



Systematics, Morphology and Biogeography

Immatures of *Epilachna* Chevrolat (Coleoptera, Coccinellidae, Epilachninae)

Sonia A. Casari^{a,*}, Edson P. Teixeira^b^a Museu de Zoologia, Universidade de São Paulo (MZUSP), São Paulo, SP, Brazil^b Instituto Agronômico de Campinas (IAC), Campinas, SP, Brazil

ARTICLE INFO

Article history:

Received 6 October 2014

Accepted 13 March 2015

Available online 11 April 2015

Associate editor: Lúcia M. Almeida

Keywords:

Eggs

Neotropical region

Pupae illustrations

ABSTRACT

Description of the pupa and redescription of the larva of *Epilachna vigintioctopunctata* (Fabricius), collected for the first time on *Brugmansia suaveoleus* (Humb. and Bonpl. ex Willd.) Bercht. and J. Presl (Solanaceae) (trombeteiro), in the state of São Paulo (Brazil), is presented. The diagnoses of the described pupae of *E. clandestina* (Mulsant), *E. paenulata* (Germar) and *E. spreta* (Mulsant), based on specimens examined, and that of *E. cacica* Guérin, based on the literature, are presented. A comparison among the known larvae and pupae of this genus is also presented. This is the first description of immatures of *E. vigintioctopunctata* from the Western Hemisphere.

© 2015 Sociedade Brasileira de Entomologia. Published by Elsevier Editora Ltda. All rights reserved.

Introduction

The treatment of coccinellid larvae by LeSage (1991) states that they are extremely diverse and cannot be completely distinguished by only one or two characters, as is the case in many beetle families. On the other hand, larvae of each tribe show a distinctive general habitus and have morphological features that are shared by all members of the tribe. He stated that many galerucine and some alticine larvae (Chrysomelidae) superficially resemble larvae of coccinellids. However, chrysomelid larvae have short legs, no stemmata or only one pair, no setose process on the body (rarely with processes covered by fine setae), and mandibles without mola; whereas coccinellid larvae that could be mistaken for chrysomelids, have long legs, three pairs of stemmata, large setose body processes covered by robust setae, and a mandibular mola. According to LeSage, coccinellid larvae usually have a reduced mola on the mandible (absent in Epilachninae), a gular area between the labium and thorax, and the median epicranial stem (coronal suture) absent in most genera. He considers that the most distinctive coccinellid larval characteristics are: great development of body armature into the setose process in most tribes; a campodeiform and usually brightly colored body; and mandibles of a predaceous type in most of the tribe (acute at apex). Except in Epilachnini and Psylloborini, they are usually very active predators.

Epilachninae larvae were treated by Kapur (1950), who presented an extensive study on its biology and external morphology. He provided a general description of the larva of this subfamily, keys to the genera of Epilachninae and to species of *Epilachna* Chevrolat, and discussed the relationship to other coccinellids. For *Epilachna*, he described or redescribed in detail at least the fourth-instar larva, and included biological notes on the following species: *E. argus* (Geoffroy), from Central and Southern Europe and North Algeria, briefly described by Mulsant (1846), Chapuis and Candèze (1853), Döbner (1862) and Schmidt (1922) (apud Kapur, 1950); *E. borealis* (Fabricius), from Central and North Africa, first described by Gage (1920); *E. chrysomelina orientalis* Zimmermann from eastern Mediterranean, northeast Africa, central Asia and across India, to as far as Indo-China, first described by Grandi (1913) and redescribed by Klemm (1930) (apud Kapur, 1950); *E. dentulata* Dieke from India; *E. eusema* (Weise), from Argentina; *E. flavosciata* (Laporte), widely distributed in South America from Colombia to Bolivia, briefly described by Candèze (1861) as *E. proteus* (apud Kapur, 1950); *E. hirta* (Thunberg), widely distributed on the African continent; *E. varivestis* Mulsant from Central America and the USA; and *E. vigintioctopunctata* (Fabricius), widely distributed throughout southeast Asia and Australia. The descriptions/redescriptions are very detailed, especially regarding to coloration and size, shape, number of branches and position of scoli in each region of the body. He illustrated the dorsal head, antenna, mandible, scoli and claw of all species, and the dorsal habitus and early instars of some species. The following was illustrated for *E. vigintioctopunctata*: dorsal habitus of the last instar larva and dorsal view of head for 1st, 3rd and 4th instars, antenna for 1st, 2nd and 4th instars, epipharynx,

* Corresponding author.

E-mail: casari@usp.br (S.A. Casari).

mandible and claw for 1st and 4th instars, and scoli for 1st, 2nd, 3rd and 4th instars. In relation to mouthparts, he described only the mandible and epipharynx of this species and compared the maxilla and labium to other described species.

According to Kapur (1950), the larvae of Epilachninae are herbivorous and have the general appearance of a porcupine. The head is connected to the thorax by a moderately long membrane that allows limited movement in all directions. Larvae of *Epilachna* show a great variety of structures, but were defined in the generic key as having: mandibles elongate and significantly narrowed distally, with not more than five teeth, two of which are large; clypeus mostly sclerotized [membranous in known *Epilachna*], shorter than labrum which is not expanded distally; galea oval, with long, dense setae; when the branches and setae are equally short, the former arises bilaterally from the main-stem; dorsal abdominal scoli on eighth segment much shorter than on the seventh. *Epilachna vigintioctopunctata*, recorded as being from "Asia", was defined in the key as having: scoli as long as the width of the head with twelve rather close branches; strumae on the underside with bases not strongly sclerotized or pigmented brown; prosternum with a single struma in the middle; ventral and subventral strumae on eighth segment fused.

Other known immatures of *Epilachna*: *E. cacica* Guérin (egg, larva, pupa) described by Almeida and Ribeiro (1986); *E. clandestina* (Mulsant) (egg, larva, pupa) described by Fonseca and Autuori (1931) as *Solanophila clandestina* and redescribed by Ribeiro and Almeida (1989) and (larva, pupa) redescribed by Costa et al. (1988); *E. paenulata* (Germar) described by Margheritis (1961) (apud Ribeiro and Almeida, 1989) and (egg, larva, pupa) redescribed by Ribeiro and Almeida (1989); *E. spreta* (Mulsant) (egg, larva, pupa) described by Ribeiro and Almeida (1989); *E. varivestis* Mulsant (larva) described by Kapur (1950) and redescribed by LeSage (1991).

Araujo-Siqueira and Almeida (2004) studied the behavior and life cycle of *E. vigintioctopunctata* in the laboratory feeding on *Lycopersicon esculentum* Mill. (Solanaceae), described its eggs, and illustrated the larva, pupa and adult of this species. The eggs of *Epilachna cacica*, *E. clandestina*, *E. paenulata*, *E. spreta* and *E. vigintioctopunctata* are known. Up to now, the pupae of *E. cacica*, *E. clandestina*, *E. paenulata* and *E. spreta* have been described.

In this work, the pupa of *Epilachna vigintioctopunctata* (Fabricius) is described and the first instar and mature larvae are redescribed based on material collected in Brazil on *Brugmansia suaveoleus* (Humb. and Bonpl. ex Willd.) Bercht. and J. Presl (Solanaceae) (trombeteiro). This species is a serious agricultural pest in Asia, and was recorded for the first time in Brazil in 1990 (Schroder et al., 1993).

Besides the pupa of *E. vigintioctopunctata*, those of *E. clandestina*, *E. paenulata*, and *E. spreta* were also studied.

Material and methods

The studied immatures and the adults are preserved in alcohol 70° GL and deposited in the Immature Coleoptera Collection of the Museu de Zoologia, Universidade de São Paulo (MZSP), except three adults, dried and pinned, deposited in the Collection of the Instituto Agrônomo de Campinas (IACC), and two in the Departamento de Zoologia, Universidade Federal do Paraná (DZUP) and MZSP.

The observations of *E. vigintioctopunctata* occurred in the field, in Campinas, and also in laboratory. The egg-layings were collected and kept in Petri-dishes until the eclosion, and the first instar larvae were fixed in alcohol. Larvae of different instars, pupae and adults were collected and kept in laboratory, in an attempt to determine the period of each stage.

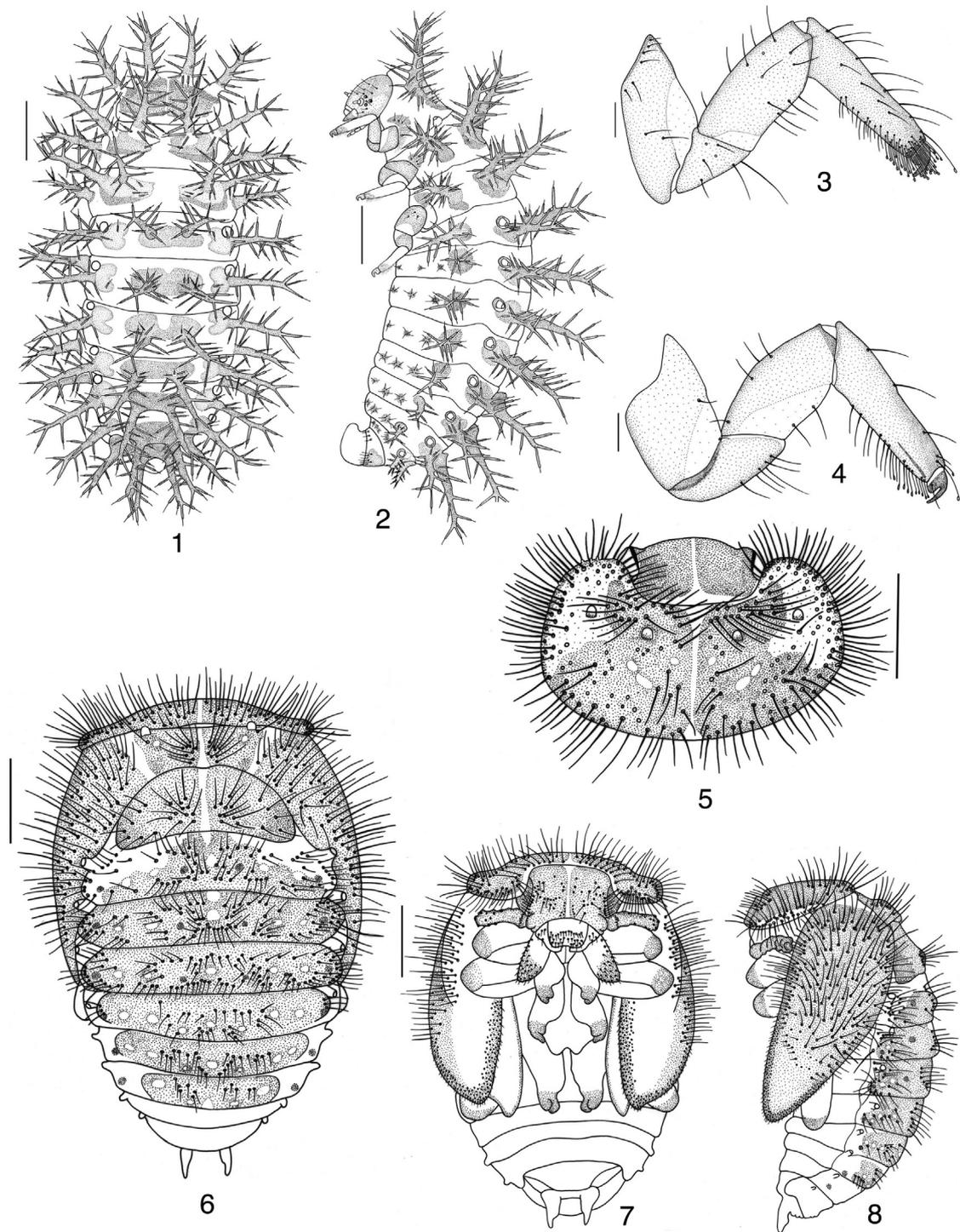
Terminology follows Kapur (1950) and LeSage (1991).

Epilachna vigintioctopunctata (Fabricius) (Figs. 1–23)

Redescription of fourth instar larva

The fourth instar larvae have a length of 6–8 mm. After fixation, cream coloration in general with brown scoli and brownish or cream strumae, lateral scoli clearer than dorsal can be noted. Scoli brown, clearer at base, with cream-colored basal and brownish distal ramifications; apical spine of each ramification brown and a few whitish; as well as two last lateral pairs of scoli on abdomen smaller and clearer too can be noticed. Dorsal scoli along with at least twelve branches arising from a rounded brown area; branches moderately long, except for a few shorter ones situated near base; each branch bearing, in addition to the apical spine, and two or three short and very thin setae are some of the features. Head remains partially yellowish or brownish except in lateral areas; besides a band on frontal sutures, cream; frontal area with several brown patches; dorsal area outside frontal sutures brownish; dorsal region with brownish irregular spots near base; stemmata black, located on a brownish small area; antennae brownish except at antennifer; labrum with brownish narrow band lateral and anterior; mandibles brown with black apex; maxilla and labium partially brownish-cream with palpi slightly darker, brownish; legs brownish, darker in direction of apex; and distal region of tibiae and claws brown (Figs. 1–3) too can be noticed.

Head (Figs. 2, 9–11) is not visible dorsally, but is rounded with several long setae; epicranial suture marked by wide cream band; frontal arms V-shaped; coronal suture long; frontoclypeal suture complete; frons with seven pairs of setae: an inclined row of five on each side near frontoclypeal suture, and two pairs near middle, one near anterior margin and one near base; four setae inside or near cream band of each frontal arm; each epicranial half with two setae near stemmata, two setae laterally below stemmata (one basal shorter) and an inclined row of four short setae on each side near base of epicranial suture are some of the features. Ventrally, two setae near each antenna, two laterally below anterior and three setae on each side, near gular area can be found. Occipital foramen remains large and sub-rounded. Stemmata (Figs. 9 and 11) is very prominent and black, with three on each side on a brownish patch. Antennae (Figs. 9, 14–16) are long; antennifer may be membranous and with three antennomeres: antennomeres I and II elongate; I shorter than II, almost as long as wide, with two campaniform sensilla dorsally near base; II is slightly narrower, which is almost twice as long as wide, with one long lateroexternal seta near apex, and at apex antennomere III, one long spine-like ventral seta and one laterointernal microseta; antennomere III remains (Figs. 15 and 16) very short, band-like, with six short setae (two longer) at apex. Clypeus (Fig. 12) is transverse, trapezoidal and translucent with two setae and one campaniform sensilla on each side, forming an inclined row, and anterior seta shorter. Labrum (Fig. 12) is transverse, with fore angles rounded, almost twice as long as wide, sclerotized in a narrow basal band, six pairs of setae and two of campaniform sensilla (three pairs longer). Epipharynx is membranous with narrow band of fine setae near anterior margin. Mandible (Fig. 13) remains symmetrical, much narrowed near apex, with five apical teeth [plant feeders]; mola and penicillus absent; and with one lateral long seta. Maxilla (Figs. 17 and 18) with mala transverse, wider than stipes, with anterior margin rounded; six ventral setae near middle; curved, longer, stout setae internally at apex; dorsally with setae, longer, denser and stouter near apex and microspines near middle; cardo is fused to stipes; stipes elongate, membranous laterally, with two long lateral setae, one at middle and one near anterior margin; palpifer lays transverse with one seta ventral; palpi remains with three palpomeres. Its basal palpomere is short, wider than long, with a campaniform ventral sensilla; palpomeres 2 and 3 elongate, 3 longer than 2; palpomere 2 with two long setae near apex, one dorsal and one ventral, and

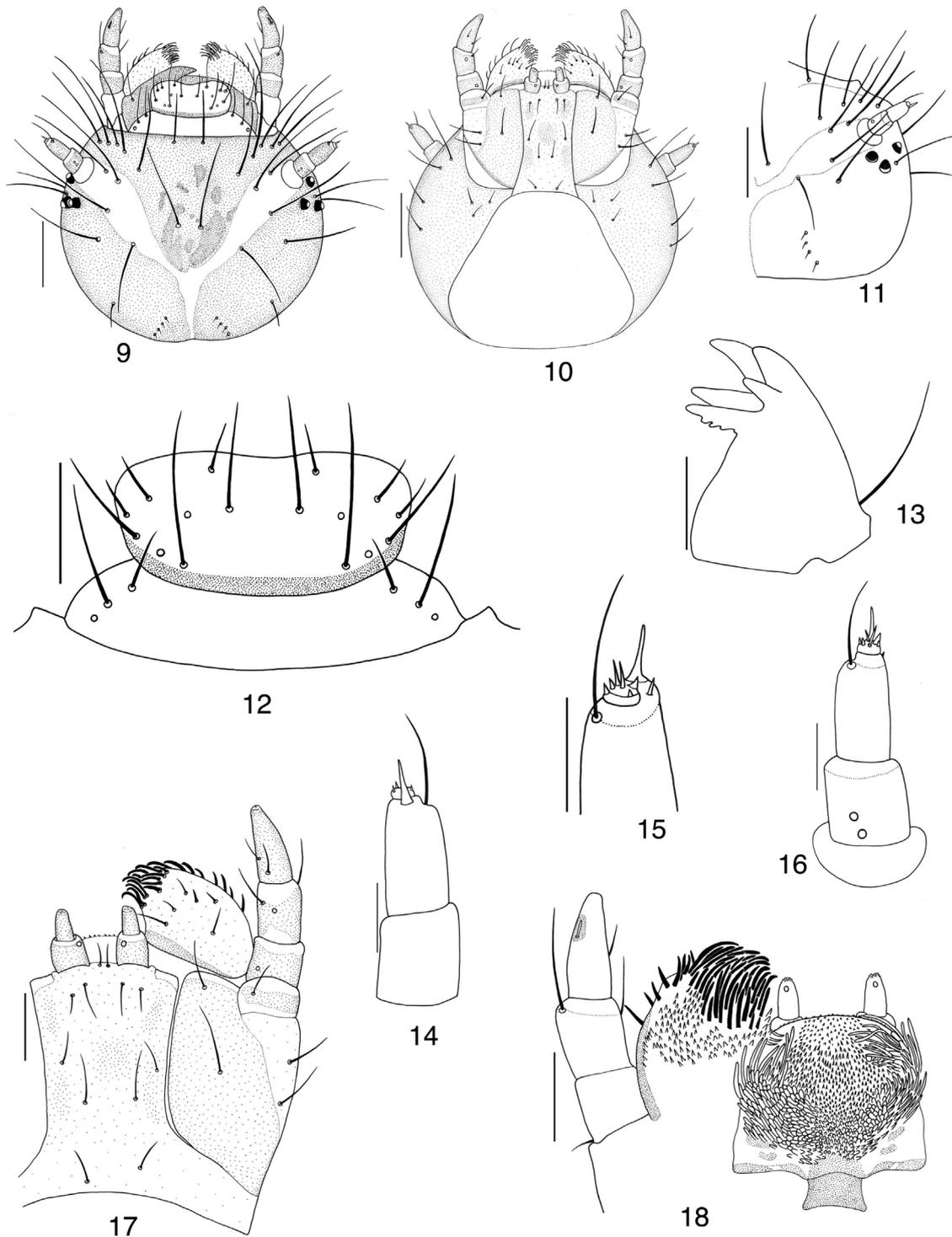


Figs. 1–8. *Epilachna vigintioctopunctata* (Fabricius). Larva: 1, 2, habitus (dorsal, lateral); 3, 4, leg (external, internal view). Pupa: 5, head and pronotum; 6–8, habitus (dorsal, ventral, lateral). Bars = 1 mm.

one campaniform ventral sensillum, near middle; distal palpomere narrowed apicad, with two ventral setae and one peg-like dorsal in groove, and several microsensilla at apex. Labium (Fig. 17) is fused to gula, almost totally translucent, formed by a unique piece with two setae at middle, near anterior margin, and another ten setae: a row of four near anterior margin, four near middle and two near base; palpi with two palpomeres elongate: basal palpomere with one ventral laterointernal campaniform sensillum near apex; distal palpomere narrower with one large dorsal campaniform sensillum near apex; microsensilla at apex can be noted. Hypopharynx is

(Fig. 18) densely setose laterally and microspined at middle; lateral setae longer.

Prothorax (Figs. 1 and 2) appears dorsally with two well-developed sclerotized plates, almost covering whole pronotum; each plate with one pair of scoli and three or four short chala-zae near base, and one very long chalaza near anterior margin; laterally with one clear brownish well-developed struma near procoxa; ventrally with one cream struma between procoxae. Meso- and metathorax have with two dorsally sclerotized plates, smaller than those of pronotum; each plate with one pair of scoli;

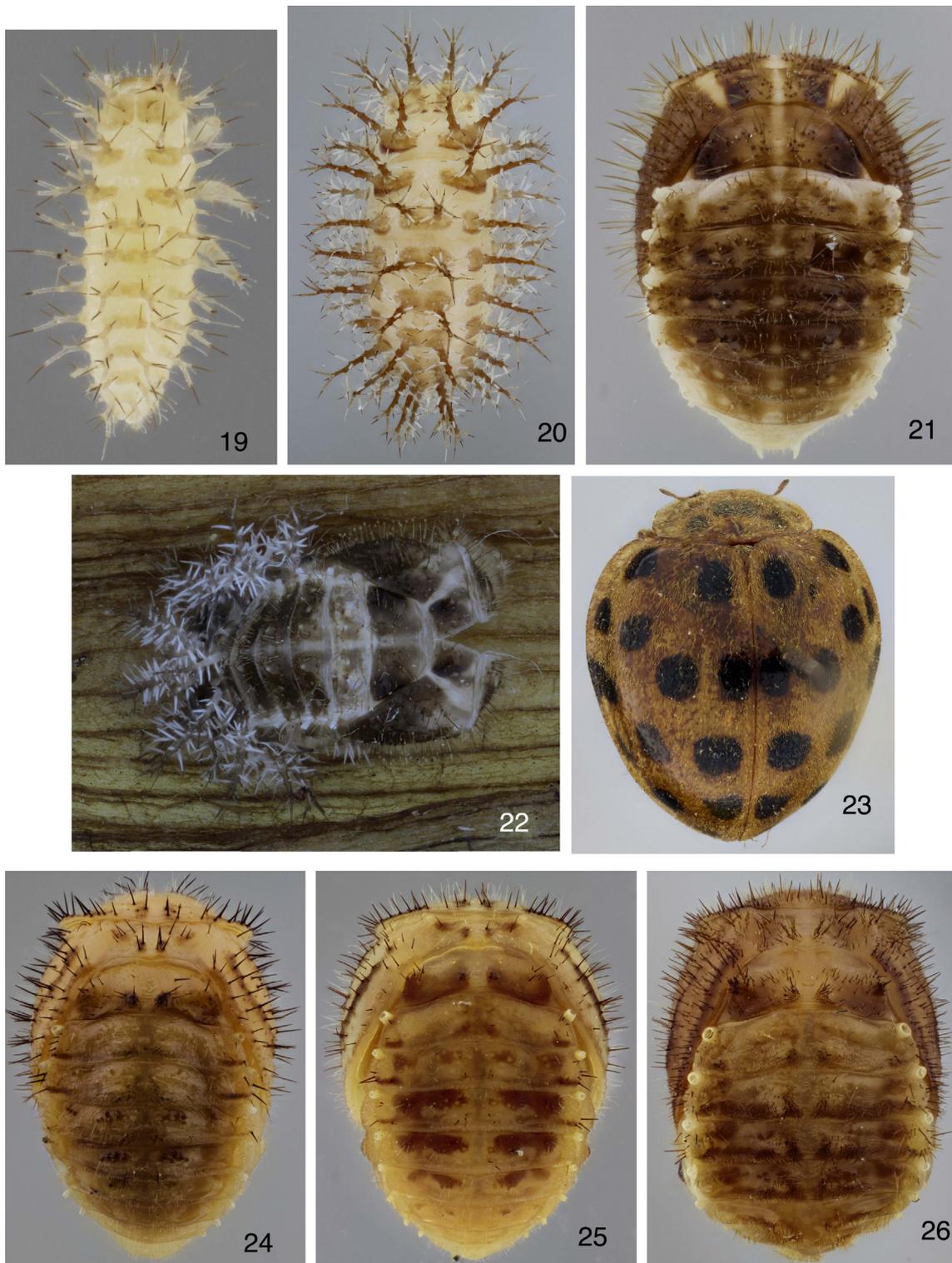


Figs. 9–18. *Epilachna vigintioctopunctata* (Fabricius). Larva: 9–11, head (dorsal; ventral, mandibles removed; laterodorsal, mouthparts removed); 12, clypeus and labrum; 13, mandible (dorsal); 14–16, antenna (ventral, apex dorsolateral, dorsal); 17, labium and maxilla; 18, apex of maxilla and hypopharynx. Bars = 1 mm, except Figs. 9–11 = 2 mm; Figs. 14–16 = 0.5 mm.

laterally, each side with one scoli and one sclerotized struma near each coxa; ventrally, each with two cream strumae between coxae. One pair of rounded latero-anterior spiracles on mesothorax is there. Legs (Figs. 3 and 4) are setose; setae brownish; distal setae of tibiae clubbed; coxae elongate, inserted distant from middle line of thorax; trochanter triangular; femur and tibia elongate; tibia narrower and brown near apex, with longitudinal narrow sclerotized band near margin of internal face, and densely

setose laterally on distal third of posterior face; dark-brown claws inserted on anterior face of tibia, with one seta at base of anterior face.

Its abdomen shall be described as: for segments I–VII, each bearing dorsally two median scoli inserted in one sclerotized plate and one scoli on each side of plate (dorsolateral); laterally, each segment with one scoli on each side; scoli decreasing slightly in size in direction of apex; ventrally with 6–7 strumae on each segment;



Figs. 19–26. *Epilachna vigintioctopunctata* (Fabricius). 19–20, larvae (first and fourth instars); 21, pupa (dorsal); 22, larval and pupal exuviae; 23, adult. Figs. 24–26. Pupae: 24, *Epilachina clandestina* (Mulsant); 25, *E. paenulata* (Germar); 26, *E. sprete* (Mulsant).

strumae more sclerotized and larger in direction of apex. This is for segments I–VIII, each with one rounded spiracle anterolaterally on each dorsolateral scoli. Segment VIII type has with four short dorsal scoli, one large sclerotized lateral verruca, and six ventral smaller sclerotized chalazae. Segment IX is narrower with one sclerotized dorsal plate, having several setae and six small ventral verrucae, each with one seta. Segment X tubular, sucker-like protuberance.

First instar larva (Fig. 19)

First instar larvae have a length of 1.8–2.0 mm., which are similar to last instar larva, but with clearer coloration: cream with head and dorsal plates yellowish and scoli brownish or cream. Scoli appear with about five ramifications; apical spine of each ramification very long and brownish; each ramification comes with several short setae, besides distal spine. Pronotum is paired with egg-bursters near base. Legs are very long in relation to body size.

Pupa description (Figs. 5–8, 21)

Its length: 5–6mm. General coloration of integument is yellowish-white, especially ventrally; dorsally almost totally brown; dorsal darker areas remain clearly apicad. Pubescence can be described as (Figs. 5–8): dense, short and yellowish-white on cream integument; long, spiniform, brownish and bristle on brown integument; and brown setae inserted in coarse darker punctures or microtubercles.

Head is (Figs. 5, 7 and 8) not visible dorsally; brown, with cream-colored longitudinal, median, narrow band; with many long setae on each side (some represented by punctures) and one longitudinal row internally on each eye; labral area is cream with many short setae on distal half; antennae brown, glabrous with small darker tubercles; mandibles cream with brown apex and four setae near base; maxillary lobe too is cream with brown apex and many short setae laterally and at distal third.

Pronotum (Figs. 5–8) is partially visible dorsally; declivous frontally; wider than long; fore angles prominent and rounded; posterior margin is slightly rounded; brown, with longitudinal median band cream and fore angles slightly brownish; each side with two clear tubercles on anterior half and three small cream patches near middle: one small and rounded, and two elliptical; many very long setae are inserted in small tubercles (some setae represented by punctures). Mesonotum is brown, with one longitudinal and one inclined cream band on each side; densely setose, especially on scutellar area; two well-developed clear tubercles present near anterior margin and two small rounded cream patches below anterior; anterior pterothecae brown and densely setose dorsally and cream with yellowish-white margins ventrally; cream area is marginated by band of short setae inserted in brown punctures. Metanotum is brown, darker laterally on hind angles; longitudinal median band cream; many long setae are more concentrated near cream band. Legs are cream with apices of femora and tarsi brown.

Abdomen is decreasing in width in the direction of apex; lateral margins are rounded; each side of segments I–VIII is bearing one rounded laterodorsal spiracle inserted in a tubular, cream prolongation; tubular prolongation of spiracle is decreasing in size in direction of apex; tergites I–VI totally or partially brown. Tergite I is with irregular brown basal band, wider laterally and near middle; tergites II–IV are brown; tergites I–IV, each with one pair of cream, rounded patches on each side and four small rounded protuberances: two near middle and one at each side; protuberances are decreasing in size, especially in height, in direction of apex; median protuberance of tergite IV is appearing as a white patch; tergites I–II are with two rounded cream patches at middle; tergites III–IV are with one median cream patch, densely setose; setae more concentrated laterally. Tergite V is narrower and lighter brown, with one tubercle on each side in cream area; with three rounded cream patches on each side and one at center; setae are sparser and more concentrated at middle. Tergite VI is with brownish narrow area and rounded well-developed median cream patch at base; each side with one or two rounded cream areas and one lateral tubercle on cream area. Tergites VII–VIII are totally cream with many short and yellowish-white setae (not represented), each with a lateral scar. Tergite IX is narrow with two laterally compressed urogomphi. Pleural cream areas are covered by short and yellowish-white setae (not represented), ventrally, abdomen cream, covered by short and yellowish-white setae (not represented).

Material examined is Brazil. São Paulo: Campinas, 18.III.2010, E. P. Teixeira col., 2 first instar larvae, 2 mature larvae, 1 larval skin, 2 pupae, 2 adults (MZSP); 3 adults (IACC); 2 adults (DZUP); São Paulo (Engenheiro Goulart), III–IV.2009, J. Fuhrmann col., 7 eggs, 20 first instar larvae, 18 mature larvae, 7 pupae, 3 adults (MZSP).

Remarks

Through comparison of the studied pupae, it was verified that they are very different, especially in relation to coloration and chaetotaxy. To facilitate their recognition, a diagnosis is hereby presented, as well as an illustration for each of these species. The diagnosis of *E. cacica* is based on the literature.

Epilachna cacica (from Almeida and Ribeiro, 1986). General coloration is yellowish with brownish areas on thorax, anterior pterothecae and parts of abdomen; apices of femora and bases of tibiae darker. Frons are with dense row of setae; apical antennomeres are with papillae. Pronotum with setae sparse and two tufts near base. Meso- and metanotum, each with two tufts of setae on each side: one near middle and the other lateral. Anterior pterotheca is with longitudinal grooves and dispersed setae. Abdomen: tergites I–V, each with two tufts of setae on each side: one near middle and the other lateral; tergites VI–VII, each with two small tufts at middle.

Epilachna clandestina (Fig. 24). Its body is gradually narrowed anteriorly and apicad, making lateral margins (from mesothorax) rounded. General coloration is yellowish-cream with brownish patches and long dark-brown setae inserted in small brown tubercles, yellowish-white and finer setae on clearer areas. Head is totally cream. Pronotum is darker on narrow bands on anterior and posterior margins and two basal median tubercles; long setae near margins and basal median. Mesonotum is darker on four small patches each with 3–4 setae. Metanotum is darker on large basal area of hind angles, continuous with two rounded small darker patches near middle, forming small tubercles; sparse setae on each side, more concentrated on tubercles. Anterior pterothecae with four narrow brown or brownish longitudinal bands, each with irregular band of setae. Abdomen: tergites I–II, each with small transverse darker band on each side near basal margin; tergites III–IV, each with transverse larger band on each side near anterior margin; few setae, more concentrated on tergites I–IV.

Material examined. Brazil. São Paulo: São Paulo, 27.III.1982, C. Costa col., 2 pupae (MZ6762).

Epilachna peanulata (Fig. 25). Body gradually narrowed anteriorly and apicad, making lateral margins (from mesothorax) rounded. General coloration is yellowish with brown or brownish patches; dark-brown setae are usually on brown patches and yellowish-white setae in remaining areas. Head remains darker on two small patches on frons, eyes, antennae, apices of mandibles, palpi and basis of femora. Pronotum is darker on two transverse bands near posterior margin and two triangular-elongate areas near anterior margin, almost reaching base; dark-brown setae are at margins and on brown patches, and whitish setae are on remaining areas. Mesonotum is darker on four small patches near middle, each with one or two dark-brown setae. Metanotum is darker in a transverse basal band including hind angles; two darker tubercles are near middle; a few dark-brown setae are on each side and three on each median tubercle. Anterior pterotheca with three longitudinal darker bands and four irregular rows of dark-brown setae. Abdomen is described so. Tergite I with two small darker patches laterally near posterior margin; tergite II, with four small darker patches on each side; tergites III–V, each with one large transverse darker band on each side; tergite VI with two small darker patches on each side; each tergite with a few dark-brown setae on each side and many yellowish-white setae.

Material examined. Brazil. Paraná: Curitiba. R. Marinoni col., 3 pupae (MZ6776).

Epilachna spreta (Fig. 26). Abdomen abruptly narrowed at apex making lateral margins straight until tergite IV and narrowed apicad thereafter. General coloration is brownish-yellow with brownish or reddish-brown areas or patches and reddish-brown setae, short or moderately long. Head is with darker eyes and

a longitudinal narrow band at frons, near each eye. Pronotum is brownish except on lateral band, one-fourth pronotum width, brownish-yellow; one triangular brown patch on each side, near middle; setae at margins and on two rounded areas near middle. Meso- and metanotum are brownish except on longitudinal median band, brownish-yellow; setae are distributed by almost all tergites, except on longitudinal median band. Anterior pterotheca is with four darker longitudinal bands, each with one irregular row of setae. Abdomen is described as: tergite I with three darker patches on each side; tergites II–IV, each with one darker transverse band on each side near posterior margin and one or two small bands near anterior margin; tergites I–IV with two weak tubercles near middle; tergite V with one small transverse band on each side, near posterior margin; setae of abdomen more concentrated near middle and posterior margin. Each abdominal tergite is with two rounded clearer spots near middle.

Material examined. Brazil. Paraná: Curitiba, R. Marinoni col., 2 pupae (MZ6756).

Epilachna vigintioctopunctata (Figs. 6–8, 21). Body gradually narrowed anteriorly and apically, making lateral margins (from mesothorax) rounded. General coloration is yellowish-white with dorsal areas and setae are brown or brownish. Head (except narrow longitudinal median band), eyes, antennae, apices of palpi, femora and tarsi are darker. Pronotum is brown, clearer laterally, with four small tubercles on anterior half, disposed in a transverse line; densely covered by setae, more concentrated near margins. Mesonotum is totally brown, darker at hind angles; narrow longitudinal yellowish-white band at median and each side at base of pterotheca; two tubercles are near anterior margin; setae are more concentrated near middle on basal half. Metanotum is brown, darker at hind angles; narrow, longitudinal, median yellowish-white band at middle; setae are more concentrated near middle on basal two-thirds. Anterior pterotheca is brown and densely setose dorsally and laterally; setae are very long. Abdomen: tergites I–VI are almost totally brown and densely setose; each with two whitish patches and two scars on each side. This species is the darkest, with the longest setae on anterior pterotheca.

Biological notes

Species of *Epilachna* feed especially on Cucurbitaceae and Solanaceae; *E. vigintioctopunctata* is a pest of solanaceous crops, such as potatoes and eggplants in Asia. This species was reported for the first time in Brazil in the state of Paraná, feeding on wild cucurbit vines (species unknown). In 1991 it was collected on pepper plant (*Piper nigrum* L.) and *Solanum americanum* Mill. (maria-preta) (Schroder *et al.*, 1993). Araujo-Siqueira and Almeida (2004) reared this species in laboratory, where it fed on *Lycopersicon esculentum* Mill. (Solanaceae) (tomato plant).

The studied material was collected from *Brugmansia suaveoleus* (Humb. and Bonpl. ex Willd.) Bercht. and J. Presl (Solanaceae) (angel's trumpet), in Campinas and São Paulo. The observations, in the field and in laboratory, were based on material collected on one plant from Campinas. This is the first record of this species as host of *E. vigintioctopunctata*.

The first observation of *E. vigintioctopunctata* on *Brugmansia suaveolens* occurred in Campinas, on 18.3.2010, when five egg-layings were found on the underside of the leaves, which agrees with the observations of Fonseca and Autuori (1931) and Parker *et al.* (1995). Two egg-layings were collected, one with 24 eggs (3 already eclosed) and the other with 23. On the following day, all eggs of the first egg-laying eclosed and all the first instar larvae were kept in Petri dishes (13.5 cm diameter). Two first instar larvae were fixed in alcohol, and some days later the remainders disappeared. The eggs of the second egg-laying eclosed five days later and all larvae died. Based on observations of Nakamura *et al.* (2004)

it was assumed that part of the larvae suffered cannibalism. According to them, “*Epilachna vigintioctopunctata* is a common species in Indonesia and it is highly cannibalistic when population density is high, and cannibalism can account for between 11% and 30% of total mortality in this species”.

Besides these two egg-layings, larvae of different instars, adults and pupae were collected together from this same plant, corroborating that egg-laying, larvae, pupae and adults occur at same time, as already observed by Parker *et al.* (1995).

In laboratory, pupation occurred from 7 to 12 days after collecting; the pupal period lasted from 5 to 7 days. Adults lived about a hundred and ten days, corroborating with Nakamura *et al.* (2004) who observed that: “Adult beetles can live for more than 80 days (females, 57.7 ± 5.6 days; males, 87.3 ± 4.9 days) and females lay, on average, batches of approximately 13 (ca. 770/57.7) eggs per day over their lifetime”.

In another field observation, made on 28.3.2010, one adult was found feeding on the buds; adults usually eat the leaves of the plant. In laboratory, larvae and adults ate both sides of leaves; pupae were found on the underside of leaves, and were attached to the leaves by last larval skin wrinkled at apex of abdomen (Fig. 22).

Discussion

The larval stages of most coccinellids, including *Epilachna* species, consist of four instars (Kapur, 1950; LeSage, 1991). According to Kapur (*l. c.*), the earlier instars of *E. vigintioctopunctata* differ from those of the final or fourth instar in having lighter coloration and simpler armature. In the first instar larva, scoli are shorter with a small number of branches and longer setae; underside, each segment has a pair of ventral setae; the basal tooth of claws smaller and triangular. These characters were observed in the studied material (Fig. 19). According to Kapur, in the third instar the scoli have about twelve branches, and the fourth and last instars have more than fourteen moderately long branches, except for a few shorter ones situated close to the base. This information allows us to confirm that the two studied larvae belong to the fourth or last instar (Fig. 20).

According to Kapur (1950), the classification of *E. vigintioctopunctata* based on the adults is also divisible into several subspecies, but exemplars of larvae examined by him from India (type-locality) and Taiwan (formerly known as Formosa) do not show differences in structure, except that some of those from Taiwan have darker coloration. We also found differences in coloration when material from the state of São Paulo was compared to illustrations by Araujo-Siqueira and Almeida (2004) (based on material from the state of Santa Catarina).

Coloration of the studied material was probably modified after fixation, but the larvae are cream-colored totally, except for brown scoli and sclerotized basal plates. This same coloration was observed by Kapur: “general color pale yellow except for the more sclerotized parts and areas round bases of scoli which area brown”. The live larva illustrated by Araujo-Siqueira and Almeida (2004, Fig. 2) is cream-colored, having yellowish-orange large patches with dorsal scoli and sclerotized plates from reddish-brown to dark-brown (darker at apices), lateral scoli, totally cream.

Through comparison of Kapur's description of the last instar larva of *E. vigintioctopunctata*, based on material from Taiwan and India, and the studied material from Brazil (state of São Paulo), some differences were verified, especially related to chaetotaxy of the head (Kapur's description parenthesized): frons with 14 setae (18 setae); median region of frons with two pairs of long setae: one near anterior margin and one near base (three pairs of long setae and one pair of short setae); clypeus translucent with two long setae and one campaniform sensilla on each side (slightly darker toward base with two setae on each side); labrum sclerotized in

narrow basal band, with six pairs of setae and two campaniform sensilla (without sclerotized basal band, with 3–4 pairs of setae); epipharynx with narrow band of fine setae near anterior margin (bands of fine setae longitudinal median and near anterior margin); mala transverse (elongate), and with six ventral setae (several setae); setae at apex of mala stout and curved (not curved); stipes with four setae (two or three setae); segment VIII with six small sclerotized chalazae (chalazae fused).

Besides the larva, we found outstanding differences between the pupa studied here and the illustration of Araujo-Siqueira and Almeida (2004, Fig. 3), especially related to coloration and chaetotaxy. The pupa of *E. vigintioctopunctata* herein described is clearly bicolored with well visible bristle setae inserted in darker punctures or small tubercles, while that presented by Araujo-Siqueira and Almeida (2004, Fig. 3) is almost unicolor, apparently with few setae on the abdomen; dorsally it is brownish-yellow with brown tubercles on meso- and metanotum; metanotum darker laterally; setae bristle and dark-brown more concentrated on thorax, anterior pterothecae and laterally on first two abdominal segments; anterior pterotheca with longitudinal brown bands. The abdomen is glabrous (except laterally), different from the pupa studied here, and densely setose dorsally. The pupa illustrated in Araujo-Siqueira and Almeida (2004, Fig. 3) is very similar to that of *E. clandestina*.

Based on data from the literature and the studied material, we characterized *Epilachna* larvae as having the following features. Head is directed ventrally, and is rounded dorsally; three sub-conical and dark stemmata are on each side, disposed in triangle below antenna; epicranial suture clearer; frontal arms are V-shaped; coronal suture is long; frons with 12–24 setae: *E. vigintioctopunctata* (12 or 16); *E. borealis* (14); *E. chrysomelina* (24); clearer area of each frontal arm with four setae in *E. vigintioctopunctata* and many setae in *E. argus*. Each epicranial half with four long setae plus a row of four short ones in *E. vigintioctopunctata*, five setae in *E. borealis* and *E. varivestis*, and eight setae in *E. argus* and *E. chrysomelina*. Antennae with three antennomeres; third antennomere is reduced with several conical sensilla at apex. Clypeus with 4–8 setae: four in *E. argus*, *E. flavosciata* and *E. vigintioctopunctata*; six in *E. eusema* and *E. hirta*; 6–8 in *E. borealis* and *E. chrysomelina*. Labrum with 3–16 setae: six in *E. argus*; four in *E. flavosciata*; 6–8 in *E. vigintioctopunctata*; eight in *E. cacica*; 12 in *E. clandestina* and *E. eusema*; 12–16 in *E. chrysomelina*; 16 in *E. borealis*. Mandibles with 3–5 teeth at apex: three large and two or three small teeth in *E. varivestis*; four large and several small teeth in *E. borealis*, *E. cacica* and *E. chrysomelina*; four teeth in *E. clandestina* (description of Costa et al., 1988); five teeth in *E. clandestina* (description of Fonseca and Autuori), *E. flavosciata*, *E. hirta* and *E. vigintioctopunctata*. Labium with two setae between palpi and 5–12 setae on mentum: five in *E. borealis*, nine in *E. chrysomelina* and *E. vigintioctopunctata* and 12 setae in *E. clandestina* (description of Costa et al., 1988). All described species present four scoli on prothorax, six each on meso- and metathorax, and six each on abdominal segments I–VIII. Abdominal segments I–VIII, each with one spiracle anteriorly, dorsolateral scoli. Claws with one basal seta in *E. argus*, *E. chrysomelina*, *E. clandestina*, *E. eusema*, *E. varivestis* and *E. vigintioctopunctata*; without basal seta in *E. borealis*, *E. cacica*, *E. flavosciata* and *E. hirta*.

Based on examined material and descriptions, we characterize *Epilachna* pupae as follows. General coloration is clear with darker

patches on thorax and abdomen; setae are spiniform, stout, dense and bristle dorsally. Distal antennomeres are with small tubercles. Frons, labrum and apices of palpi and femora are with setae, finer and clearer than dorsal. Abdominal spiracles are at apex of a tubular prolongation. Last abdominal segment is with two elongate projections to fit on last larval skin.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

Our appreciation is extended to Juarez Fuhrmann and Daniela Bená (MZSP) for having donated material from São Paulo (Engenheiro Goulart); Gabriel Biffi (MZSP) for his patience in teaching and helping with photographic techniques; Guilherme Ide M. dos Santos (MZSP) for assisting and offering hints on photography; Antonio Santos-Silva (MZSP) for his partnership related to photographic techniques; and the reviewer for comments and suggestions.

References

- Almeida, L.M., Ribeiro, C.S., 1986. Morfologia dos estágios imaturos de *Epilachna cacica* Guérin 1844 (Coleoptera, Coccinellidae). Rev. Bras. Entomol. 30, 43–49.
- Araujo-Siqueira, M., Almeida, L.M., 2004. Comportamento e ciclo de vida de *Epilachna vigintioctopunctata* (Fabricius) (Coleoptera Coccinellidae) em *Lycopersicon esculentum* Mill. (Solanaceae). Rev. Bras. Zool. 21, 543–550.
- Candèze, E., 1861. Histoire des metamorfoses de quelques coléoptères exotiques. Mém. Soc. R. Sci. Liège 16, 1–86.
- Chapuis, F., Candèze, E., 1853. VIII. Catalogue des Larves des Coléoptères. connues jusqu'à ce jour avec la description de plusieurs espèces nouvelles. Mém. Soc. R. Sci. Liège 8, 347–653.
- Costa, C., Vanin, S.A., Casari-Chen, S.A., 1988. Larvas de Coleoptera do Brasil. Museu de Zoologia, Universidade de São Paulo.
- Döbner, E.P., 1862. Beiträge zur Entwicklungsgeschichte einiger Coleopteren Berlin. Ent. Ztschr. 6, 67–68.
- Fonseca, J.P., Autuori, M., 1931. Contribuição para a biologia de *Solanophyta clandestina* (Muls.) (Coleoptera Coccinellidae). Rev. Entomol. 1, 219–224.
- Gage, J.H., 1920. The larvae of the Coccinellidae. III. Biol. Monogr. 6, 1–62.
- Grandi, G., 1913. Studi sui Coccinellidi Bollettino del Laboratorio di Zoologia Generale e Agraria della R. Sc. Superiore Agric. Portici 7, 267–302.
- Kapur, A.P., 1950. The biology and external morphology of the larvae of *Epilachna* (Coleoptera Coccinellidae). Bull. Entomol. Res. 41, 161–208.
- Klemm, M., 1930. Beitrag zur Morphologie und Biologie der *Epilachna chrysomelina* Fabr (Coleopt.). Z. Wiss. Insekt. Biol. 24, 238–245.
- LeSage, L., 1991. Coccinellidae (Cucujoidea). In: Stehr, F.W. (Ed.), Immature insects, vol. 2. Kendall/Hunt, Dubuque, pp. 485–494.
- Margheritis, A., 1961. *Epilachna paenulata* su ontogenia y destrucción. Rev. Facult. Agron. Univ. Nacl. La Plata 1, 148–158.
- Mulsant, E., 1846. Histoire naturelle des Coléoptères de France 4. Sulcicollis-Sécuirpalpes, Paris.
- Nakamura, K., Hasan, N., Abbas, I., Godfray, H.C.J., Bonsall, M.B., 2004. Generation cycles in Indonesian lady beetle populations may occur as a result of cannibalism. Proc. R. Soc. Lond. 271, 501–504.
- Parker, B.L., Talekar, N.S., Skinner, M., 1995. Field Guide: Insect Pests of Selected Vegetables in Tropical and Subtropical Asia. Asian Vegetable Research and Development Center, Taiwan.
- Ribeiro, C.S., Almeida, L.M., 1989. Descrição dos estágios imaturos de *Epilachna spreta* (Muls., 1850) (Coleoptera Coccinellidae), com redescricao, comentários e chave para três outras espécies. Rev. Bras. Zool. 6, 99–110.
- Schmidt, E., 1922. Festschr.50. Jubil. Lehranst. Obst- u. Gartenb. Geisenheim, 512–514.
- Schroder, R.F.W., Athanas, M.M., Pavan, C., 1993. *Epilachna vigintioctopunctata* (Coleoptera: Coccinellidae), new record for Western Hemisphere, with a review of host plants. Entomol. News 104, 111–112.