

RESEARCH ARTICLE

# The way to maturity: taxonomic study on immatures of Southern Brazilian Coccinellini (Coleoptera: Coccinellidae) species important in biological control

Nathália Del G. da R. Celli<sup>1</sup> , Lúcia M. Almeida<sup>1</sup> , Daniel S. Basílio<sup>1</sup> , Camila F. Castro<sup>1</sup>

<sup>1</sup>Laboratório de Sistemática e Bioecologia de Coleoptera, Departamento de Zoologia, Universidade Federal do Paraná. Caixa Postal 19030, 81531-980 Curitiba, RP, Brazil.

Corresponding author: Camila Fediuk de Castro ([camifc@yahoo.com](mailto:camifc@yahoo.com))

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**ABSTRACT.** Among the predatory ladybird beetles (Coccinellidae: Coleoptera), members of the Coccinellini, predators of aphids and psyllids, stand out. Although the beneficial status of these beetles has been acknowledged by biological control researchers, there are no keys or detailed studies on the immature stages of South American Coccinellidae, especially Coccinellini. We provide descriptions and illustrations of the immatures and adults of major predatory Coccinellini species in southern Brazil along with an identification key for fourth instar larvae and pupae. The following species are included: *Cycloneda sanguinea* (Linnaeus, 1763), *Eriopis connexa* (Germar, 1824), *Harmonia axyridis* (Pallas, 1773), *Hippodamia convergens* Guérin-Méneville, 1842 and *Olla v-nigrum* (Mulsant, 1866). The morphological study, which included the use of scanning electron microscopy, revealed new characters such as the type of tarsal claws, spiracles, chalazae, parascoli and strumae. The identification key provided here may be useful in biological control programs.

**KEY WORDS.** Adults, Coccinellinae, larvae, morphology, predators, pupae.

## INTRODUCTION

The ladybird beetles of Coccinellidae (Coleoptera) are known as the most efficient predators, mainly of aphids and of the first larval instars of Lepidoptera, Coleoptera and Hymenoptera, small Diptera and Thysanoptera (Hodek and Honek 1996, Araújo-Siqueira and Almeida 2006, Hodek et al. 2012, Zazycki et al. 2015, Escalona et al. 2017, Boopathi et al. 2019). The beneficial status of these insects has rich history, which is acknowledged by the general public and researchers in biological control programs (Hodek 1973, Hodek et al. 2012, Bouvet et al. 2019).

Among the predatory species of Coccinellidae, Coccinellini stand out, feeding mainly on aphids and psyllids, considered harmful insects, because they suck the sap of numerous cultivated plants (Hodek et al. 2012, Bouvet et al. 2019). For Brazil, 98 species of Coccinellini are listed, in 24 genera (Almeida et al. 2021), 58 with potential for biological control in the south of the country.

The Coccinellini species *Cycloneda sanguinea* (Linnaeus, 1763), *Olla v-nigrum* (Mulsant, 1866), *Eriopis connexa* (Germar, 1824), *Harmonia axyridis* (Pallas, 1773) and *Hippodamia convergens* Guérin-Méneville, 1842 are particularly important in southern Brazil, because they have potential for use in biological control

programs (Cardoso and Lázari 2003, Castro et al. 2011, Santos et al. 2014).

In general, the larvae of Coccinellidae have the shape of the body variable from oval to elongated; integument usually pigmented and with tubercles, covered with processes or branched spines, sometimes coated with waxy secretion; head usually pigmented; epicranial suture with V-shaped frontal arms; coronal suture long, absent in most genera; frons with setae; antennae short and robust, usually tri-articulated, mandible almost always uni- or bidentate, reduced mola; labium with 2 or rarely 1-segmented palps; legs generally long and well separated from each other; abdomen with nine tergites, ventral or posterolateral abdominal segment 10, sometimes terminal and rounded with annular spiracles (Costa et al. 1988, Booth et al. 1990, LeSage 1991, Ślipiński 2007).

The pupae are oval to elongated, integument usually pigmented with bristles, wings are usually lightly sclerotized with intersegmental membranes exposed, membranous and tapered apically; in Coccinellini the 4 to 7 abdominal terga are sclerotized; terga 1 to 8 with abdominal spiracles; tergum 9 modified into a pair of urogomphi that anchor the pupa to the substrate (Phuoc and Stehr 1974, Ślipiński 2007).

There are few studies presenting data on the immature forms of Coccinellidae. Stehr (1991) presented an identification key for to the larvae of North American tribes, suggesting that Coccinellini differ from the other tribes by the abdominal structures in segment 9, body with different colorations and antennae with third antennomere reduced.

Vandenberg (1992) revised the species of *Olla* Casey, 1899 from the New World, including comparison with species allocated in *Cycloneda* Crotch, 1871 and *Eriopsis* Mulsant, 1850. Also described immature forms and adults, including a key for adults based on genitalia characters. Gordon and Vandenberg (1993) described the North American species of *Cycloneda*, including an immature key and characters of adults and larvae, comparing with other Coccinellini as *Olla* and *Hippodamia* Dejean, 1837. The most relevant papers using characters of immature stages were the field guide of Gordon & Vandenberg (1991) and Rees et al. (1994), although, both used only North American species.

There are no keys or detailed studies on the immature stages of South American Coccinellidae, especially of Coccinellini. Therefore, the aim of this study is to provide, for the first time, descriptions, and keys for fourth instar larvae and pupae of the main predators known from southern Brazil.

## MATERIAL AND METHODS

Adults of *Cycloneda sanguinea*, *Eriopsis connexa*, *Harmonia axyridis*, *Hippodamia convergens* and *Olla v-nigrum* were manually collected in Curitiba and Palotina, state of Paraná, and Recife, state of Pernambuco, Brazil and reared in 500 mL plastic dishes in rearing chambers (BOD) at  $25 \pm 1$  °C,  $70\% \pm 10\%$  R.H. and 12:12 h L:D. The food, *Anagasta kuehniella* (Zeller, 1879), was supplied daily to maintain the population stock.

After eclosion, the larvae were placed individually in Petri dishes lined with filter paper and a cotton swab moistened with a drop of honey and reared in the same conditions. The larvae and pupae were fixed in Kahle-Dietrich. For morphological studies, the specimens were boiled in 10% KOH for a few seconds, washed in distilled water and then dissected.

The morphological studies were performed using stereomicroscopes ZEISS Stemi SV6 and Stereo Discovery V20. The larvae were photographed using a Sony Cyber-Shot (DSC-W300) digital camera coupled in microscope Standard M 20, stereomicroscopes ahead mentioned. The larvae and pupae measurements were made in stereoscopic microscope Wild 15. Scanning electron microscopy (SEM) images were produced with a JEOL JSM-6360LV scanning electron microscope in the Electronic Microscopy Center, Universidade Federal do Paraná.

Short descriptions and illustrations of adults were included to associate immatures and adults of each species. The terminology used in the descriptions follows Gordon and Vandenberg (1991), Rees et al. (1994), Ślipiński (2007), Ślipiński and Tomaszewska (2010).

## TAXONOMY

### Diagnostics characters

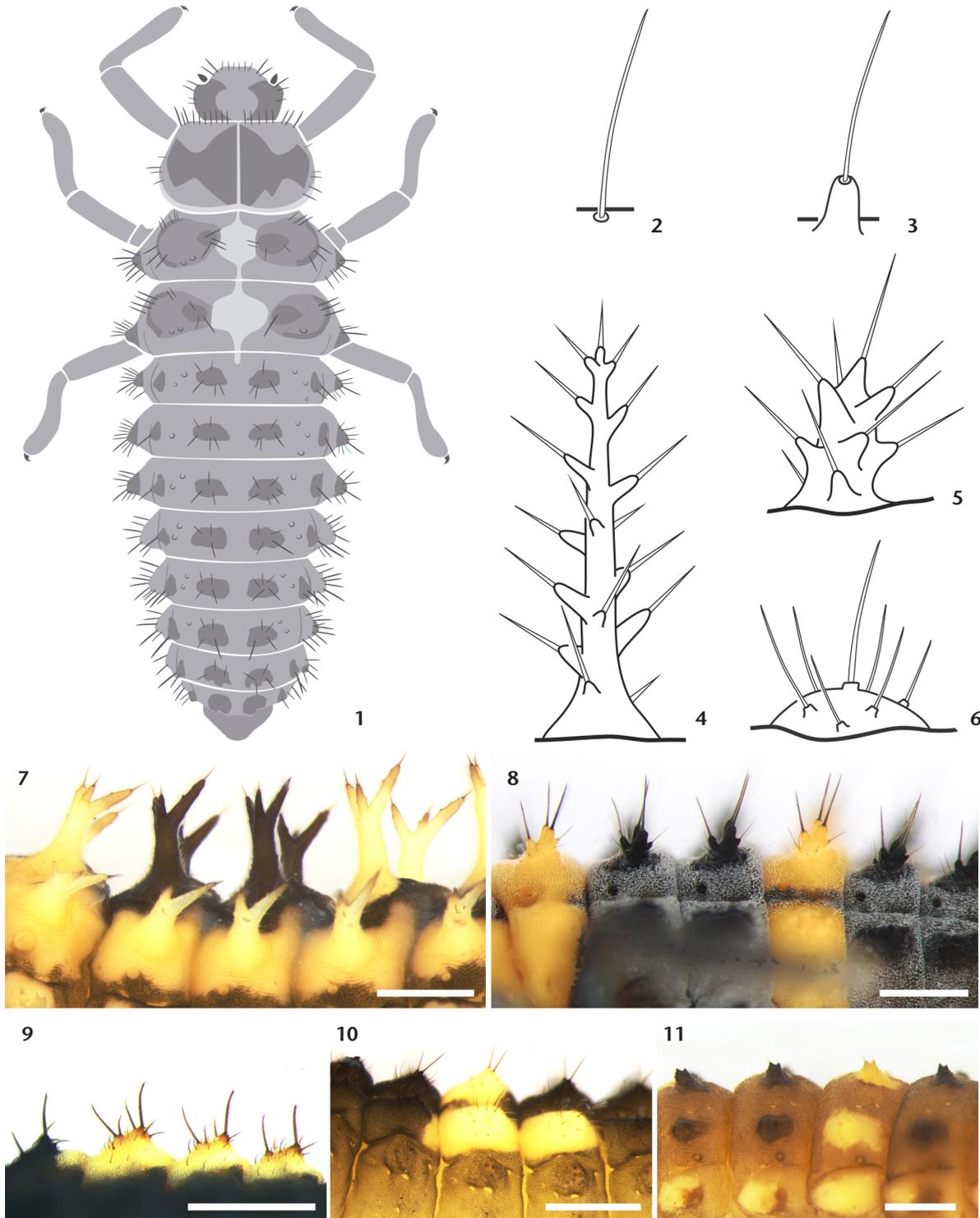
The color and structures of the immature stages such as parascoli, strumae, chalazae (Figs 1–11) and tarsal claws (Figs 24, 37, 50, 63, 77) were used to distinguish the species. The presence and shape of these structures were the main differences found in the species studied, in addition to coloration and spots on the body. Fourth instar larvae were described in more detail, as they provide clear-cut diagnostic characters at the species level.

### Key to fourth instar larvae of the main predatory species of Coccinellini from South Brazil

1. Body with parascoli (Figs 7, 8) ..... 2
- 1'. Body without parascoli (Figs 9, 10, 11)..... 3
2. Parascoli long and robust (Figs 7, 15); tarsal claw with basal tooth (Fig. 24) ..... *Harmonia axyridis* (Pallas, 1773)
- 2'. Parascoli short and slender (Figs 8, 28); tarsal claw lacking basal tooth (Fig. 37).....  
.....*Hippodamia convergens* Guérin-Méneville, 1842
3. Pronotum without spots, with yellowish central longitudinal band and lateral margins (Fig. 41); tarsal claw with basal tooth (Fig. 50).....  
.....*Cycloneda sanguinea* (Linnaeus, 1763)
- 3'. Pronotum with spots, without central longitudinal band (Figs 54, 68); tarsal claw with or without basal tooth (Figs 63, 77)..... 4
4. Abdominal tergites 2 to 8 with the same spotted pattern (Fig. 54); tarsal claw lacking basal tooth (Fig. 63).....  
.....*Eriopsis connexa* (Germar, 1824)
- 4'. Abdominal tergite 4 with different spots pattern, with yellow banded spots (Fig. 68); tarsal claw with basal tooth (Fig. 77).....*Olla v-nigrum* (Mulsant, 1866)

### Key to pupae of the main predatory species of Coccinellini from South Brazil

1. First abdominal tergite with defined dark spots; second and third abdominal tergites with central and lateral spots merged (Figs 29, 30).....  
.....*Hippodamia convergens* Guérin-Méneville, 1842
- 1'. First abdominal tergite lacking dark spots; second and third abdominal tergites with only one spot or more than one spot distinctly separated (Figs 16, 42, 55, 69)..... 2
2. Second and third abdominal tergites with only one spot on each side (Figs 16, 55)..... 3
- 2'. Second and third abdominal tergites with more than one spot on each side (Figs 42, 69) ..... 4
3. Abdomen with oval spots, separated each other and not extending to central area (Figs 16, 17).....  
.....*Harmonia axyridis* (Pallas, 1773)
- 3'. Abdomen with subtriangular spots, connected on the central area (Figs 55, 56) ...*Eriopsis connexa* (Germar, 1824)
4. Fourth and fifth abdominal tergites with no more than one spot on each side (Figs 42, 43) .....  
.....*Cycloneda sanguinea* (Linnaeus, 1763)
- 4'. Fourth and fifth abdominal tergites with more than one spot on each side (Figs 69, 70) .....  
.....*Olla v-nigrum* (Mulsant, 1866)



Figures 1–11. (1–6) Schematic drawing of Coccinellidae larvae and its body structures: (1) larva, dorsal view; (2) seta; (3) chalaza; (4) scolus; (5) parascolus; (6) struma (Modified from Rees et al. 1994). (7–11) Structures of body: (7–8) Parascoli: (7) *Harmonia axyridis*; (8) *Hippodamia convergens*. (9–11) Strumae: (9) *Eriopis connexa*; (10) *Cycloneda sanguinea*; (11) *Olla v-nigrum*. Scale bars = 500  $\mu$ m.

## Descriptions

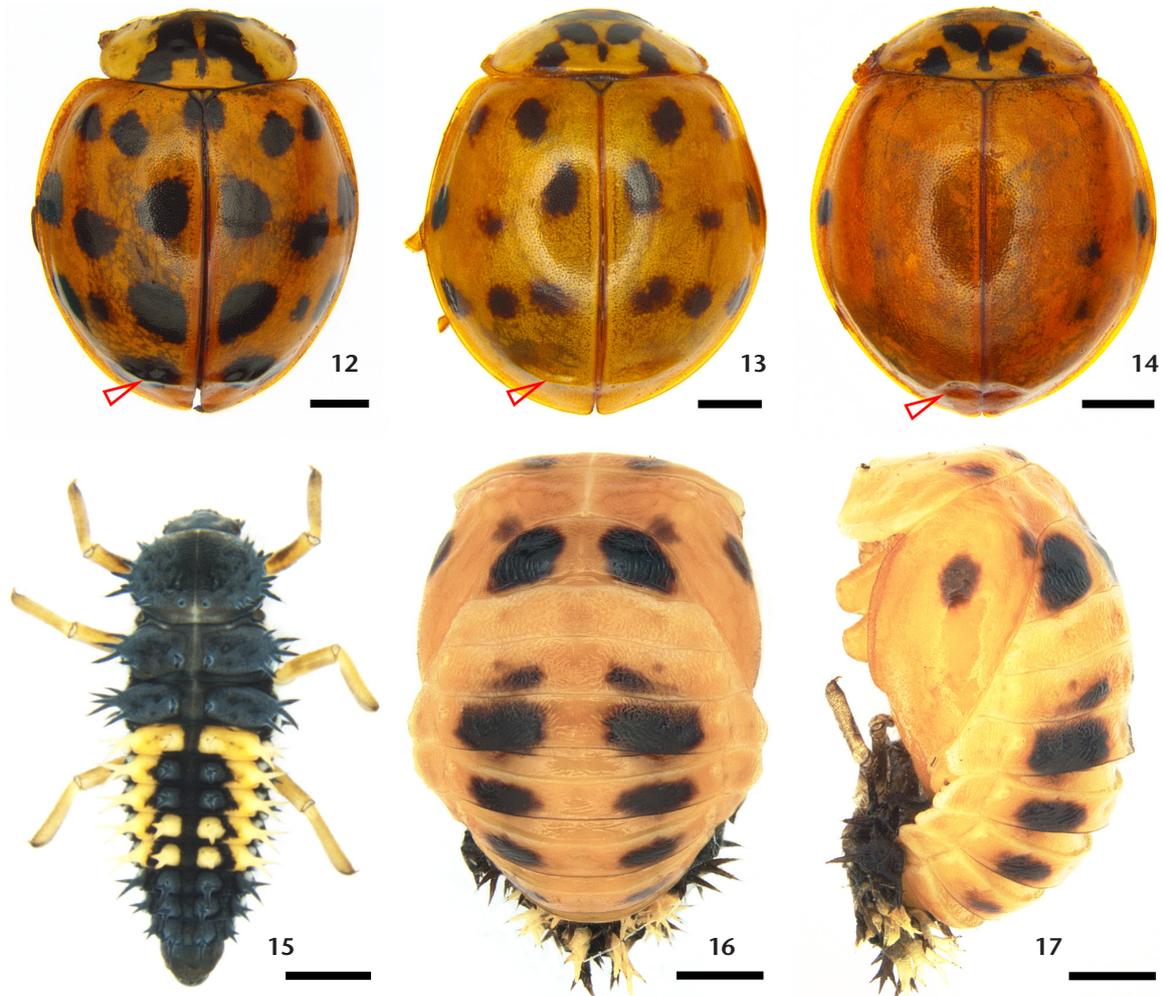
*Harmonia axyridis* (Pallas, 1773)

Figs 7, 12–26

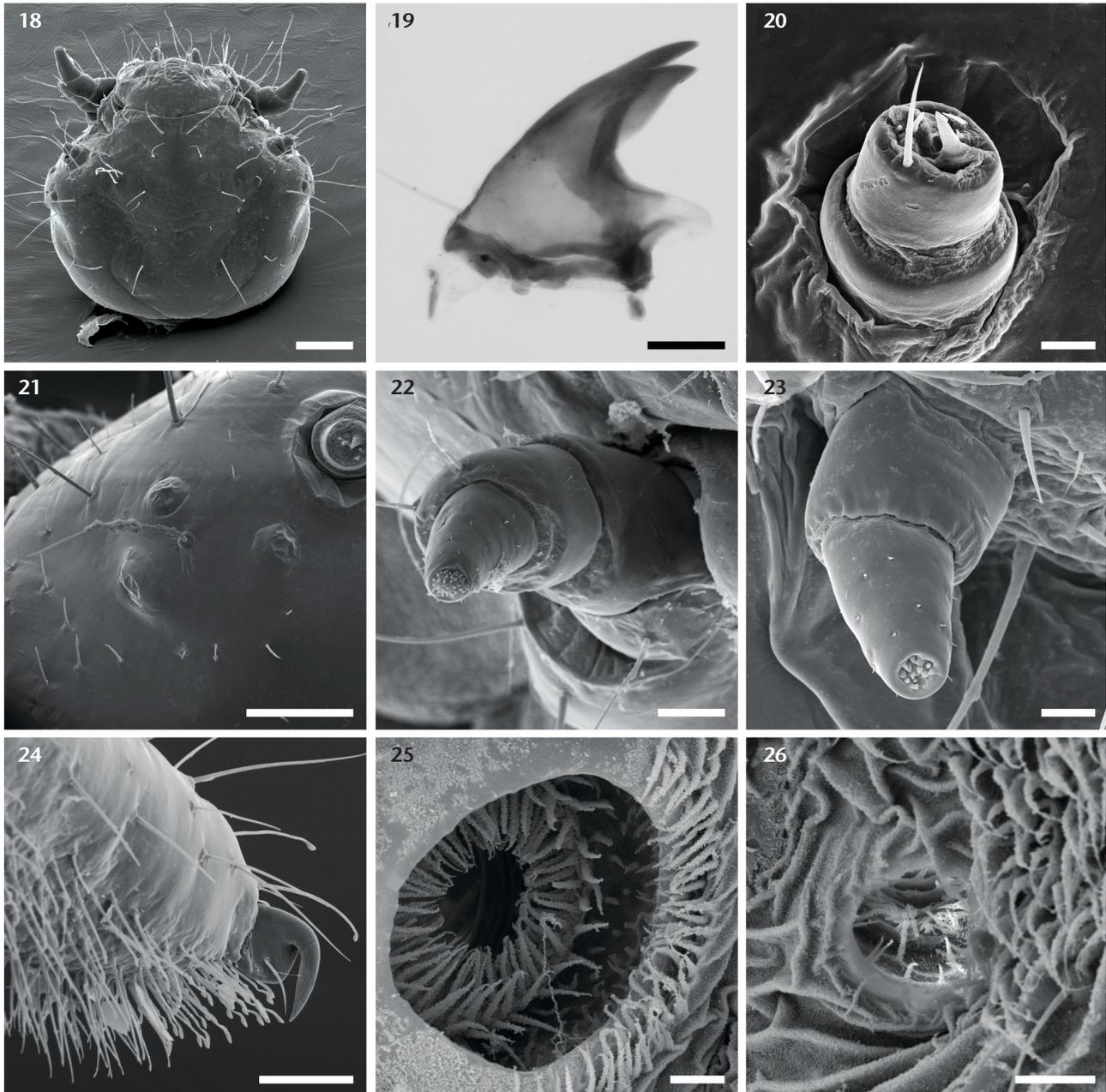
Adult (Figs 12–14). Length 6.00–7.67 mm; width 4.83–6.33 mm. Rounded, glabrous and convex body. Generally, male head yellow with black spot and female head black with yellow spot. Frons and labrum black in females and yellowish-white in males. Pronotum usually pale yellow with four black spots often joined in M-shape that may or may not be joined forming an “M” in the central region (Figs 12–14). Color of elytra ranging from yellow to red with or without black spots. Each elytron with 0 to 10 black spots, shape, and location variable. Elytral apex with transverse fold (Figs 12–14).

Fourth instar larva (Figs 15, 18–26). Length 8.00–9.67 mm; width 1.67–3.33 mm. Body elongate, cylindrical and tapered,

with parascoli, tegument of dark brown color and yellow-orange areas well demarcated from abdominal segments 1 to 5. Head: dark brown with some lighter areas, triangular with rounded edges and thin light and sparse bristles. Epicranial suture with frontal arms U-shaped (Fig. 18). Three sub-conical stemmata, light brown, dorsolateral, near the base of the antennae (Fig. 21). Antennae with 3 antennomeres; antennomere 1 wider and approximately twice as short as antennomere 2; the latter with two apical bristles and a series of papillae; antennomere 3 broad spine shaped with rounded apex (Fig. 20). Labrum with sparse bristles (Fig. 18). Well-sclerotized mandible with two apical teeth and mola with acuminate tooth, with a long bristle above the condyle (Fig. 19). Maxilla with mala (galea + lacinia) largely trapezoidal, rounded at apex, with numerous long curved bristles and outer margin with thin and short bristles. Maxillary palp with three palpomeres, sparse bristles and apex



Figures 12–17. *Harmonia axyridis*. (12–14) Adult, dorsal view, three elytral variations, arrows point to elytral apex with a terminal transverse fold. (15) Fourth larval instar; (16) pupa, dorsal view; (17) pupa, lateral view. Scale bars = 1 mm.



Figures 18–26. *Harmonia axyridis*, fourth larval instar: (18) head, dorsal view; (19) mandible; (20) antenna; (21) stemmata; (22) maxillary palpus; (23) labial palpus; (24) tarsal claw; (25) mesothoracic spiracle; (26) abdominal spiracle. Scale bars: 18 = 200  $\mu\text{m}$ ; 19, 21, 24 = 100  $\mu\text{m}$ ; 20, 23, 25, 26 = 20  $\mu\text{m}$ ; 22 = 50  $\mu\text{m}$ .

with sensillae (Fig. 22). Labium with distinct ligula, terminal palpomere with small stout sensillae at apex (Fig. 23). Thorax: pronotum with two semi-oval, sclerotized plates, separated by narrow band (Fig. 15). Lateral margin of plates with about ten long and five short chalazae; central surface and marginal areas of the plates without parascoli. Meso and metanotum plates dark

brown (Fig. 15) with semi-circular lateral margins; straight inner margin; each lateral margin of the plate with one parascolus; anterior and posterior margins without parascoli; dorsal surface of the plate with two pairs of chalazae. A pair of circular anterolateral spiracles with lamellae at the opening (Fig. 25). Legs light brown, long (Fig. 15), with sparse pale yellow hairs; tibia

with dorsal and lateral rows of bristles; ventrally with bristle brush, most of them tapered and many spatulate at the apex near the tarsal claw; tarsal claw with a subquadrate basal tooth and with a long lateral bristle on the external face of the tooth (Fig. 24). Abdomen: ten segments, S9 facing downwards. S1 to S8 with a pair of circular anterolateral spiracles similar to those of the mesothorax (Fig. 26). S1 dorsally dark brown with two dorsolateral yellow-orange spots, separated by a narrow band, each with a parascolus divided into three branches; S2 and S3 with yellow-orange spots, with half the width of the spots of S1, each with a parascolus with two branches; central area with a parascolus divided in three branches; S4 and S5 with the same spots as S2 and S3, and additional two smaller, rounded yellow-orange spots; each spot with a parascolus with three branches; S6 to S8 dark brown, each with two parascoli, with two and three brancheds, respectively (Figs 7, 15). S1 laterally with yellow-orange chalazae; S2 and S3 with 1 to 3 branched parascoli; S4 to S8 with strumae and chalazae with long and short bristles. Ventrally each segment with transverse rows of bristles. Dorsal area of S9 with approximately 100 chalazae with many short bristles; most of them in the apical third of the segment.

First instar larva. Length 1.98–2.20 mm; width 0.56–0.71 mm. Body elongate, cylindrical and tapered, with whitish integument, and brown head and thoracic plates. Head similar to fourth instar larva. Parascoli short and thick. Abdominal parascoli similar in size and color to these on the meso and metanotum.

Second instar larva. Length 3.30–3.94 mm; width 1.08–1.17 mm. Parascoli darker than those on the first instar. Head structures similar to the first instar larva; thoracic and abdominal parascoli proportionally larger.

Third instar larva. Length 5.42–6.83 mm; width 1.67–1.75 mm. Larva similar to the fourth instar. Abdomen with segments 1 to 4 yellow in the dorsolateral regions; parascoli of the same color as the integument.

Pupa (Figs 16–17). Length 5.50–5.67 mm; width 4.00–4.50 mm. Orange with black spots. Body oval, convex and truncate in the anterior region. Pronotum with a rounded spot on each side. Mesonotum usually without spots. Number and shape of elytral spots variable. Metanotum with a big oval spot on each side. Abdominal segments: S1 and S7 without spots; S2 to S4 with one spot on each side, spot on S2 smaller than these on S3; S5 and S6 usually with one spot on each side (Figs 16–17).

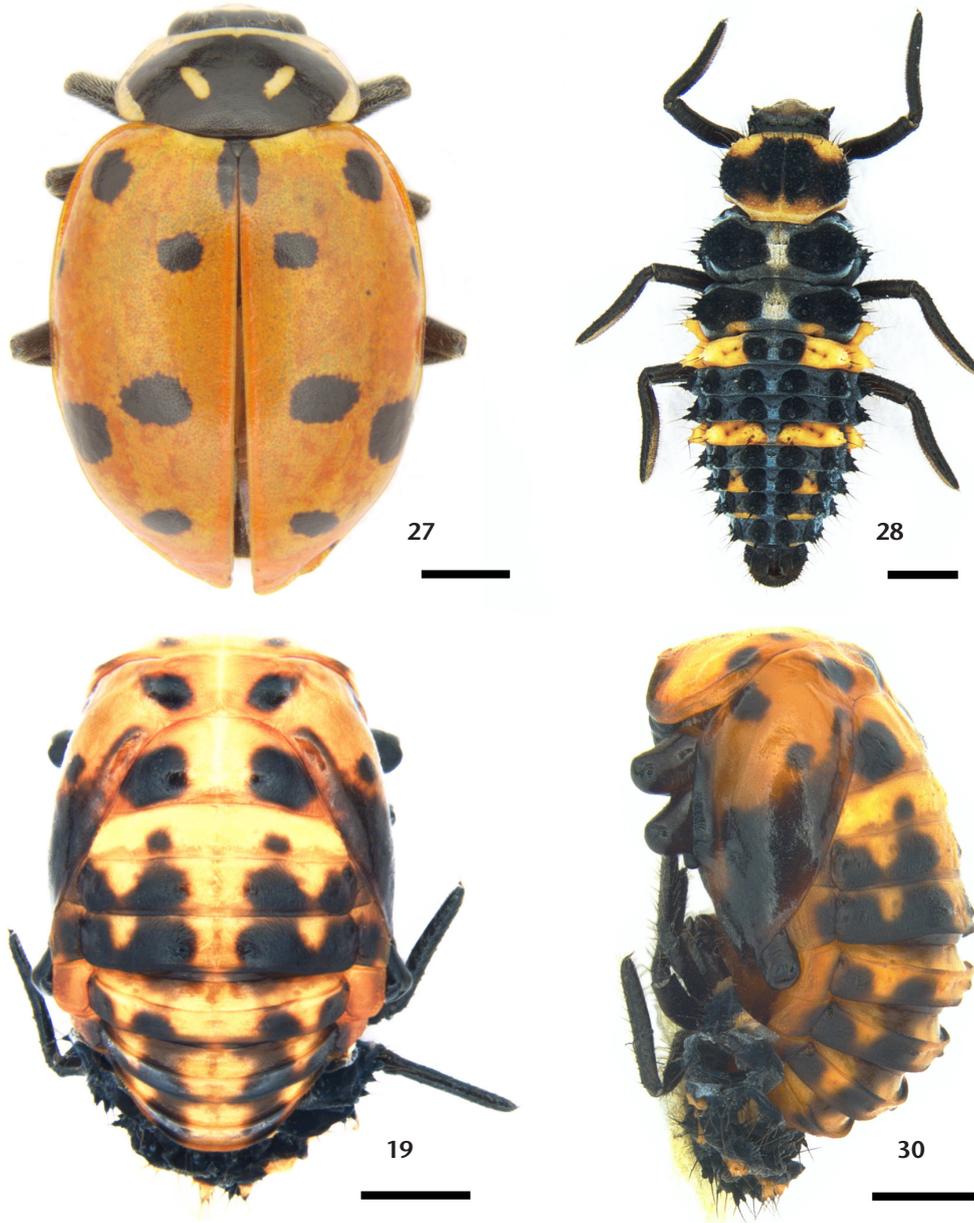
### *Hippodamia convergens* Guérin-Ménéville, 1842

Figs 8, 27–39

Adult (Fig. 27). Length 6.17–7.50 mm; width 3.83–4.67 mm. Body elongate-oval, glabrous and slightly convex. Black head with a whitish-yellow transverse spot. Antennae with subrectangular scape, twice the length of the pedicel. Pronotum black, with narrow yellowish-white border around lateral and anterior margins and two oblique yellowish-white oval

spots on disc. Elytra orange; each elytron usually with seven black spots, four smaller, in basal third of elytron and three larger, oval, in the apical third. Four smaller: one elongate, near suture at base of elytra; one rounded on humeral callus; and two rounded, parallel in anterior part of elytron, below first two spots. Three larger elongate and transverse, two near suture and one at apex.

Fourth instar larva (Figs 28, 31–39). Length 6.17–7.83 mm; width 1.67–2.50 mm. Body elongated, cylindrical and tapered, with strumae, dark brown and yellow spots well demarcated in the abdominal segments 1 and 4. Head: dark brown with some lighter areas, triangular with rounded edges and thin light and sparse bristles. Epicranial suture with frontal arms U-shaped (Fig. 31). Three sub-conical stemmata, light brown, dorsolateral, near the base of the antennae (Fig. 34). Antennae with 3 antennomeres; antennomere 1 wider and approximately twice as short as antennomere 2; the latter with two apical bristles and a series of papillae; antennomere 3 broad spine shaped with rounded apex (Fig. 33). Labrum with sparse bristles (Fig. 31). Well-sclerotized mandible with two apical teeth and mola with an acuminate tooth, with a long bristle above the condyle (Fig. 32). Maxilla with mala (galea + lacinia) largely trapezoidal, rounded at apex, with numerous long curved bristles, and on the outer margin thin and short bristles. Maxillary palp with two terminal palpomeres with sparse bristles; apex of terminal palpomere with sensillae (Fig. 35). Labium with distinct ligula, last palpomere with small sparse and stout sensillae at apex (Fig. 36). Thorax: Pronotum with two sclerotized plates, semi-oval, light brown with two dark brown spots, one on the outer margin and one on the inner margin, separated by a narrow band (Fig. 28). External margin of the plates with about 24 long-bristle chalazae; central surface of the plates without chalazae; marginal areas with shallow bristle chalazae. Meso and metanotum plates dark brown with black spots (Fig. 28) with the semi-circular outer margin; lateral margin of the plate with chalazae; dorsal chalazae with short bristles. Laterally mesonotum with parascoli with 1 to 3 branches with long bristles and two with short bristles; metanotum laterally yellow with parascoli of approximately seven long-bristle chalazae. A pair of circular anterolateral spiracles with lamellae at the opening (Fig. 38). Legs developed, dark brown (Fig. 28), with sparse yellow-white hair; with row of dorsal and lateral bristles in the tibia; ventrally with a bristle brush, most of them tapered and some spatulate at the apex near the tarsal claw; tarsal claw with a subquadrate basal tooth with a long lateral bristle on the tooth (Fig. 37). Abdomen: ten segments, S9 facing downwards. S1 to S8 with a pair of anterolateral spiracles similar to those of the mesosternum (Fig. 39). Dorsally the first segment with a dark brown area in the center and two dorsolateral yellow areas; S2 and S3 dark brown; S4 dark brown in the center and yellow in the dorsolateral areas; S5 dark brown; S6 and S7 dark brown with two small yellow areas on the posterior margin; S8 and S9 dark brown (Fig. 28). Laterally, S1 and S4 yellow and the others dark brown (Fig. 28). Prosternum,



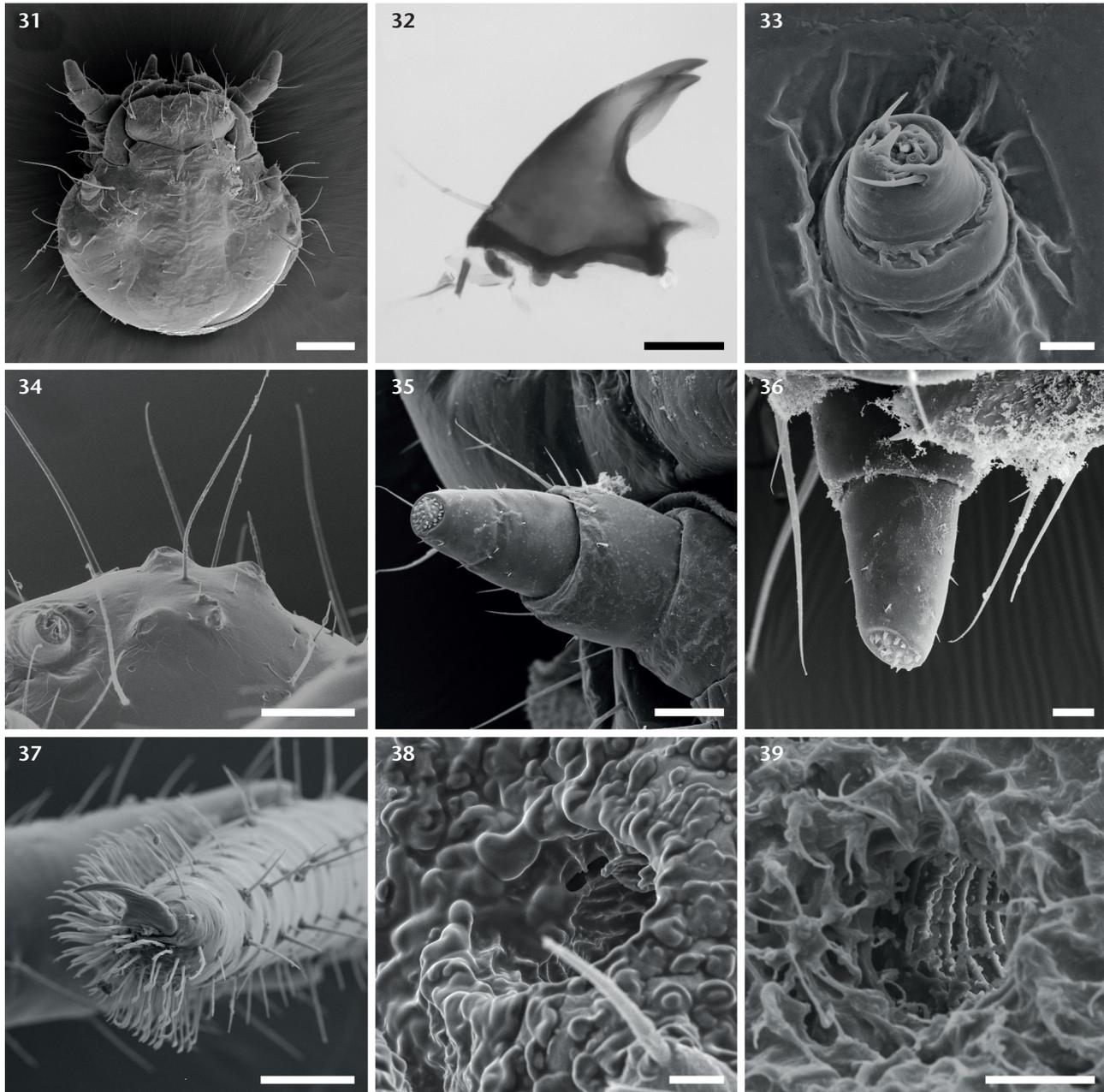
Figures 27–30. *Hippodamia convergens*: (27) adult, dorsal view; (28) fourth larval instar; (29) pupa, dorsal view; (30) pupa, lateral view. Scale bars: 1 mm.

mesosternum and metasternum with a pair of strumae; S1 to S8 with two pairs of parascoli. Dorsal and dorsolateral parascoli with 2 to 3 long bristle branches (Figs 8, 28); lateral parascoli with 3 to 6 long bristle branches and approximately ten small chalazae with short bristles on the surface. S9 with approximately 100 long-bristle chalazae; dorsally with two long-bristle chalazae; most chalazae confined to the apical third of the segment.

First instar larva. Length 1.80–2.00 mm; width 0.60–

0.72 mm. Body elongated, cylindrical and tapering, with whitish integument with the head and light brown thoracic plates. Head similar to the fourth instar larva. Prothoracic and abdominal strumae with long chalazae, with color similar to the other instars.

Second instar larva. Length 3.33–3.92 mm; width 0.08–1.17 mm. Chalazae smaller than those of the first instar. Head structures similar to those of the first instar larva.



Figures 31–39. *Hippodamia convergens*, fourth larval instar: (31) head, dorsal view; (32) mandible; (33) antenna; (34) stemmata; (35) maxillary palpus; (36) labial palpus; (37) tarsal claw; (38) mesothoracic spiracle; (39) abdominal spiracle. Scale bars: 31 = 200  $\mu$ m; 32, 34, 37 = 100  $\mu$ m; 33, 36, 38, 39 = 20  $\mu$ m; 35 = 50  $\mu$ m.

Third instar larva. Length 4.75–5.83 mm; width 1.50–1.67 mm. Strongly similar to fourth instar.

Pupa (Figs 29–30). Length 4.83–5.33 mm; width 3.17–3.50 mm. Yellowish color with black spots. Body with oval, convex contour, and truncated anterior region. Pronotum with two small, rounded spots on each side next to mesonotum and an elongated

spot covering the lateral border. Sometimes the elongated spot merged with the first rounded one. Mesonotum and metanotum with a rounded spot on each side. Apical half of elytra black and basal half yellowish with variable spots pattern. Abdominal segments: S1 with a rounded spot on each side; S2 to S3 with two merged spots on each side; S4 to S7 with two spots, sometimes merged (Figs 29–30).

*Cycloneda sanguinea* (Linnaeus, 1763)

Figs 10, 40–52

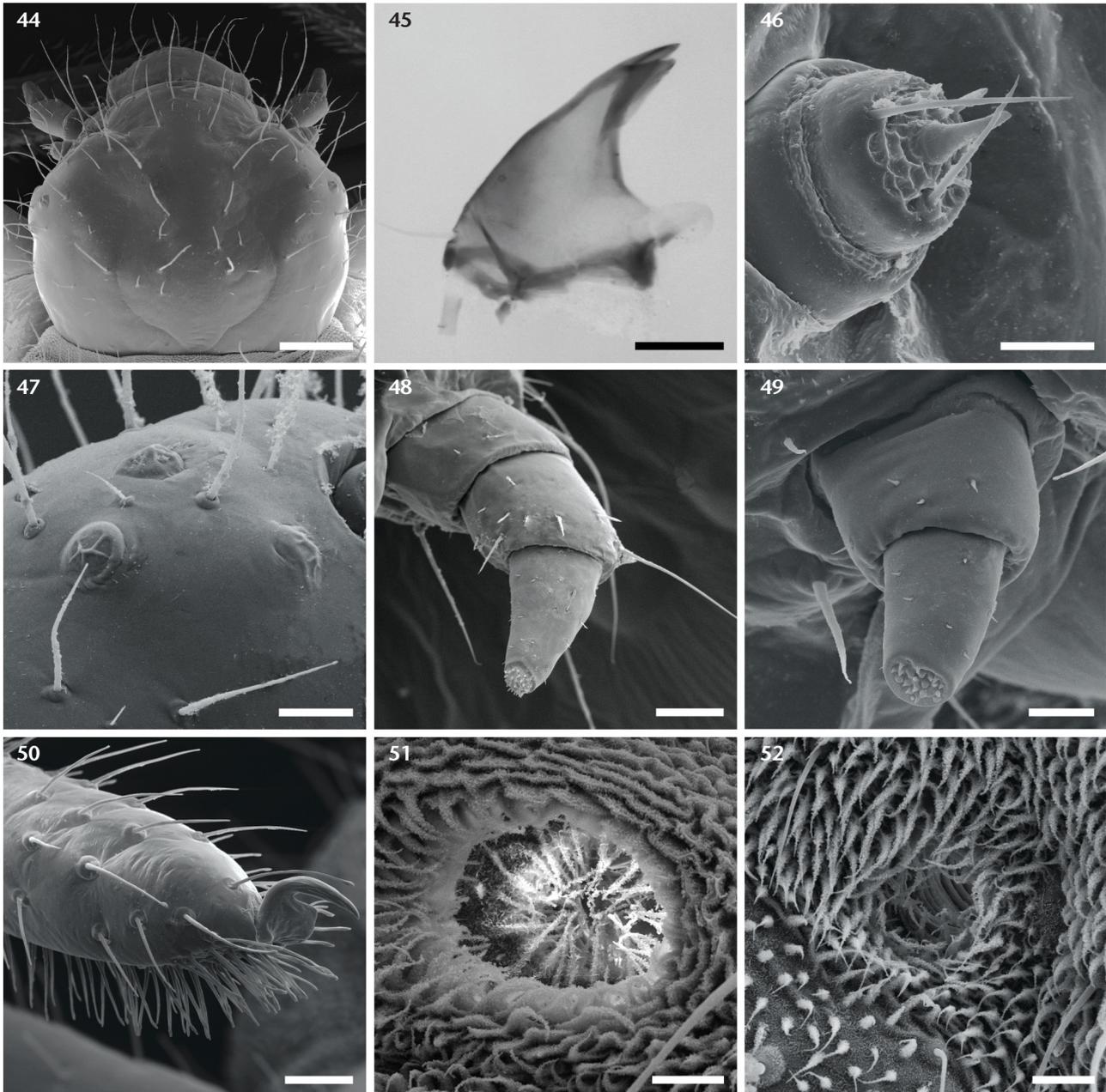
Adult (Fig. 40). Length 4.83–6.50 mm; width 4.00–5.67 mm. Body rounded, glabrous and convex. Elytra reddish without spots. Head dark brown to black; males with horizontal large white spot between the eyes; females with the same spot divided into two with a dark brown central line. Pronotum in males black with a narrow yellowish white stripe skirting the lateral and anterior margins, projecting in the middle toward

the base; two pale yellow rounded spots on each side; in females the same narrow yellowish white stripe skirting the lateral and anterior margins, but without projection in the middle and the two pale yellow rounded spots attached to border.

Fourth instar larva (Figs 41, 44–52). Length 6.50–7.50 mm; width 1.50–2.50 mm. Body elongated, cylindrical and tapered, with strumae, tegument of dark brown color and yellow spots well demarcated in the mesonotum, metanotum, and abdominal segments 1 and 4. Head: Dark brown with lighter areas, quadrangular with rounded edges, thin light, and sparse bristles.



Figures 40–43. *Cycloneda sanguinea*: (40) adult, dorsal view; (41) fourth larval instar; (42) pupa, dorsal view; (43) pupa, lateral view. Scale bars: 1 mm.



Figures 44–52. *Cycloneda sanguinea*, fourth larval instar: (44) head, dorsal view; (45) mandible; (46) antenna; (47) stemmata; (48) maxillary palpus; (49) labial palpus; (50) tarsal claw; (51) mesothoracic spiracle; (52) abdominal spiracle. Scale bars: 44 = 200  $\mu$ m; 45 = 100  $\mu$ m; 46, 49, 51, 52 = 20  $\mu$ m; 47, 48, 50 = 50  $\mu$ m.

Epicranial suture with frontal arms U-shaped (Fig. 44). Three sub-conical stemmata, light brown, dorsolateral, close to the base of the antennae (Fig. 47). Antennae with 3 antennomeres; antennomere 1 wider and approximately twice as short as antennomere 2; the latter with two apical bristles and a series of papillae; antennomere 3 broad, spine shaped with rounded

apex (Fig. 46). Labrum with sparse bristles (Fig. 44). Sclerotized mandible with two apical teeth and mola with acuminate tooth-shaped; with a long bristle above the condyle (Fig. 45). Maxilla with mala (galea + lacinia) largely trapezoidal, rounded at apex, with some long-curved bristles on the outer margin. Maxillary palp with three palpomeres, the last two with sparse

bristles; apex of the last palpomere with sensillae (Fig. 48). Labium with distinct ligule, the last palpomere with small sparse and stout sensillae at the apex (Fig. 49). Thorax: pronotum with two sclerotized plates, semi-oval, dark brown, separated by a narrow band (Fig. 41). External margin of the plates with approximately 25–30 long-bristle chalazae; central surface of the plates almost without chalazae, and approximately 20 chalazae of very short bristles in the marginal areas. Dark brown meso and metanotum plates with oval or triangular central area light yellow (Fig. 41). Small mesonotum plate with semi-circular outer margin; truncated inner margin; lateral margin of the plate with approximately 20 long chalazae and short bristles; anterior margin without chalazae; posterior margin with six long chalazae and short bristles; dorsal struma with two long chalazae, five of them short; dorsal surface of the plate with approximately 15 short-bristle chalazae. Mesothorax with two chalazae with long bristles in the lateral struma and 10 to 13 chalazae with short bristles. A pair of circular anterolateral spiracles with lamellae at the opening (Fig. 51). Metanotum with transverse plate, semi-circular outer margin; rounded inner margin; lateral margin of the plate with approximately 16 chalazae with long and short setae; anterior margin without chalazae; posterior margin with a long-bristle chalaza, four short-bristled chalazae; dorsal struma with three long-bristle chalazae and two with short-bristles; plate surface with approximately ten chalazae with very short bristles; lateral strumae of the metathorax with two long-bristle chalazae and approximately ten with short-bristles. Developed legs dark brown (Fig. 41), with sparse whitish yellow bristles; with row of dorsal and lateral bristles on the tibia; ventrally with a bristle brush, most of them tapered and some spatulate at apex near the tarsal claw; tarsal claw with subquadrate basal tooth with a long lateral bristle on the external face of the tooth (Fig. 41). Abdomen: ten segments, S9 facing downwards. S1 to S8 with a pair of anterolateral spiracles similar to those of the mesosternum (Fig. 52). Dorsally, S1 with a dark brown area in the center, separated by a light-yellow band and with two light yellow areas dorsolateral; S2 and S3 dark brown with a small light yellow center area; S4 light yellow with four thin dark brown longitudinal lines; S5 with yellow pleura and from S6 to S7 dark brown with a small light yellow area in the center; S8 dark brown. Laterally each segment with a pair of strumae; S1, S4 and S5 light yellow and in the others dark brown (Fig. 41). Ventrally each segment with transverse rows of bristles; S4 to S8 with longer bristles, longer than the previous segments. Dorsal struma of the abdomen (Figs 10, 41) with a large median chalaza, two large chalazae in the posterior half, all with long bristles; 10 to 12 small chalazae with short setae distributed on the surface; dorsal-lateral struma with two large median chalazae with long bristles, one chalaza slightly smaller than the median ones with bristle close to the lateral margin; 6 to 8 small chalazae with short bristles distributed over the entire surface; lateral struma with two large long-bristle chalazae, one median, one posteromedian, four slightly smaller chalazae with short bristles, 6 to 10 small

chalazae with short bristles on the entire surface. Dorsum of S9 with approximately 100 chalazae with short bristles; most of them confined to the apical third of the segment.

First instar larva. Length 1.52–2.60 mm; width 0.52–0.72 mm. Elongated, cylindrical, and tapering body, with whitish integument; head and thoracic plates light brown. Head like the fourth instar larva. Prothoracic and abdominal strumae with long chalazae, with color similar to the other instars.

Second instar larva. Length 2.50–3.50 mm; width 0.67–1.08 mm. Chalazae smaller than those of the first instar. The head structures similar to those of the first instar larva.

Third instar larva. Length 3.58–4.92 mm; width 1.17–1.42 mm. Chalazae with size similar to those of the second instar and color similar to that of the fourth instar.

Pupa (Figs 42–43). Length 4.17–4.67 mm; width 2.33–3.83 mm. Yellowish with black spots. Body with oval, convex contour, and truncated anterior region. Pronotum with at least four spots on each side, two on base and two on apex; mesonotum and metanotum with a rounded spot on each side; elytra without defined spots. Abdominal segments: S1, S6 and S7 without defined spots; S2 with two spots on each side; S3 with three spots on each side; S4 and S5 sometimes with one spot on each side (Figs 42–43).

### *Eriopsis connexa* (Germar, 1824)

Figs 9, 53–65

Adult (Fig. 53) Length 5.67–6.67 mm; width 3.00–3.50 mm. Body oval-elongated, glabrous, and slightly convex. Dark brown to black head. Antennae with regular and elongated scape, twice the length of the pedicel. Pronotum hexagonal, black, with a yellowish-white or orange stripe on the lateral margins, a small triangular spot on the anterior margin and a twice larger spot at base. Elytra black with seven yellowish-white or orange rounded spots: four spots parallel to the suture, the first juxtaposed to the base and the last at apex of elytra; the second and third rounded spots, close to the elytral suture; the fifth and sixth spots juxtaposed to the elytral outer margin, joined to a narrow marginal band of the same color as the spots.

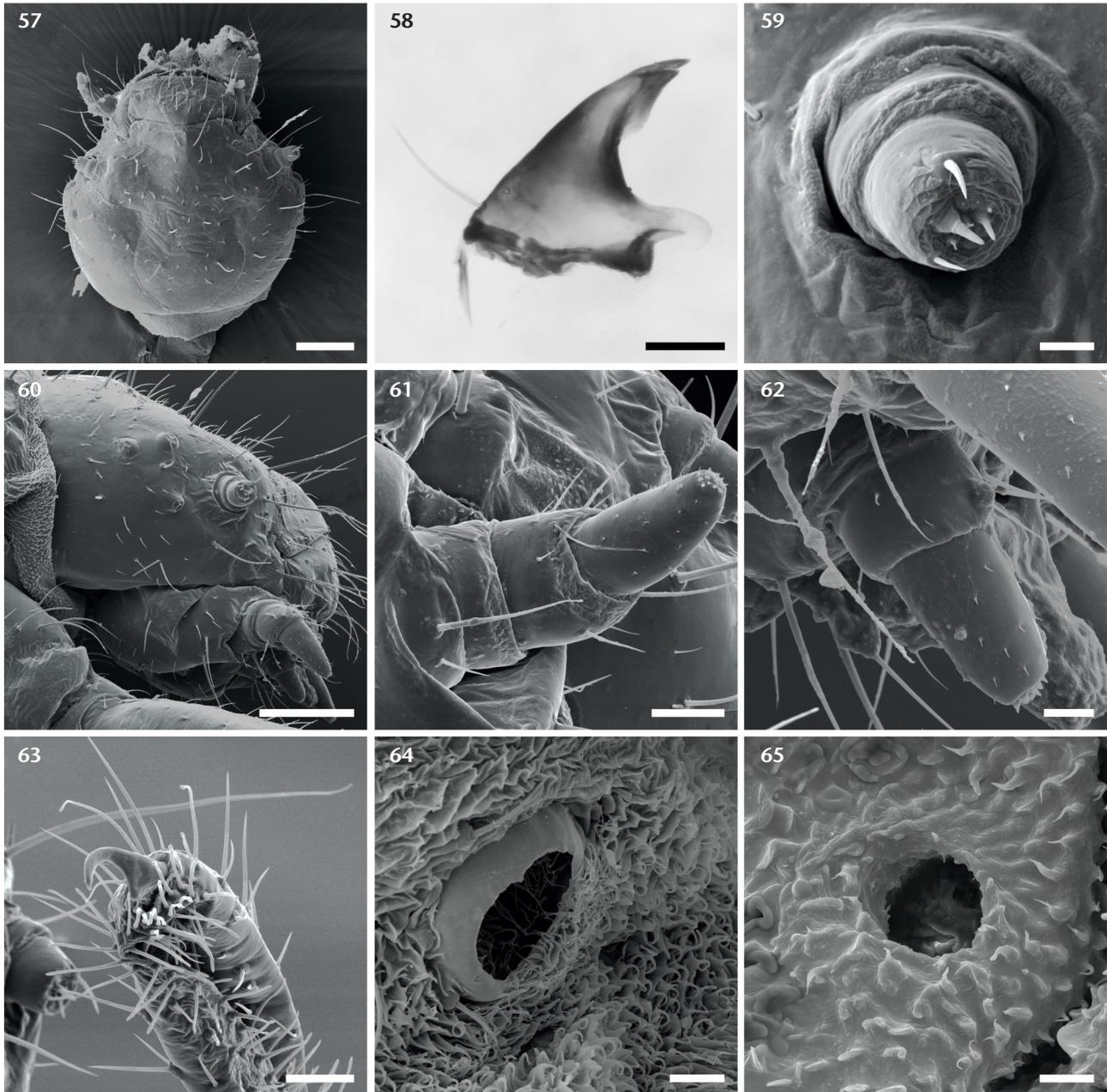
Fourth instar larva (Figs 54, 57–65). Length 6.67–7.17 mm; width 1.67–2.00 mm. Body elongated, cylindrical and tapered, with strumae, dark brown tegument and well-demarcated whitish yellow areas of mesonotum, metanotum, and abdominal segments 1 to 8. Head: dark brown with some light areas, triangular with rounded edges and thin light and sparse bristles. Epicranial suture with frontal arms U-shaped (Fig. 57). Three pairs of subconical stemmata, dark brown, dorsolateral, near the base of the antennae (Fig. 60). Antennae with 3 antennomeres; antennomere 1 wider and approximately twice as short as antennomere 2; this one with two apical bristles and a series of papillae; antennomere 3 broad spine shaped with rounded apex (Fig. 59). Labrum with sparse bristles (Fig. 57). Well-sclerotized mandible with two apical teeth and mola with acuminate tooth,



Figures 53–56. *Eriopis connexa*: (53) adult, dorsal view; (54) fourth larval instar; (55) pupa, dorsal view; (56) pupa, lateral view. Scale bars: 1 mm.

with a long bristle above the condyle (Fig. 58). Maxilla with mala (galea + lacinia) largely trapezoidal, rounded at apex, with some long-curved bristles on the outer margin. Maxillary palp with three palpomeres; the last two with sparse bristles; the apex of the last palpomere with sensillae (Fig. 61). Labium, with distinct ligule, the last palpomere with small sparse and stout sensillae at apex (Fig. 62). Thorax: pronotum with two sclerotized, semi-oval, light brown plates with two longitudinal dark brown spots, the internal ones longer than the external ones, separated by a narrow band (Fig. 54). External edge of the plates with about

28 long-bristle chalazae; central surface of the plates without chalazae and with few sparse short bristles in the marginal areas. Dark brown meso- and metanotum plates with oval central area light yellow (Fig. 54). Mesonotum plate with truncated anterior margin without chalazae, semi-circular outer margin with four chalazae each one with long and short bristles; truncated inner margin; posterior margin with a chalaza with short bristle; dorsal struma with two long chalazae; dorsal surface of the plate with approximately six chalazae with long bristles. Mesothorax with four chalazae with short bristles on the lateral yellowish struma. A



Figures 57–65. *Eriopis connexa*, fourth larval instar: (57) head, dorsal view; (58) mandible; (59) antenna; (60) stemmata; (61) maxillary palpus; (62) labial palpus; (63) tarsal claw; (64) mesothoracic spiracle; (65) abdominal spiracle. Scale bars: 57, 60 = 200  $\mu$ m; 58 = 100  $\mu$ m; 59, 62, 64, 65 = 20  $\mu$ m; 61, 63 = 50  $\mu$ m.

pair of circular anterolateral spiracles with lamellae at the opening (Fig. 64). Metanotum with transverse plate, semi-circular outer margin; truncated inner margin; lateral margin of the plate with approximately four chalazae with long setae; anterior margin without chalazae; posterior margin with one chalaza of short bristle; dorsal struma with four long-bristled chalazae; dorsal

surface of the plate with approximately 14 chalazae with long bristles; lateral struma of the metathorax yellowish with four chalazae of short bristles. Legs developed dark brown (Fig. 54), with sparse whitish yellow hair; tibia with dorsal and lateral rows of bristles; ventrally with a bristle brush, most tapered and some spatulate at the apex near the tarsal claw; simple tarsal claw, with-

out basal tooth with bristle on the outer face (Fig. 63). Abdomen: with ten segments, S9 facing downwards. S1 to S8 with a pair of anterolateral spiracles similar to those of the mesosternum (Fig. 65). S1 yellowish with a yellowish spot surrounded by dark brown. Dorsally from S2 to S8 dark brown; S2 with a white oval spot in the center, and four black dorsal-lateral strumae; S3 to S8 dark brown with a trapezoidal central spot; S9 dark brown with four black strumae (Fig. 54). Laterally, each segment with a pair of strumae; S1, S4, S5 and S6 with whitish yellow strumae and in the other dark brown (Fig. 54). Ventrally each segment with four strumae with 4 to 5 chalazae, arranged transversely. Dorsal strumae of the abdomen with two chalazae with long bristle; 5 to 7 small, short bristles distributed on the surface; dorsal-lateral strumae with two chalazae with long bristle and one with short bristles; lateral strumae with three large chalazae with long bristle; about 20 small chalazae with short bristles on all the surface (Fig. 54). Tergite of S9 with approximately 100 chalazae with many long bristles; dorsally with four chalazae with long bristles; most of them confined to the apical third of the segment.

First instar larva. Length 1.44–2.32 mm; width 0.44–0.56 mm. Elongated, cylindrical, and tapering body, with whitish yellow integument, with brown head and thoracic plates brown. Head, stemmata, antennae, and mouthparts similar to fourth instar larva. Pro-thoracic strumae longer than those of the fourth instar. Abdominal strumae similar in size and color to those of the meso and metanotum.

Second instar larva. Length 4.00–4.50 mm; width 0.08–1.00 mm. Thoracic and abdominal strumae shorter than those of the first instar; head structures similar to those of the first instar.

Third instar larva. Length 4.17–5.25 mm; width 0.92–1.25 mm. Larvae with color similar to the fourth instar.

Pupa (Figs 55–56). Length 4.83–5.17 mm; width 2.83–4.50 mm. Dark brown with black spots, distributed throughout the body. Body with elongated contour, convex and truncated at anterior region. Pronotum with two spots on each side, one on base and one on apex; Mesonotum and metanotum with a big oval spot on each side; Elytra with two elongated spots, one on the full inner edge and the other on the full outer edge. Abdominal segments: S1 and S7 without defined spots; S2 to S6 with one subtriangular spot on each side, extending to central area (Figs 55–56).

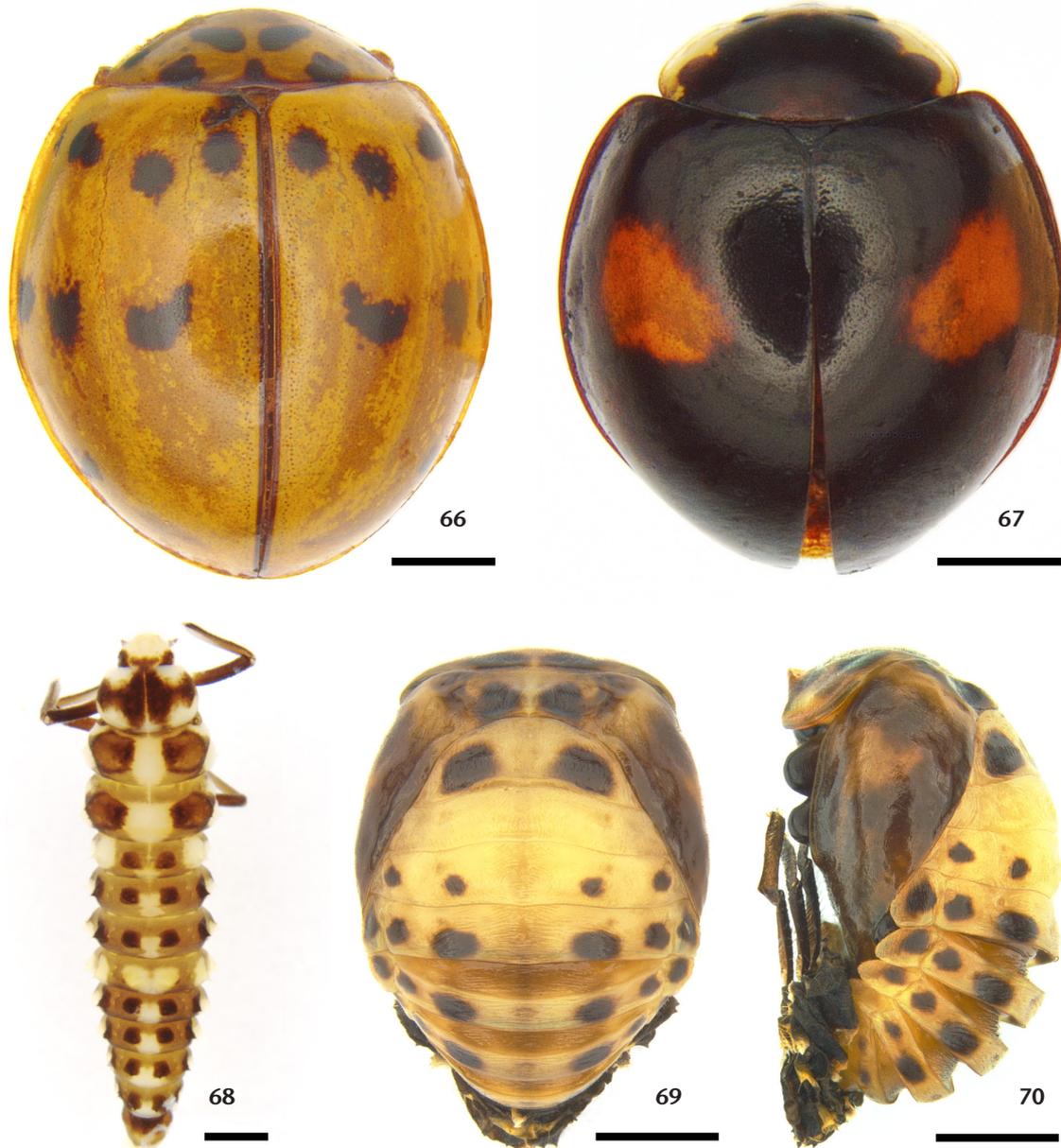
### *Olla v-nigrum* (Mulsant, 1866)

Figs 11, 66–79

Adult (Figs 66–67). Length 3.91–6.25 mm; width 3.25–5.16 mm. Body rounded, glabrous and convex. Color pattern with very distinct melanic and non-melanic forms. Melanic form with black dorsal color, pronotum with a narrow yellowish-white band on the apical margin, wider at the lateral apices. Elytra with an orange quadrate spot in the anterior third (Fig. 67). Non-melanic form with dorsal whitish yellow, pronotum with seven black spots, four of them interconnected forming an “M”, separated and of

different sizes. Elytron generally with seven spots: four rounded parallel in a transversal line at the elytral base, near the humeral callus; three parallel, in a transversal line, in the elytral half, the next in the suture, half-moon shaped, and the others rounded; the last spot in 3/4 of the elytron, closer to the lateral margin (Fig. 66).

Fourth instar Larva (Figs 68, 71–79). Length 6.17–7.83 mm; width 1.50–2.00 mm. Body elongated, cylindrical and tapered, with strumae, tegument dark brown with yellow spots well demarcated from the mesonotum, metanotum, and abdominal segments 1 to 8. Head: light yellow with some darker areas, quadrangular with rounded edges and thin light and sparse bristles. Epicranial suture with frontal arms U-shaped. Three sub-conical stemmata, light brown dorsolateral, near the base of the antennae (Fig. 74). Antennae with 3 antennomeres; antennomere 1 wider and approximately twice as short as antennomere 2; the latter with two apical bristles and a series of papillae; antennomere 3 broad spine shaped with rounded apex (Fig. 73). Labrum with sparse bristles (Fig. 71). Mandible well sclerotized with two apical teeth and mola with an acuminate tooth, with a long bristle above the condyle (Fig. 75). Maxilla with mala (galea + lacinia) largely trapezoidal, rounded at apex, with numerous long curved bristles, and outer margin with thin and short bristles. The last two maxillary palpomeres with sparse bristles; apex of the last palpomere with sensillae (Fig. 76). Labium with separate ligula, last palpomere with small, sparse and sensilla bristles at apex (Fig. 76). Thorax: pronotum with two sclerotized, semi-oval, yellow-white plates, with two dark brown longitudinal spots extending to the lateral margins, the inner ones longer than the outer ones, separated by a narrow band (Fig. 68). External edge of the plates with about 12 long-bristle chalazae; central surface of the plates without chalazae, and very short bristle chalazae in the marginal areas. Meso and metanotum plates dark brown with oval central area light yellow (Fig. 68). Lateral margin of the plate with yellow spots and about four short-bristled chalazae; anterior and posterior margin without chalazae; dorsally with 2 to 3 chalazae of very short bristles; dorsal surface of the plate with approximately six short-bristle chalazae; laterally with very short bristle chalazae. A pair of circular anterolateral spiracles with lamellae at the opening (Fig. 78). Legs developed, dark brown (Fig. 68) with yellow-white sparse setae; with row of dorsal and lateral tibial bristles; ventrally with a bristle brush, most of them tapered and some spatulate at apex near tarsal claw; tarsal claw with a subquadrate basal tooth, long lateral bristle on the external face of the tooth (Fig. 77). Abdomen: ten segments, S9 facing downwards. S1 to S8 with a pair of circular anterolateral spiracles similar to those of the mesosternum (Fig. 79). S1 dorsal light brown with yellow circular areas strumae on the sides; and smaller central dark brown areas. From S1 to S8 with yellow spots on the central region; S2 and S3 with two pairs of dark brown strumae; S4 brown with two pairs of rounded strumae on yellow spots; S5 to S8 light brown and two pairs of rounded strumae on dark brown spots, one pair next to central yellow spot and second lateral segment; S9 dark brown (Fig. 68). Laterally each segment

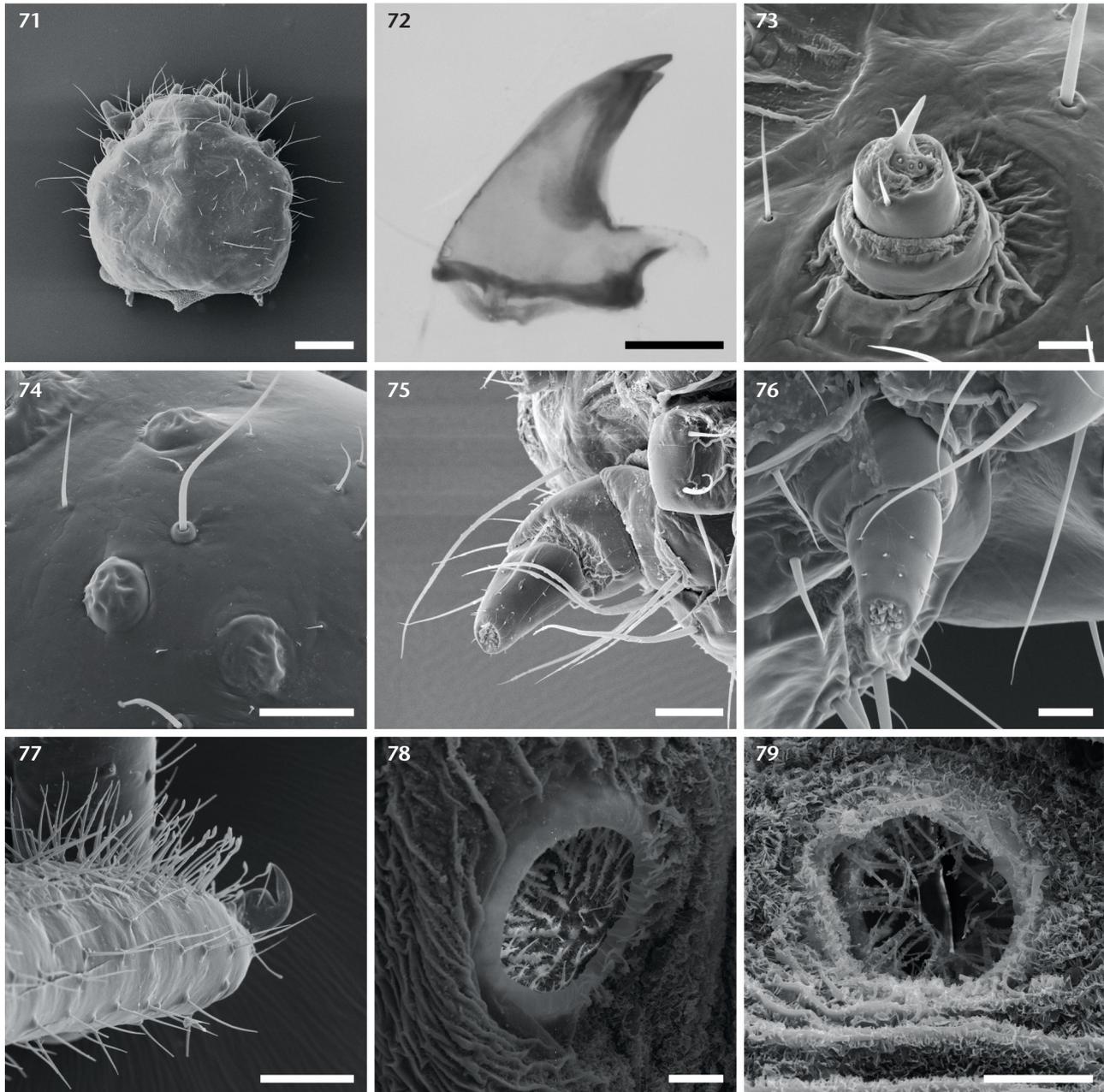


Figures 66–70. *Olla v-nigrum*: (66) adult, non-melanic form, dorsal view; (67) adult, melanic form, dorsal view; (68) fourth larval instar; (69) pupa, dorsal view; (70) pupa, lateral view. Scale bars: 1 mm.

with a pair of whitish yellow strumae (Fig. 68). Ventrally each segment with transversal rows of bristles. Abdominal dorsal strumae with two large long-bristled chalazae; 6 to 10 small chalazae with short bristles; strumae dorsal-lateral with the same pattern of chalazae and bristles; lateral strumae with approximately 20 chalazae with short-bristles distributed over the entire surface. Tergum of S9 with approximately 100 chalazae with short bristles; most of them on the side edges of the segment.

First instar larva. Length 1.20–2.00 mm; width 0.48–0.64 mm. Body elongated, cylindrical and tapered, with whitish yellow integument. Head, thoracic plates and legs brown. Head similar to fourth instar larva, with epicranial suture, stemmata, antennae and mouthparts similar to the fourth instar. Prothoracic and abdominal strumae with short-bristled chalazae.

Second instar larva. Length 3.33–4.58 mm; width 0.83–1.17 mm. Head and strumae similar to the first instar.



Figures 71–79. *Olla v-nigrum*, fourth larval instar: (71) head, dorsal view; (72) mandible; (73) antenna; (74) stemmata; (75) maxillary palpus; (76) labial palpus; (77) tarsal claw; (78) mesothoracic spiracle; (79) abdominal spiracle. Scale bars: 71 = 200  $\mu\text{m}$ ; 72, 77 = 100  $\mu\text{m}$ ; 73, 76, 78, 79 = 20  $\mu\text{m}$ ; 74, 75 = 50  $\mu\text{m}$ .

Third instar larva. Length 4.00–5.42 mm; width 1.00–1.42 mm. Larva similar to the fourth instar in structure and color; pronotum with small-defined spots.

Pupa (Figs 69–70). Length 4.00–4.83 mm; width 2.83–3.83 mm. Dark brown color on the lateral margins. Oval, convex and truncated anterior region. Pronotum with

at least four spots on each side, two on base and two on apex. Mesonotum and metanotum with a big spot on each side. Elytra spot pattern variable. Abdominal segments: S1 without defined spots; S2, S6 and S7 with two rounded spots on each side; S3 to S5 with three spots on each side (Figs 69–70).

## DISCUSSION

Despite the acknowledged importance of Coccinellidae larvae and adults in biological control programs, the focus of the main studies still is on the adults, which creates a gap in the knowledge about immature stages of the ladybird beetles.

According to LeSage (1991), most coccinellids have four immature stages, and in the first instar larvae, scoli are shorter having a small number of branches and longer setae, and this was confirmed in the present study.

Rees et al. (1994) presented an identification key for larvae of 46 North American Coccinellidae genera including characters like dorsal plates of the body and their structures, such as bristles, scoli, parascoli, strumae and chalazae. Many taxa were included in the key only at generic level. From the five species treated in the present study, only *O. v-nigrum* was included in that key.

The morphological characteristics of immatures differ according to their performance as a predator. The presence of parascoli is one of the main differences observed in the studied species. *Harmonia axyridis* is the only species that has more sclerotized, long, and robust parascoli, in addition to the larger body size, which may indicate greater aggressiveness comparing to native species (Guedes 2013). However, larvae of some phytophagous species, e.g., *Epilachna spreta* Mulsant, 1850 (Ribeiro and Almeida 1989) also have these structures well-developed (Kapur 1950, LeSage 1991).

*Hippodamia convergens* also has parascoli, however smaller and slender. Such structures act in defense and therefore are considered important in species more efficient as agents in biological control. It is the case of these two species, *Hippodamia convergens* and *Harmonia axyridis*, which were introduced to control aphids in different regions of the world (Eigenbrode et al. 1998, Provost et al. 2006). Among the other species, *Eriopis connexa* and *Cycloneda sanguinea* are the most aggressive and have strumae with longer bristles, different from *Olla v-nigrum*, which is less aggressive, and the bristles are shorter (C.F. Castro pers. obs.).

Based on the mouthparts' morphology of the species, it is possible to evidence the type of preferred food, both for larvae and adults, as their mandibles are generally morphologically similar, robust, with acute apical teeth and with developed mola (Hodek 1973, Almeida and Ribeiro-Costa 2012, Hodek et al. 2012). According to Escalona et al. (2017), the evolution of food preferences in ancestral Coccinellini accompanied strong changes in larvae dorsal ornamentation.

The Coccinellini species present characters comparable to *Eupalea reinhardti* Crotch, 1874 (Coccidulini) by the type of parascoli on the abdomen and the distribution of the tergal plates. The spiracles of the studied larvae are annular like in all Coccinellidae however they differ from those of *Eupalea reinhardti* by having chalazae (Ferreira and Almeida 2000).

*Eriopis connexa* and *Hippodamia convergens* do not have a basal tooth in the tarsal claw, which differentiates these species from the others, which have a tarsal claw with a subquadrate basal tooth.

Some larval characters like the type of tarsal claws, spiracles, chalazae, parascoli and strumae were studied for the first time thanks to the use of the electron microscope. This enriches our knowledge about morphology of Coccinellini immature stages.

The identification key provided here, being an addition to the existing biology articles, may help in future biological control programs, as complementing the knowledge of species of common predators in the southern region of Brazil.

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