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Keys for Identification of Immature Insects

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Chapter 2 Keys for Identification of Immature Insects

Patricia J. Thyssen

2.1 Introduction

The study of immature insects is important in forensic entomology, because the identification of the involved species is a crucial step in calculating the post-mortem interval (PMI) and because it is the insect life stage most frequently collected from corpses. The immature stage consists of the egg, nymph or larva with its average of three or four development instars, and the pupa.

Decomposition of a dead body starts through the action of bacteria and fungi, followed by the action of a series of arthropods with a predominance of the dipteran insects (e.g., Carvalho et al. 2000, 2004). Therefore, the anatomical features of the immature stages of these insects are described and taxonomic keys utilized to identify order, family, and species of most of Brazil forensic relevant insects, with emphasis on larvae of the major Diptera families, are presented.

2.2 Anatomical Features of Dipteran Immature Stages

The most recent classification recognizes two suborders, Nematocera and Brachycera (the latter suborder include dipterans that are known as Cyclorrhapha – for classification consult McAlpine et al. 1981). The descriptions below apply to dipterans of the suborder Brachycera and the infraorder Muscomorpha.

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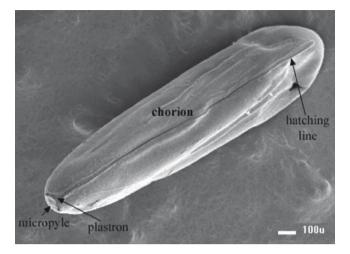


Fig. 2.1 Scanning electron micrograph of the egg of a calliphorid. Scale = $100 \mu m$

2.2.1 Egg Morphology

The egg is the first stage of development in which a series of changes occurs before the hatching of the larvae, since the embryos contain cells and developmental programs for larval structures. A typical egg has the following external characteristics (Fig. 2.1):

- Chorion: outer covering of the egg;
- Micropyle: a pore at the anterior end of the egg that permits entrance of the spermatozoa;
- Plastron: a cell membrane inside and next to the chorion and surrounding the cytoplasm;
- Hatching line: a longitudinal strip that splits off to let the larva emerge.

2.2.2 Larval Morphology

During growth the number of molts varies among insect groups, but in some insect orders this number is rather constant (e.g., two in the muscomorph diptera). The interval between molts is known as stadium, and the form assumed by an insect during a particular stadium is termed an instar (e.g., when an insect hatches from the egg, it is said to be in its first instar) (Fig. 2.2).

The commonest shape of the larvae of muscomorph flies is basically cylindrical, with the anterior end tapering gradually to a slender, pointed head and the posterior end bluntly rounded or somewhat truncated (Fig. 2.3). The body comprises 12 segments: a head (segment I), followed by a prothoracic (segment II), a mesothoracic segment (segment III), a metathoracic segment (segment IV), followed by eight

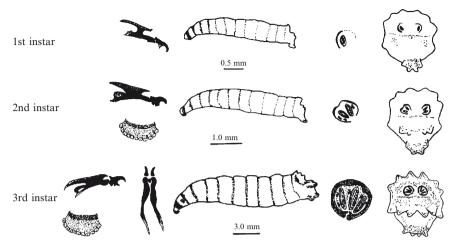


Fig. 2.2 Development of a generalized larvae

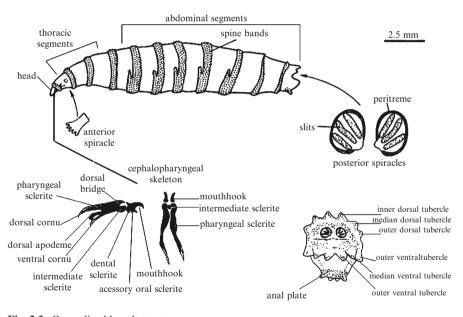


Fig. 2.3 Generalized larval structures

abdominal segments (V-XII). Posterior spiracles are found on the last abdominal segment; and in each spiracle there is a number of slits (according to larval stage) surrounded by a structure called a peritreme. Inside the larval head and extending into the thorax is a chitinous cephalopharyngeal skeleton, which consists of a number of distinct sclerites. Anterior spiracles (when present – generally appear from the second instar) are located on each side of the prothoracic segment and protrude from the body wall.

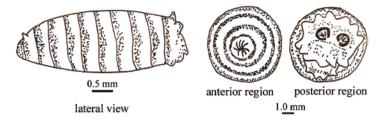


Fig. 2.4 Puparium of a calliphorid

2.2.3 Puparium Morphology

The puparium of the Muscomorpha is most commonly barrel-shaped, heavily sclerotized (formed by hardening of the third instar larval cuticle), with the morphology similar to the previous instar, but smaller in length due to a retraction in the body segments (Fig. 2.4).

2.3 Taxonomic Keys

2.3.1 Key to Larvae and/or Nymphs of Hexapod Orders

This key was modified and adapted from Chu and Cutkomp (1992) (Fig. 2.5a, b and e).

1.	Wing pads usually external, nymphs or naiads	2
1′.	Wing pads usually internal, larvae	21
2.	Chewing mouthparts	3
2′.	Sucking mouthparts	18
3.	Abdomen 6-segmented, a spring-like organ on the fourth	Collembola
3′.	Abdomen at least 9-segmented, no spring-like structure	4
4.	Terrestrial	5
4′.	Aquatic	13
5.	Cerci absent	6
5′.	Cerci present	10
6.	Parasitic on birds and some mammals; one tarsal claw	Mallophaga
6′.	Not parasites; two tarsal claws	7
7.	Antennae long, longer than body	Psocoptera
7′.	Antennae often inconspicuous	8
8.	Cerci minute; social insects	Isoptera

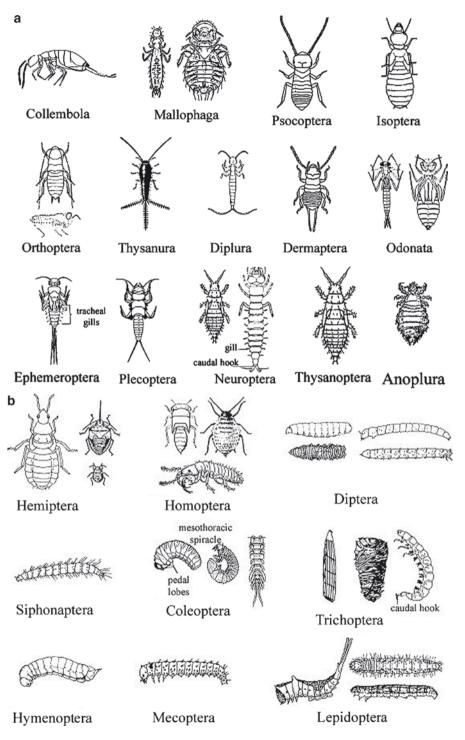


Fig. 2.5 (a) Larval morphology of the different Insecta orders (adapted from Chu and Cutkomp, 1992). (b) Larval morphology of the different Insecta orders (Adapted from Chu and Cutkomp 1992)

8′.	Cerci absent; solitary insects	9
9.	Mandibles not sickle-shaped	17
9′.	Mandibles sickle-shaped	17
10	Cerci short; with compound eyes	Orthoptera
10′.	Cerci long without compound eyes	11
11.	Three-filamented cerci on the end of the abdomen	Thysanura
11′.	Two appendages on the end of the abdomen	12
12.	Body not distinctly sclerotized; no wing pads	Diplura
12′.	Body distinctly sclerotized; with wing pads	Dermaptera
13.	(4') Labium much elongated as a spoon	Odonata
13′.	Labium normal	14
14.	Cerci conspicuous	15
14′.	Cerci inconspicuous	16
15.	Seven or eight abdominal tracheal gills; one tarsal claw	Ephemeroptera
15′.	With thoracic gills; two tarsal claws	Plecoptera
16.	With anal hooks; mandible normal; case bearing	26
16′.	Without anal hooks; mandibles sickle-like	17
17.	(9) Labial palpi 2-segmented; if gills present, on the sides	of the abdomen 24
17′.	(9') Labial palpi if present, more than two segments; if g ventral side of abdomen	
18.	(2') Tarsi without claws	Thysanoptera
18′.	Tarsi with claws	19
19.	Single tarsal claw	Anoplura
19′.	Two tarsal claws	20
20.	Proboscis arising from frontal margin of head	Hemiptera
20′.	Proboscis arising from hind margin of head	Homoptera
21.	(1') Legless	22
21′.	With thoracic legs	25
22.	Head capsule not well developed; maggots	Diptera
22′.	Head capsule developed	23
23.	Without eyes	Siphonaptera
23′.	Eyes present	24
24.	(17) Prothorax without spiracles	25
24′.	Prothorax with spiracles	27
25.	(24) No distinct prolegs	Coleoptera
251	With distinct prolegs	26

26.	(16) Only one pair of prolegs on abdomen, located on	
	segment	Trichoptera
26′.	Two or more pairs of prolegs on abdomen	27
27.	(24') One large ocellus on each side of head	Hymenoptera
27′.	Two or more small ocelli on each side of head	28
28.	Six to eight pairs of prolegs, without crochets	Mecoptera
28′.	Two to five pairs of prolegs, with crochets	Lepidoptera

2.3.2 Key to larvae of major Diptera families

Thi	s key was modified and adapted from Chu and Cutkomp (1992) (Fig. 2.6a-c).
1.	Mandibles move horizontally; head complete or, if not, the posterior portion with deep longitudinal incisions, or the thorax and abdomen together consisting of 13 segments (Suborder Nematocera)
1′.	Mandibles move vertically; head incomplete, without a strongly developed upper arched (arcuate) plate
2.	Maxillae well developed, palpi distinct; mandibles normally sickle-like; antennae well developed on the upper surface of a slightly arcuate, sclerotized dorsal plate (Suborder Brachycera)
2′.	Maxillae poorly developed, palpi visible only in a few larvae; mandibles short and hook-like; antennae poorly developed or absent, when present situated upon a membranous surface (Suborder Brachycera, old Suborder Cyclorrhapha)28
3.	Head incomplete; thorax and abdomen combined consist of 13 segments; fleshy, pointed paired prolegs on 7 or more abdominal segments; usually with a sclerotized plate on ventral surface of mesothorax
3′.	Less than 13 segments; other characters differ4
4.	Head and thorax and first and second abdominal segments fused; larvae with minute abdominal spiracles; abdomen with a ventral longitudinal series of sucker-like discs
4′.	Head free, or if retracted within or fused with prothorax the other thoracic segments are distinct
5.	Head complete; mandibles opposed6
5'.	Head incomplete posteriorly, either with three deep wedge-shaped slits (two on dorsum and one on venter), or ventral surface very poorly sclerotized and the dorsal surface posteriorly in the form of four slender heavily sclerotized rods, with a weakly sclerotized divided plate on anterior half of the dorsum (in part, see also 15)
6.	Thoracic segments fused, flattened, and wider than abdominal segments, forming a complex mass
6′.	Thoracic segments distinct and not wider than abdomen

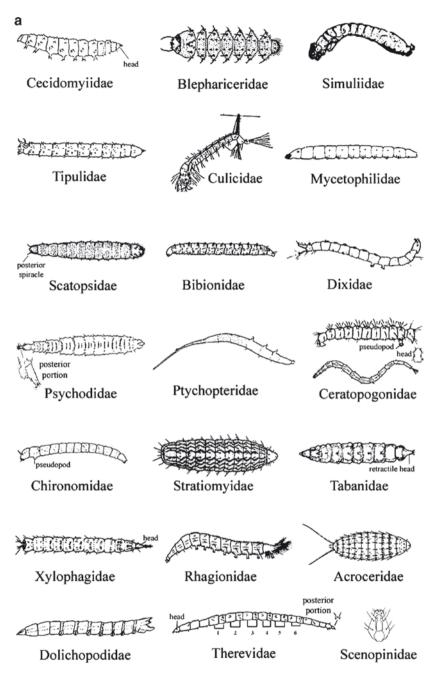


Fig. 2.6 (a) Larval morphology of the different Diptera families (adapted from Chu and Cutkomp, 1992). (b) Larval morphology of the different Diptera families (adapted from Chu and Cutkomp, 1992). (c) Larval morphology of the different Diptera families (Adapted from Chu and Cutkomp 1992)

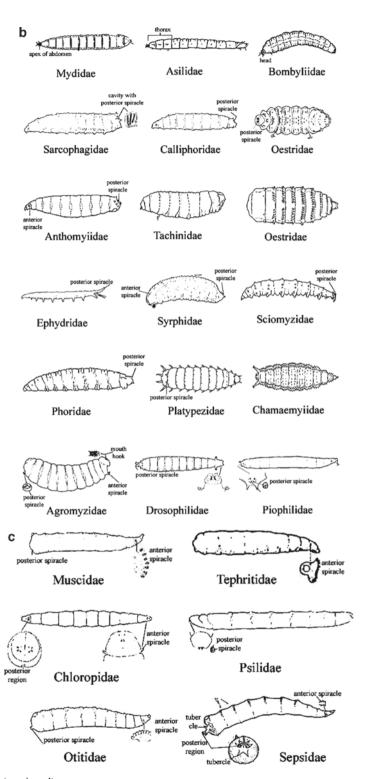


Fig. 2.6 (continued)

7.	Larvae with a row of spiracles on each side of body (peripneustic), or with
	at least rudimentary abdominal spiracles8
7′.	Larvae with spiracles on the prothorax and terminal abdominal segment (amphipneustic) or only on the terminal segment (metapneustic)
8.	Larvae with rudimentary abdominal spiracles; mouth with a large articulated
	process on each side that bears a number of long hairs and closes fan-like when
	at rest; posterior abdominal segments dilated, the last one armed on venter with a
	sucker-like disc that bears concentric series of bristlesSimulidae
8′.	Larvae with distinct though sometimes small abdominal spiracles; mouth
	without fan-like processes; posterior abdominal segments not noticeably
	dilated, the last one without sucker-like disc9
9.	Antennae elongate; body armed with conspicuous bristles or hairs10
9′.	Antennae usually short and inconspicuous, sometimes apparently absent; body without conspicuous bristles
10.	Posterior spiracles at the apices of a pair of long stalk-like processes
	Scatopsidae
۱۵	Posterior spiracles not noticeably elevated, situated near base of dorsal surface
	of posterior segment
11.	One pair of prolegs on each of first and second abdominal segments;
11.	body U-shaped when at rest
1.17	· · · · · · · · · · · · · · · · · · ·
ΙΙ΄.	No prolegs on abdominal segments, with possible exception on posterior
	segment
12.	All or some of the dorsal segments with narrow, sclerotized strap-like
	transverse bands; or the apical posterior segment in the form of a short,
	sclerotized tube; rarely, the ventral abdominal segments bear a central series
	of sucker-like discs
12′.	Dorsum without narrow; sclerotized, strap-like bands; posterior segment not in
	the form of a short sclerotized tube; ventral abdominal segments with sucker-
	like discs
13.	Antennae undeveloped, appearing as pale round spots on side of head with sclerites
	contiguous anteriorly, widely separated posteriorly Mycetophilidae
13′.	Not as described above
14.	Antennae stalked (pedunculate) usually well developed; ventral surface
	of head with sclerites contiguous for entire length, not separated widely
	posteriorly
14'	Antennae greatly reduced; abdomen elongated and inconspicuously segmented;
	three pairs of prolegs with hooks present
15.	
15′.	•
16	
16.	Aquatic larvae very slender, tapering toward both ends; without thoracic or
	anal foot-like appendages (pseudopods) or surface hairs (except about eight
	at apex of abdomen) or terrestrial larvae stout, with well-defined segments
	that are armed with strong, bristles, some of which are lanceolate; pseudopods
	present

16′.	Larvae rarely very slender, generally of an almost uniform thickness, rarely with the thoracic segments appreciably swollen but not fused; abdominal
	and thoracic segments frequently with rather noticeable soft hairs, the last segment almost invariably with a conspicuous tuft of hairs on dorsum near the apes; pseudopods almost always present, sometimes (very rare) only the
	thoracic one is distinguishable in terrestrial formsChironomidae
17.	Posterior spiracles close together, situated in a terminal or subterminal cleft or chamber, usually concealed; body surface roughened and leathery, or wholly or in part longitudinally striated
17′.	Posterior spiracles rather widely separated, visible, situated on apical segment, which may be truncated, sclerotized, or armed with apical processes; or spiracles on second to last (penultimate) or third to last (antepenultimate) are most body surface not revenue and are visible stricted.
18.	segment; body surface not roughened or visibly striated
10.	with lateral abdominal spiracles, without vestigial pseudopods; spiracular fissure transverse, sometimes rather small; pupae enclosed in larval
	skinStratiomyiidae
18′.	Head retractile; body cylindrical, surface not roughened, usually longitudinally striated; abdomen with a girdle of pseudopods on each segment; spiracular fissure vertical
10	Posterior spiracles situated on apical segment
	Posterior spiracles situated on apreal segment24
20.	Projecting portion of head and flattened apical plate of terminal abdominal
	segment heavily sclerotized, the former cone-shaped, entirely closed except at
	extreme apex and not retractile; the apical plate obliquely truncate and with
	projecting processesXilophagidae
20′.	Projecting portion of head more or less retractile, not cone-shaped, the movable portion not enclosed; apical abdominal segment without a heavily sclerotized flattened terminal plate
21.	Apical abdominal segment ending in two long processes that are fringed with long soft hairs; abdomen with paired pseudopods and fleshy dorsal and lateral appendages (in part, see also 22)
21′.	Apical abdominal segment not as described above; paired abdominal pseudopods usually absent; other abdominal appendages always absent22
22.	Apical abdominal segment ending in four short pointed processes or two
	fleshy lips; internal portion of head with a large, arched, sclerotized upper
	plate, the longitudinal rods and other head (cephalic) parts are on a horizontal plane
22′.	Apical abdominal segment not as described above, or the internal portion of
	head without an arched upper plate, and the longitudinal cephalic rods and other cephalic parts meet at right angles
23.	Apical abdominal segment without projecting processes, spiracles very small
	first instar with distinct segments and two long apical bristles on abdomen
	narasites of spiders Acroceridae

23'.	Apical abdominal segment frequently with projecting processes; spiracles large; species live in water, mud, earth, or decaying vegetable
	matterEmpididae or Dolichopodidae
24.	Posterior spiracles situated on the antepenultimate segment; abdominal
	segments 1–6 subdivided, the body apparently consisting of 20 segments exclusive of the head
24'	Posterior spiracles situated on penultimate segment; abdominal segments
21.	simple, the body apparently consisting of 11 or 12 segments exclusive of the head
25.	Posterior dorsal internal extension of head spatulate at apex; ventral posterior
	projections in the form of two short sclerotized rodsTherevidae
25′.	Posterior dorsal extension of head not spatulate at apex; ventral posterior
	projections absentScenopinidae
26.	Penultimate abdominal segment longer than ultimate, with a deep transverse
	depression near its apex giving it the appearance of two distinct segments;
	ultimate segment terminating in a sharp ridge with a median sharp point, on
	either side, of which dorsally and ventrally four very closely approximated
	hairs are situatedMydidae
26′.	Penultimate abdominal segment shorter than ultimate, or if longer then
	without a deep transverse depression; apical segment not as describe above,
	the hairs not closely approximated27
27.	Thoracic segments each with two long hairs, one on each side on ventrolateral
	margin; apical segment with six or eight long hairs; head well developed, forward
	protruded, and more or less cone-shaped when viewed from above, appearing flattened when viewed from side; penultimate abdominal segment usually shorter
	than ultimate or not much longer; body straight when alive
27′.	Thoracic segments without hairs or, if present, they are very weak; apical
21.	segment without distinguishable hairs; head slightly protruded, directed
	downward, not cone-shaped, with a dorsal protuberance when viewed from
	side; penultimate segment distinctly longer than ultimate; body usually
	curved in a half circle when alive
28.	Parasitic
28′.	Nonparasitic or predaceous
29.	Parasitic on insects and other arthropods
29′.	Parasitic on other animals
30.	Midle portion of body enlarged with strong spinous girdles31
	Body tapering; spines minute54
31.	Parasitic under animal skin, in nasal sinuses, or in throat; large and stout
	larvae that parasitize rabbits and rodents
31′.	Parasitic as describe above, and some species can be found in animal digestive
	system; larvae is known as bot or warble fliesOestridae
32.	Endoparasitic; portion end of body truncate with a button on posterior
	spiracles

32′.	Some species are endoparasitic	33
33.	Aquatic	34
33′.	Terrestrial	38
34.	Body smooth, without transverse folds (plicae) or tubercles	35
34′.	Body rough, with transverse plicae or tubercles	
35.	Prolegs distinct and with hooks	36
35′.	Prolegs not distinct; posterior spiracles in a cavity (see	also 54)
	Sarco	
36.	Posterior spiracles knob-like on conical projections; posterior prole	
	(see also 42')Antho	
36′.	Posterior spiracles on a prolongation of posterior segment; 6-8	
	prolegs, but ventral prolegs absent Ep	-
37.		
·	prolegs absent	myzidae
37′.	Posterior spiracles contiguous; ventral prolegs present	1 . 1
20	(see also 39')	
38.	<u>.</u>	
	Posterior spiracles not as described above	
39.	Saprophagous; body segments with processes	
	Predaceous or phytophagous; body with transverse plicae	
40.	Not associated with fungi	
	Associated with fungi	
41.	•	
	Not as described above	
42.	, , , ,	•
	Scavengers; body with spiny or setiferous processes	
43.	3	
	Anterior (prothoracic) spiracles adjacent	•
	Posterior portion truncate or rounded	
44′.	Posterior portion somewhat elongated or terminating in two subs	
	processes; if inconspicuous processes occur, the posterior spiral inconspicuous and usually light-colored	
15		
45. 45'	Posterior spiracles on two short processes	
	•	-
46.	One mouthhook; posterior spiracles sinuous (Fig.2.7)	
40 . 47.		
	Anterior (prothoracic) spiracles arranged in a circle of empse	
47. 48.		-
	Not described above	
40 . 49.		
サフ.	r osterior spiracies conspicuous, deepty pigniented	JI

49′.	Not described above
50.	Posterior spiracular slits oval
50'.	Posterior spiracular slits elongateTephritidae
51.	Posterior spiracles with a distinct pointed process on the dorsal margin
	of ring
51'.	Not as described above Otitidae
52.	Posterior spiracles on elevated projections
52'.	Posterior spiracles sessile
53.	Slits of posterior spiracles slender and long
53′.	Slits of posterior spiracles short and radial Anthomyiidae
54.	Slits of posterior spiracles nearly transverse, button almost always distinct
	(Fig.2.7); posterior spiracles not in a cavity (Fig.2.8)
54'.	Slits of posterior spiracles nearly vertical, button indistinct (Fig.2.7); posterior
	spiracles are enclosed within a deep cavity (Fig.2.8) Sarcophagidae



Fig. 2.7 Posterior spiracles of major Diptera families found on corpses

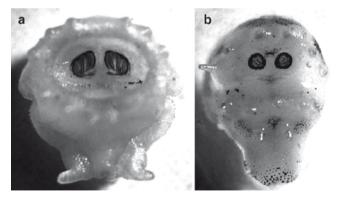


Fig. 2.8 Posterior aspect of a sarcophagid larvae (a) and a calliphorid larvae (b). The posterior spiracles of sarcophagid larvae are enclosed within a deep stigmal cavity

2.3.3 Key to Third Instar Larvae of the Most Carrion Breeding and Feeding Dipteran Species from Brazil

This key was produced by reference to specimens in the author's own collection. Other keys and descriptions by the following authors were also consulted: Zumpt (1965), Ishijima (1967), Prins (1982), Greenberg and Szyska (1984), Smith (1986), Erzinclioglu (1987), Queiroz and Carvalho (1987), Liu and Greenberg (1989), Guimarães and Papavero (1999), Wells et al. (1999), Greenberg and Kunich (2002), and Thyssen and Linhares (2007).

2.3.3.1 Nomenclature according to Fig. 2.3

1.	Larvae with an obvious head that is sclerofized and clearly differentiated from the rest of the body; body flattened; mouthhook moving vertically, parallel to each other like a pair of hooks
1′.	Larvae without an obvious head, which merges with the rest of the body although the mouthparts may be obvious
2.	Surface of the body segments with obvious fleshy or spinous processes3
2′.	Surface of the body segments without processes, although the integument itself may have strong spines
3.	Body flattened dorsoventrally and with numerous projections4
3′.	Body cylindrical5
4.	Each projections with short and broad spines on the basal portion
4′.	Each projections with large branches; lateral part of segment 12 with three feather-like large projections, with more than five forked branches
5.	Larger longer pointed fleshy processes laterally and dorsally ending up in a tuff of hairs or crown of spines strongly pigmented on the apex; posterior spiracles in a cleft on posterior face of anal segment and consisting of flattened plates with three slits
5′.	Small white, slightly flattened larvae up to 4 mm long with short processes on the dorsal and lateral surfaces; posterior spiracles on brown, sclerotized tubercles, each with a narrow opening
6.	Posterior spiracles are enclosedin a deep cavity
6′.	Posterior spiracles not enclosed in a deep cavity
7.	Peritreme of posterior spiracle complete, although sometimes weaker in the region of button
7′.	Peritreme of posterior spiracle incomplete and not enclosing button, or it is sometimes indistinct

8.	plate covered with small spines; anal plate surrounded by small spines
8′.	Slits of posterior spiracles straight or at most arcuate
9.	Slits of posterior spiracles almost parallels; anterior spiracles with 10 lobes; anal segment with ventral lobes much longer than the dorsal lobes
9′.	Peritreme with button distinct
10.	Slits of posterior spiracles bent distally (3 o'clock position); posterior margin of ventral surface of segment 10 with a row of numerous small spines; anterior margin of anal plate convex and posterior margin concave, lateral part of anal plate bent posteriorly
10′.	Accessory oral sclerite absent
11.	Posterior dorsal margin of segment 11 withoutrows of spines
11′.	Posterior dorsal margin of segment 11 with rows of spines
12.	Inner dorsal tubercles of the posterior region separated from each other by a distance approximately equal to the distance between the inner and median dorsal tubercles; very few spines present dorsal to anusLucilia sericata(Calliphoridae)
12′.	Inner dorsal tubercles of the posterior region separated from each other by a distance approximately equal to the distance between the inner and outer dorsal tubercles; strong patch of spines present dorsal to anusLucilia cuprina(Calliphoridae)
13.	Posterior margin of segment 11 without dorsal spines; in other segments of the body, spines multipointed, each with at least two teeth (sometimes up to four); the teeth are well separated from one another and having rounded tips; main tracheal trunks leaving anterior spiracles are not black-pigmented
13′.	Posterior margin of segment 11 with dorsal spines; button distinct or
	indistinct
	$All\ spines\ single-pointed\ and\ small\ \textit{Sarconesia chlorogaster} (Calliphoridae)$
14′.	Spines single or multipointed
15.	Lateral fusiform areas absent; with accessory dental sclerite16
15′.	Lateral fusiform areas present; with or without accessory dental sclerite $\dots 17$
16.	Spines single and multipointed, especially large and numerous ventrad on segment 12; peritreme is moderately sclerotized with delicate peritreme; anal plate well extended
16′.	Spine pattern follows the described above; peritremeis strongly sclerotized; a littledistinguished button; anal plate is short

17.	With accessory dental scleriteCompsomyiops spp. (Calliphoridae)
17′.	With or without accessory dental sclerite
18.	Spine pattern on anal protuberance convex or bell-shaped; mouthhook devoid of accessory oral sclerite; anterior spiracleswith 10–12 branches
18′.	Spine pattern on anal protuberance V- or U-shaped
19.	Mature third instar blue-gray; devoid of accessory oral sclerite
19′.	Mature third instar non-pigmented and with accessory dental sclerite20
20.	Mouthhook with the tooth-like apical portion longer than greatest depth of the basal portion; accessory oral sclerite present between the mouthhook; peritreme complete and posterior spiracles smaller and separated by a distance equal to or greater than the width of a single spiracle
20′.	Mouthhook with small accessory oral sclerite and comma-shaped; peritremes of the posterior spiracles separated one from the other by a distance equal to

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approximately one-third to one-half the diameter of one of the peritremes; anterior spiracles with 8–10 short branches Chrysomya megacephala(Calliphoridae)

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