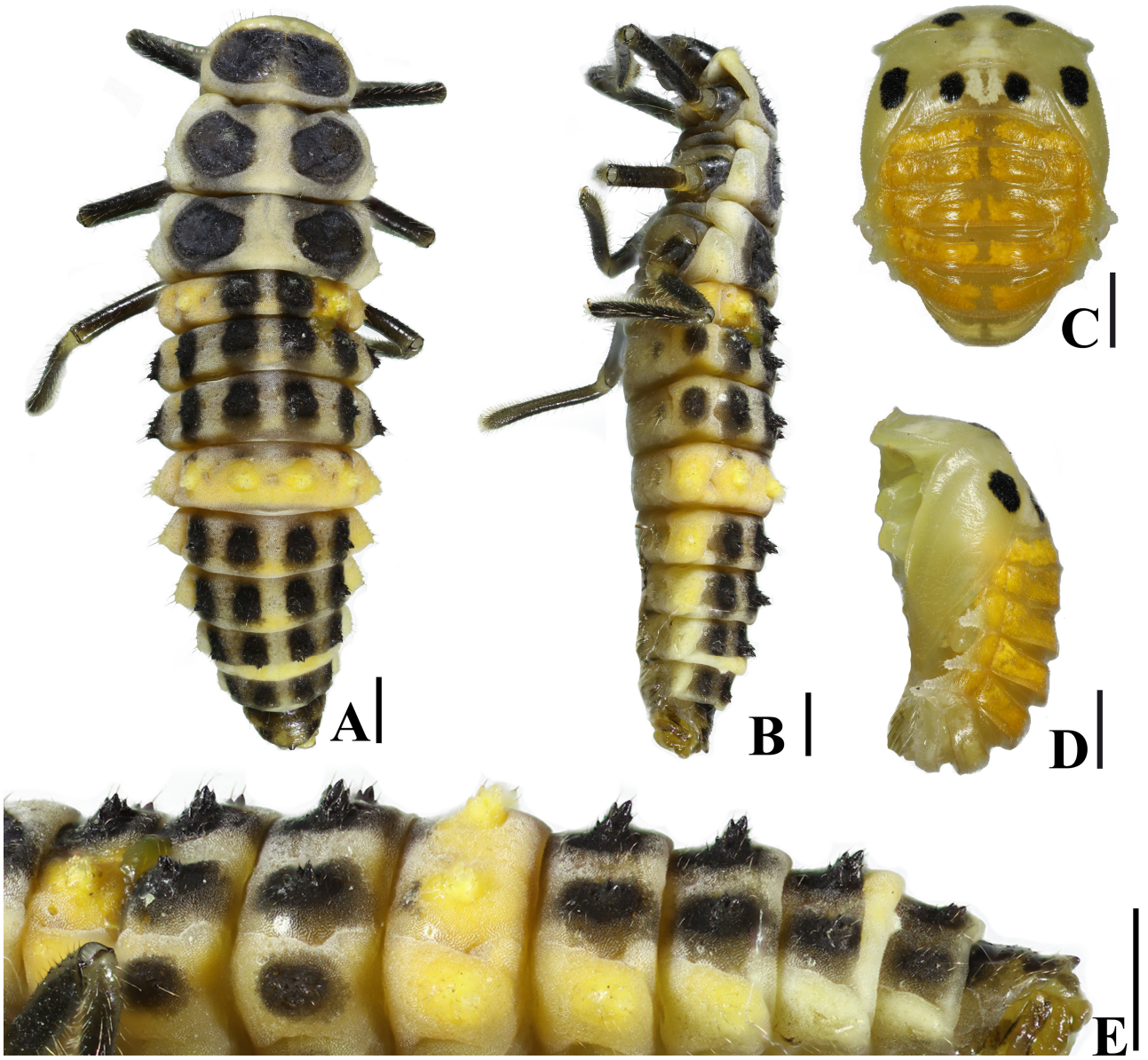

FIGURE 10. The larva and pupa of *Microcaria pupillata*. **A–B.** fourth instar larva, in dorsal (A) and lateral (B) view. **C–D.** pupa, in dorsal (C) and lateral (D) view. **E.** nine segments of abdomen of fourth instar larva, lateral view. Scale bars: 1 mm.

Pupa (Figs 10C–D). Length 4.5 mm; width 3.5 mm. Yellowish white or yellow with black spots. Body oval, convex and truncate in the anterior region. Pronotum with a pair of black spots in parallel in anterior region. Mesonotum yellowish white with a white trapezoid spot in central part and a pair of large black spots in lateral areas. Metanotum with white U-shape spot in the central region and a pair of black spots. Abdomen with seven visible segments. Segment S1 to S6 yellow with a light brown band in central part. Segment S7 yellowish white with a light brown band in central part.

Notes. The immature stages of this species are described for the first time. This species has been previously placed in the genus *Bothrocalvia* Crotch, 1874 by several authors (Yu 2010; Ren *et al*. 2009; Wang & Chen 2022). However, Ślipiński *et al*. (2020) considered *Bothrocalvia* as a synonym of *Microcaria* Crotch, 1871 and consequently transferred the species to the genus *Microcaria*.

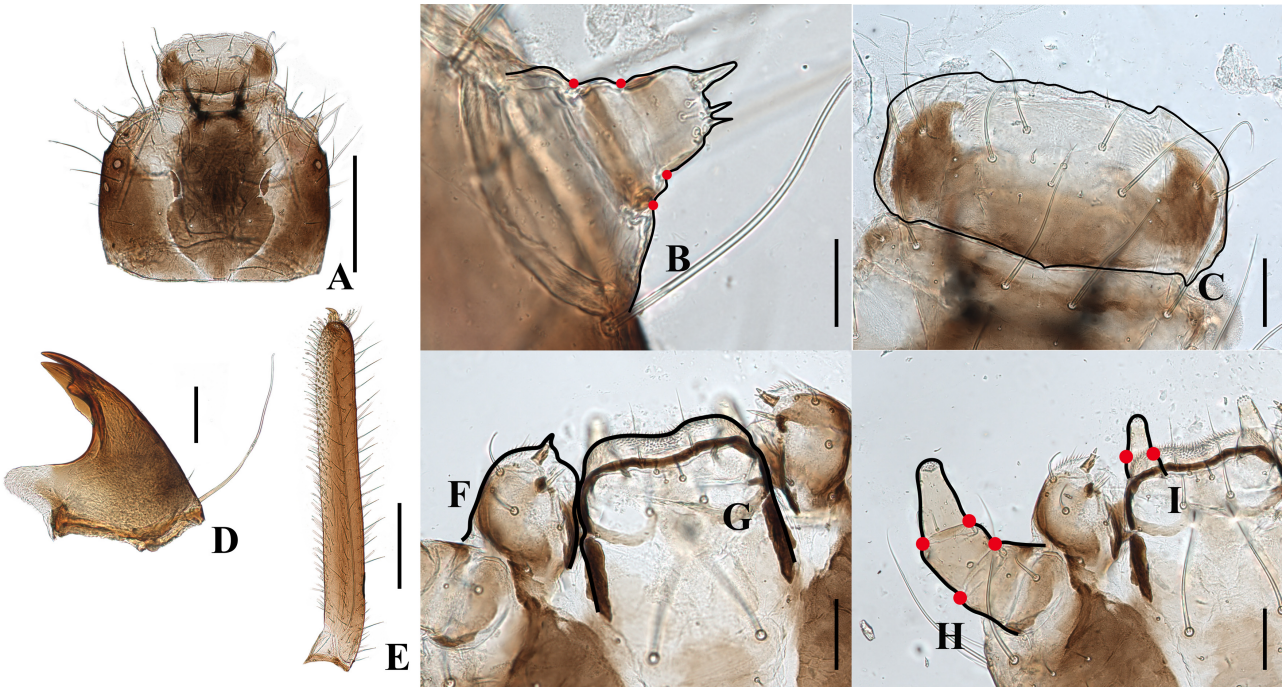

FIGURE 11. Main characters of the fourth instar larva of *Microcaria pupillata*. **A.** head, dorsal view. **B.** antenna. **C.** labrum. **D.** mandible. **E.** tibiotarsus. **F.** maxilla. **G.** labium. **H.** maxillary palp. **I.** labial palp. Scale bars: 500 μm in A, E; 100 μm in C, D, F–I; 50 μm in B. Black lines: outlines of structures; red dots: boundaries of segmentation of maxillary and labial palpi.

Phrynocaria unicolor (Fabricius, 1792)

(Figs 1I–J, 12–13)

Fourth instar larva (Figs 12A–B, 12E, 13). Length 11.5 mm; width 3.5 mm. Body elongate, cylindrical and tapered, with parascoli and chalazae, black and yellow color. Head: black, tapered (Figs 12A–B). Epicranial stem absent; frontal arms inverted omega-shaped, do not reach antennae (Fig. 13A). Three hemispherical stemmata dark, arranged in a triangle, near the base of antenna. Antenna with 3 antennomeres; antennomere 3 with preapical setae and sensilla (Fig. 13B). Labrum with setae and brown curves on each side, posterior margin V-shaped (Fig. 13C). Mandible with two apical teeth, central convex, and with one basal tooth and two setae above the condyle in dorsal side (Fig. 13D). Maxilla with mala trapezoidal, rounded at apex, with spare long curved setae and outer margin with thin and short setae in ventral side (Fig. 13F). Maxillary palp with three palpomeres (Fig. 13H). Labium with setae, posterior margin with numerous short setae, labial palpi with three palpomeres in ventral side (Figs 13G, 13I).

Thorax. Pronotum black, with two dorsal plates, semi-oval and sclerotized, separated by a narrow band and yellow stripe in anterior margin; lateral margin of plates and central surface without projection, only with dense short setae (Figs 12A–B). Mesonotum black separated by a yellow stripe. Lateral margin with a yellow struma; anterior and posterior margins of mesonotum without projection, with dense short setae (Figs 12A–B). A pair of circular spiracles with lamellae at the opening in anterolateral part of mesonotum (Fig. 12B). Metanotum black, with a yellow trapezoidal spot; lateral margins with a yellow struma; anterior and posterior margins with dense short setae (Figs 12A–B). Legs black, long, with setae; ventral part with a seta brush near the tarsal claw, and row of external setae in the tibia; tarsal claw with a subquadrate basal tooth and dense setae around the tooth (Fig. 13E).

Abdomen with nine segments (Fig. 12E). Segment S1 to S8 with a pair of anterolateral spiracles similar to those of the mesothorax (Fig. 12E). Segment S1 black, with light yellow spots in dorsolateral and lateral areas, unconnected to S2; dorsal plates with a pair of black strumae; dorsolateral and lateral projections with yellow strumae (Figs 12A–B, 12E). Segment S2 and S3 dark brown, with black strumae in dorsal, dorsolateral and lateral areas (Figs 12A–B, 12E). Segment S4 light yellow, and black between lateral and dorsolateral regions; dorsal, dorsolateral and lateral areas with light yellow strumae (Figs 12A–B, 12E). Segment S5 to S6 black, with black strumae in dorsal and dorsolateral plates and light yellow strumae in lateral areas (Figs 12A–B, 12E). Segment S7

and S8 black with yellowish white stripes in posterior margin; dorsal and dorsolateral plates with black strumae; lateral areas with light yellow strumae (Figs 12A–B, 12E). Segment S9 black with nearly 20 setae (Figs 12A–B, 12E).

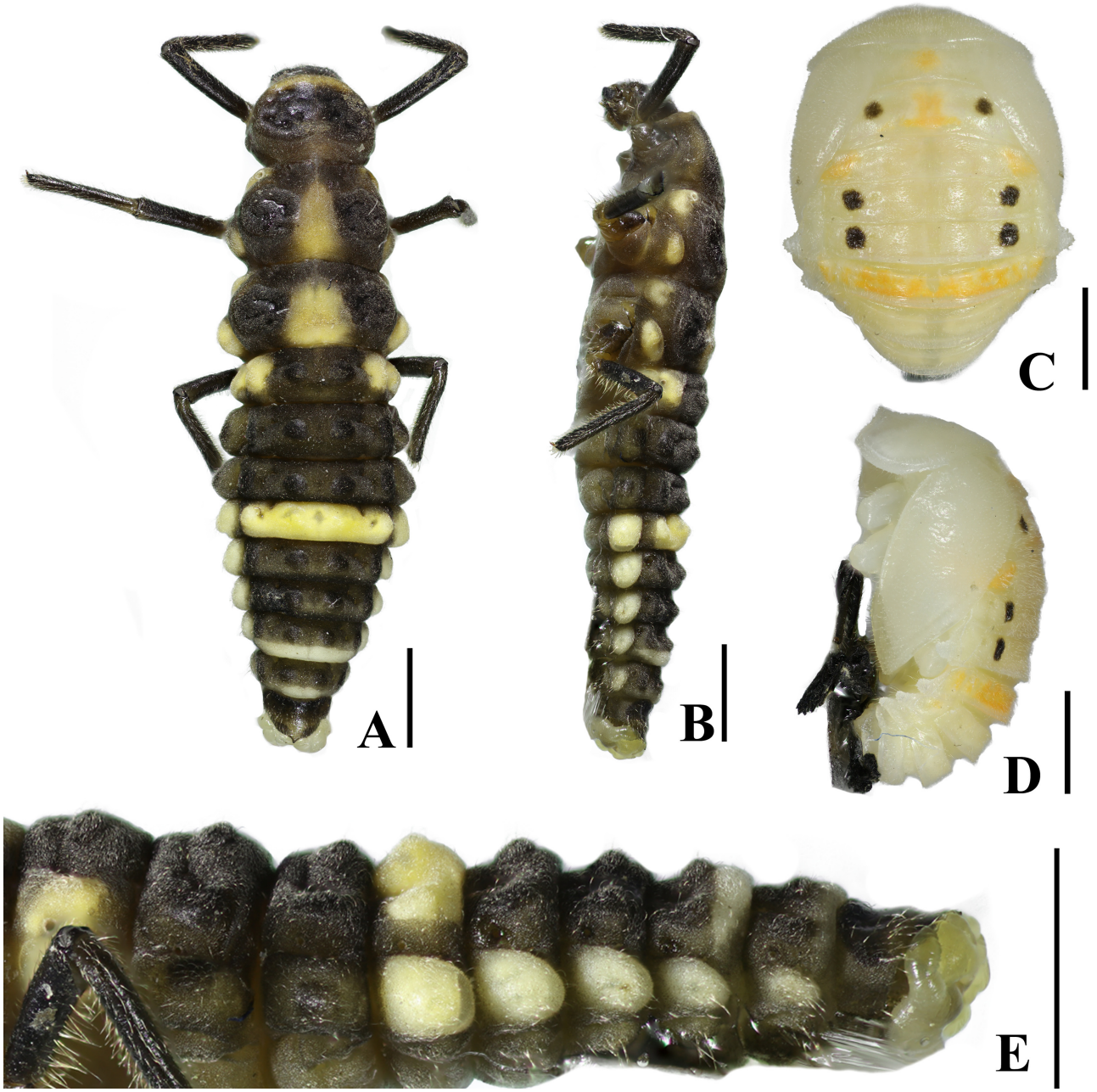

FIGURE 12. The larva and pupa of *Phrynocaria unicolor*. **A–B.** fourth instar larva, in dorsal (A) and lateral (B) view. **C–D.** pupa, in dorsal (C) and lateral (D) view. **E.** nine segments of abdomen of fourth instar larva, lateral view. Scale bars: 1 mm.

Pupa (Figs 12C–D). Length 6.2 mm; width 4.8 mm. Body yellowish white with black spots. Body oval, convex and truncate in the anterior region. Pronotum yellowish white with short setae. Mesonotum with a yellow spot in central surface. Metanotum with a central yellow stripe and a pair of black spots in anterolateral margins. Abdomen with seven visible segments. Segment S1 yellowish white, with yellow irregular stripes in lateral margins. Segment S2 and S3 yellowish white with two black spots; S4 yellow, without black spot. Segment S5 to S7 yellowish white, without black spot.

Notes. The immature stages of this species are described for the first time. Yu (2010) provided illustrations of larva and pupa for this species; however, no further descriptions regarding its immature stages were given.

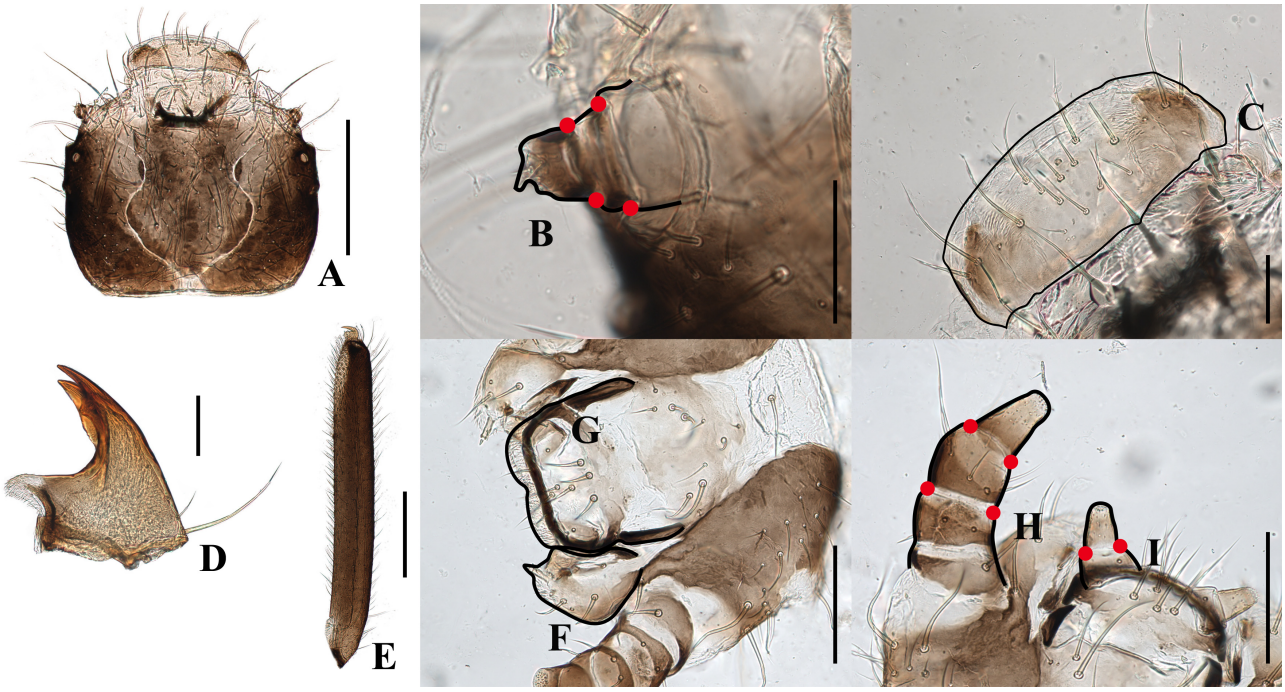

FIGURE 13. Main characters of the fourth instar larva of *Microcaria pupillata*. **A.** head, dorsal view. **B.** antenna. **C.** labrum. **D.** mandible. **E.** tibiotarsus. **F.** maxilla. **G.** labium. **H.** maxillary palp. **I.** labial palp. Scale bars: 500 μm in A, E; 100 μm in B–D, F–I. Black lines: outlines of structures; red dots: boundaries of segmentation of maxillary and labial palpi.

Discussion

Although the critical role of both larvae and adults of Coccinellidae in biological control has been recognized, current researches primarily focus on adults, resulting in a scarcity of detailed morphological descriptions for larvae and an incomplete understanding of the immature stages of ladybird beetles (Celli *et al.* 2021). Studies on the larvae of Coccinellini in China are also scanty. Huang *et al.* (2022) mentioned that *H. axyridis* has over 200 variations in elytral colour patterns, complicating species identification based solely on adult external appearance. In contrast, larval morphological characteristics remain stable can provide important characters for coccinellid identification.

Escalona *et al.* (2017) pointed out that evolutionary shifts in food preferences among ancestral Coccinellini were associated with significant modifications in larval dorsal projections. Rees *et al.* (1994) provided an identification key for the larvae of 46 North American genera within Coccinellidae, including characters such as dorsal plates and their structures, including setae, scoli, parascoli, strumae and chalazae. In some immature stages of Coccinellini, the most developed dorsal projections of larvae are typically observed on the dorsal and dorsolateral plates of segments S1–S6 (Gage 1920; Sasaji 1968; Hodek 1973; Houston 1988; Gordon & Vandenberg 1993; Rees *et al.* 1994; Ślipiński 2007; Celli *et al.* 2021). Hodek (1973) mentioned that only Coccinellini larvae have variable dorsal projections, which include parascoli (e.g., *Adalia* Mulsant, 1850, *Hippodamia* Chevrolat in Dejean, 1837), scoli (*Harmonia*) and strumae (*Propylea* Mulsant, 1846, *Coccinula* Dobzhansky, 1925). The most prominent dorsal projections of *P. unicolor*, *P. gratiosa*, *P. astronabiana* and *M. pupillata* larvae are strumae, while *C. sexmaculata* has senti (Figs 2A–B, 2E, 10A–B, 10E, 12A–B, 12E) (Hodek 1973; Houston, 1988; Ślipiński 2007; Yu 2010). *Coelophora biplagiata* and *L. saucia calypso* (Mulsant, 1856) have short parascoli, whereas *H. axyridis*, *H. dimidiata* and *H. sedecimnotata* have more pronounced parascoli in their dorsal projections (Figs 4A–B, 6A–B, 8A–B) (Sasaji 1968, 1977; Houston 1988; Singh & Phaloura 1990; Phaloura & Singh 1991; Yu 2010; Celli *et al.* 2021). Consequently, the location and morphology of dorsal projections are generally consistent within the same genus among Coccinellini larvae but vary across different genera.

Harmonia dimidiata and *H. axyridis* exhibit distinct variations in their elytral pattern (Sasaji 1977; Celli *et al.* 2021). From the fourth instar larval stage, their body surfaces are largely similar, except for the fifth abdominal segment of the nine-segmented abdomen. In this segment, *H. dimidiata* displays a black surface with parascoli,

whereas *H. axyridis* exhibits a yellow surface with parascoli (Figs 6A, 6B) (Sasaji 1977; Celli *et al.* 2021). Therefore, the fourth instar larvae of these two species can be differentiated based on differences in surface coloration.

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