Chapter 3
Key for the Identification of Third Instars of European Blowflies (Diptera: Calliphoridae) of Forensic Importance

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3.1 Introduction

In Europe larvae of blowflies are the main group of insects responsible for decomposition of exposed vertebrate remains, including the human body. This determines their high forensic importance and frequent application for estimation of PMI. The importance of proper identification of insects collected in forensic cases and experiments to the species level is underlined by all manuals of forensic entomology (e.g. Smith 1986; Byrd and Castner 2001; Greenberg and Kunich 2002). Especially difficult is the identification of the larval stages, where breeding to the adult stage or DNA-based methods are recommended. Fortunately, the available knowledge of the morphology of third instars of Calliphoridae is sufficiently good to allow the preparation of a complete identification key for at least all European species of forensic importance. Eleven species are included in the key. Most of them are widespread through Europe (Rognes 2004) and have been frequently reported from both real cases and carrion experiments, and the necessity of their inclusion into the key cannot be questioned. There are: *Calliphora vicina*, *C. vomitoria*, *Chrysomya albiceps*, *Phormia regina*, *Protophormia terraenovae*, *Lucilia caesar*, *L. illustris*, *L. sericata*. The author has also decided to add three additional species to the key: *Cynomya mortuorum*, *Chrysomya megacephala*, and *Lucilia ampullacea*. Recently, the larvae of *Cynomya mortuorum* were recorded from human corpses at least twice (Stærkeby 2001; Benecke 2002). Smith (1986) points out this species as rather a late newcomer in comparison to other blowflies, but recent research on succession shows that in spring conditions *Cynomya mortuorum* may be among the first colonizers of pig carrion (Szpila et al. 2008). *Chrysomya megacephala* is the newly discovered species in Europe, with distribution still restricted to the Spanish mainland, Canary Is. (Rognes 2004), Malta (Ebejer 2007), and Madeira (Martínez and Rognes 2008). In the tropical regions,

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this species is very common and abundant on human corpses; in continental Spain, the first record of the development of *Chrysomya megacephala* on pig carrion was recently reported (Velásquez et al. 2008). *L. ampullacea* may develop on large vertebrate carrion at least in Central European conditions (Grunwald et al. 2009) and has been reported from human corpses (Benecke 1998). A few species of *Calliphora* Robineau-Desvoidy other than *C. vicina* or *C. vomitoria* were also recorded in pig carrion experiments, but only as single adult flies and they are hence not included in this key. Also, *Lucilia cuprina* (Wiedemann, 1830) known from the Iberian Peninsula (Rognes 2004), is not included there. This facultative parasite may also develop in carrion but has not been recorded so far in any real case or carrion experiment in European conditions.

Original information concerning the morphology of third instars of European, forensically important blowflies is scattered in many papers (Knipling 1936; Hall 1948; Zimin 1948; Kano and Sato 1952; Schumann 1954, 1965, 1971; Ishijima 1967; Kitching 1976; Teskey 1981; Prins 1982; Holloway 1985, 1991; Erzinçlioğlu 1985, 1987a, b, 1988, 1990; Smith 1986, 1989; Shewell 1987; Liu and Greenberg 1989; Carvalho Queiroz et al. 1997; Fan et al. 1997; Wells et al. 1999; Wallman 2001; Povolný 2002; Grassberger et al. 2003; Sukontason et al. 2003, 2008; Zumpt 1965). Several keys have been compiled so far, but none of them cover the current complete list of species. The most comprehensive, according to this list, is the key of Ishijima (1967) where only *C. mortuorum* and *Ch. albiceps* are absent. Other important keys have been provided by Schumann (1954, 1971); especially valuable is his contribution to the knowledge of the larval stages of European species of *Lucilia* Robineau-Desvoidy. Erzinçlioğlu (1985, 1987a, b, 1988, 1990) published a series of excellent papers with descriptions and keys covering all third instars of the European, forensically important Calliphorinae and Chrysomyinae. Data about third instar morphology of Central European Calliphoridae were summarized by Draber-Mońko (2004). In her monograph (written in Polish) are accumulated all the figures useful for identification of the larvae of Central European blowflies, published before year 2003.

A critical review of the morphological characters of third instars of Calliphoridae was provided by Erzinçlioğlu (1985). Experience from the continuous work of the author on larval morphology of blowflies makes it possible to point a few morphological details that should be used for taxonomic purposes with special caution. They are the small sclerotised spot below the posterior tip of ventral cornua (used in *Lucilia* identification), the direction of the process on the postero-dorsal angle of the basal part of mouthhooks (*Lucilia*), spinulation on the last abdominal segments (*Lucilia*), and the shape of the peritreme (Chrysomyinae vs other blowflies). The most difficult part of the presented key is in distinguishing the larvae of the two closely related species *L. caesar* and *L. illustris*. It may be done only on the basis of two characters (from those listed above, see also key), both of which are difficult to see and need preparation of light microscope slides. The presence of an interrupted peritreme of posterior spiracles cannot be used as feature is characteristic only for larvae of subfamily Chrysomyinae (Erzinçlioğlu 1985; Wallman 2001). Such form of peritreme is present at least in some specimens of *C. vicina* (Erzinçlioğlu 1985; see also Fig. 3.4i). Also the value of spiracular distance factor

(SDF) may vary according to the size of the maggots and the techniques of preparation. This measure should be used only for the fully grown third instars. Moreover, Wallman (2001) recommends using this measure only for freshly killed larvae. It is also important to mention significant doubts, reported recently (Hale et al. 2008) concerning the reliability of some important morphological characters used in the present key (presence of sclerotised oral sclerite). However, the continuing extensive work of the author on larval material has not confirmed this observation so far.

The present key for the identification of third instar larvae is the first to cover all European species of forensic importance. Thanks to the opportunity provided by editors, all significant characters are illustrated in the form of color pictures, taken using a digital camera mounted on the microscopes. The black and white figures are not included here but are easily available in the references listed below. This key has been seriously tested before publication and seems to work well; in doubtful cases, however, the author recommends that the identifications should be checked against the keys and descriptions published in the listed references.

3.2 Material and Methods

Third instars of *C. vicina*, *C. vomitoria*, *P. regina*, *P. terraenovae*, *L. ampullacea*, *L. illustris*, and *L. sericata* were bred from eggs deposited by females collected in the city of Toruń in Northern Poland (53°00′N, 18°35′E). At least a part of the larvae in all the cases were bred to adult form for unquestionable species identification. Third instars of *C. mortuorum*, *Ch. albiceps,* and *L. caesar* were collected during research on the insect succession on pig carrion conducted on Biedrusko Military Range in Western Poland (52°31′N, 16°54′E) and identified as larvae using suitable references. Specimens of *C. megacephala* were available for investigation thanks to Professor Kabkaev Sukontason and Professor Kom Sukontason (Chiang Mai University) who provided larval material from Thailand.

All larvae were killed by soaking in hot water (about 95°C), and then stored in 80% ethanol. This technique of preservation is often recommended for the forensic entomologist as it is very convenient and can be used even in poorly equipped laboratories.

For preparation of slide, larvae were macerated for 24 h in a cold solution of 5% KOH. Next, the particular fragments of the body were mounted in Hoyer's medium or dehydrated through 80%, 90%, and 99.5% ethanol and mounted in Euparal. For the cephaloskeletons, concave slides, and for other morphological details, flat slides were used.

A digital Nikon 8400 camera mounted on a Nikon Eclipse E200 microscope and a Nikon SMZ1500 stereomicroscope were used for photomicrography.

Larval terminology follows Courtney et al. (2000) and Szpila and Pape (2007). The spiracular distance factor was calculated according to Erzinçlioğlu (1985) (SDF = a/b, see Fig. 3.4m).

In the references, species originally figured in a particular paper are listed in square brackets after the reference.

3.3 General Morphology

The body of larvae in necrophagous Calliphoridae follows the general pattern for Calyptrata in being divided into a bilobed pseudocephalon, three thoracic segments (termed TI–TIII below), seven abdominal segments (AI–AVII), and the anal division (AD) (Fig. 3.1a). Third instar is easily distinguishable from the other instars by the

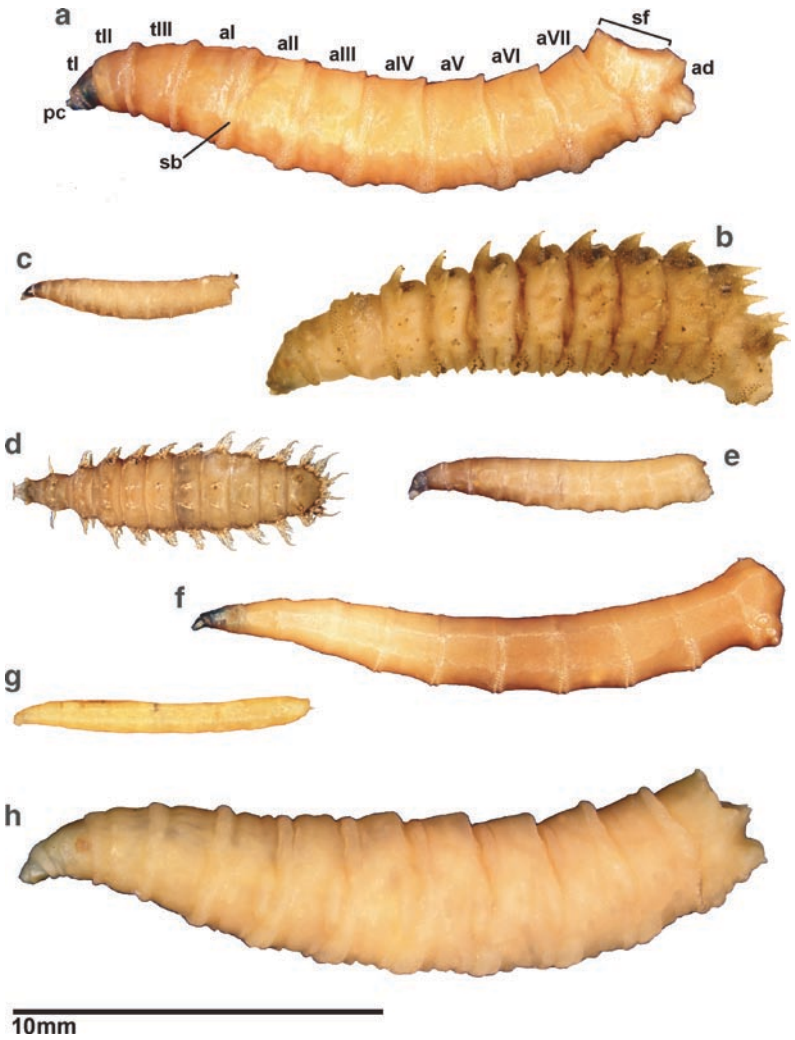

Fig. 3.1 Third instars of necrophagous flies: **a** – Calliphoridae, *Calliphora vomitoria*; **b** – Calliphoridae, *Chrysomya albiceps*; **c** – Sepsidae, *Nemopoda nitidula*; **d** – Fannidae, *Fannia coracina*, dorsal view; **e** – Heleomyzidae, unidentified species; **f** – Muscidae, *Hydrotaea dentipes*; **g** – Piophilidae, *Stearibia foveolata*; **h** – Sarcophagidae, *Sarcophaga caerulescens*. Abbreviations: ad – anal division, aI-VII – abdominal segments, pc – pseudocephalon, sb – spinose band, sf – spiracular field, tI-III – thoracic segments

presence of anterior spiracles (vs first instar) and the number of slits of posterior spiracles (vs second instar) (Fig. 3.2a–f). Each of the pseudocephalic lobes of a larva has an antennal complex with the antennal dome situated on a basal ring (Fig. 3.2g). The maxillary palpus is located on the anterior surface of the pseudocephalic lobe and has the form of a flattened protuberance with numerous sensilla. Above and lateral to the mouth opening is the ventral organ. The functional mouth opening is closed from below by a triangular labial lobe with two sensilla of labial organ. Numerous oral ridges are present on ventro-lateral surfaces of the pseudocephalon. The internal cephaloskeleton consists of massive paired mouthhooks, a small oral sclerite, small paired dental sclerites, unpaired intermediate and labial sclerites and paired basal sclerites with parastomal bar, dorsal bridge, vertical plate, and dorsal and ventral cornua (Fig. 3.4a). The

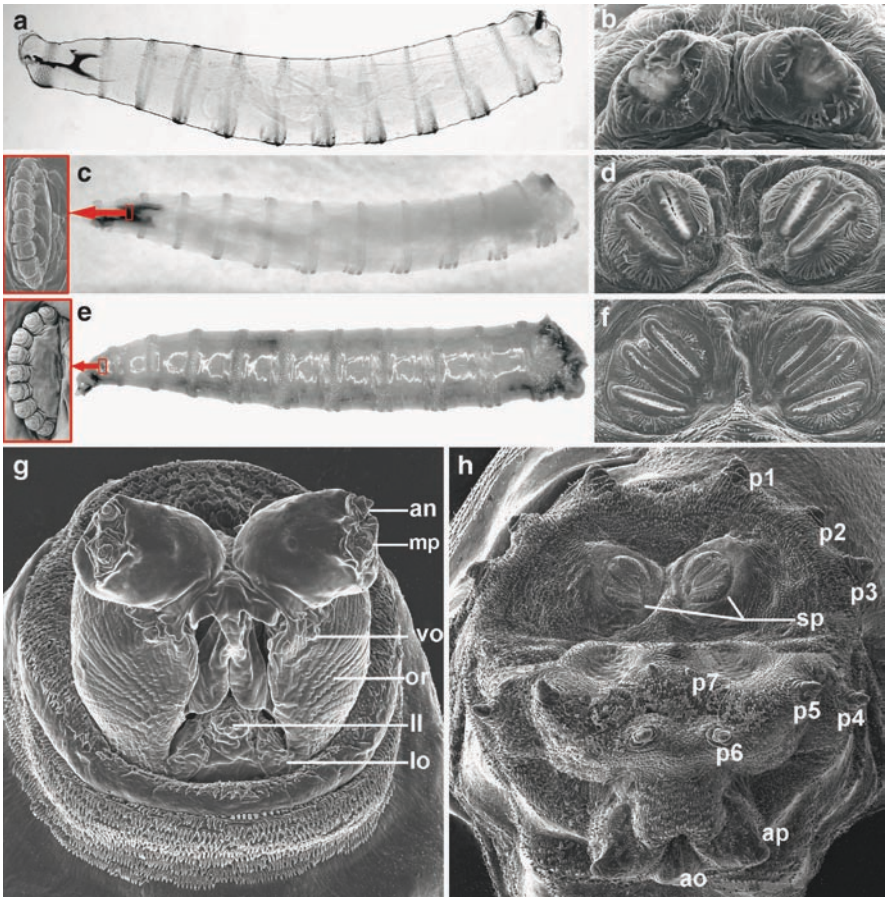

Fig. 3.2 Larvae of necrophagous Calliphoridae: **a** – first instar, habitus; **b** – first instar, posterior spiracles; **c** – second instar, habitus and anterior spiracles; **d** – second instar, posterior spiracles; **e** – third instar, habitus and anterior spiracles; **f** – third instar, posterior spiracles; **g** – third instar, pseudocephalon; **h** – third instar, anal division. Abbreviations: an – antenna, ao – anal opening, ap – anal pad, ll – labial lobe, lo – labial organ, mp – maxillary palpus, or – oral ridges, p1-7 – papillae 1-7, sp – posterior spiracles, vo – ventral organ

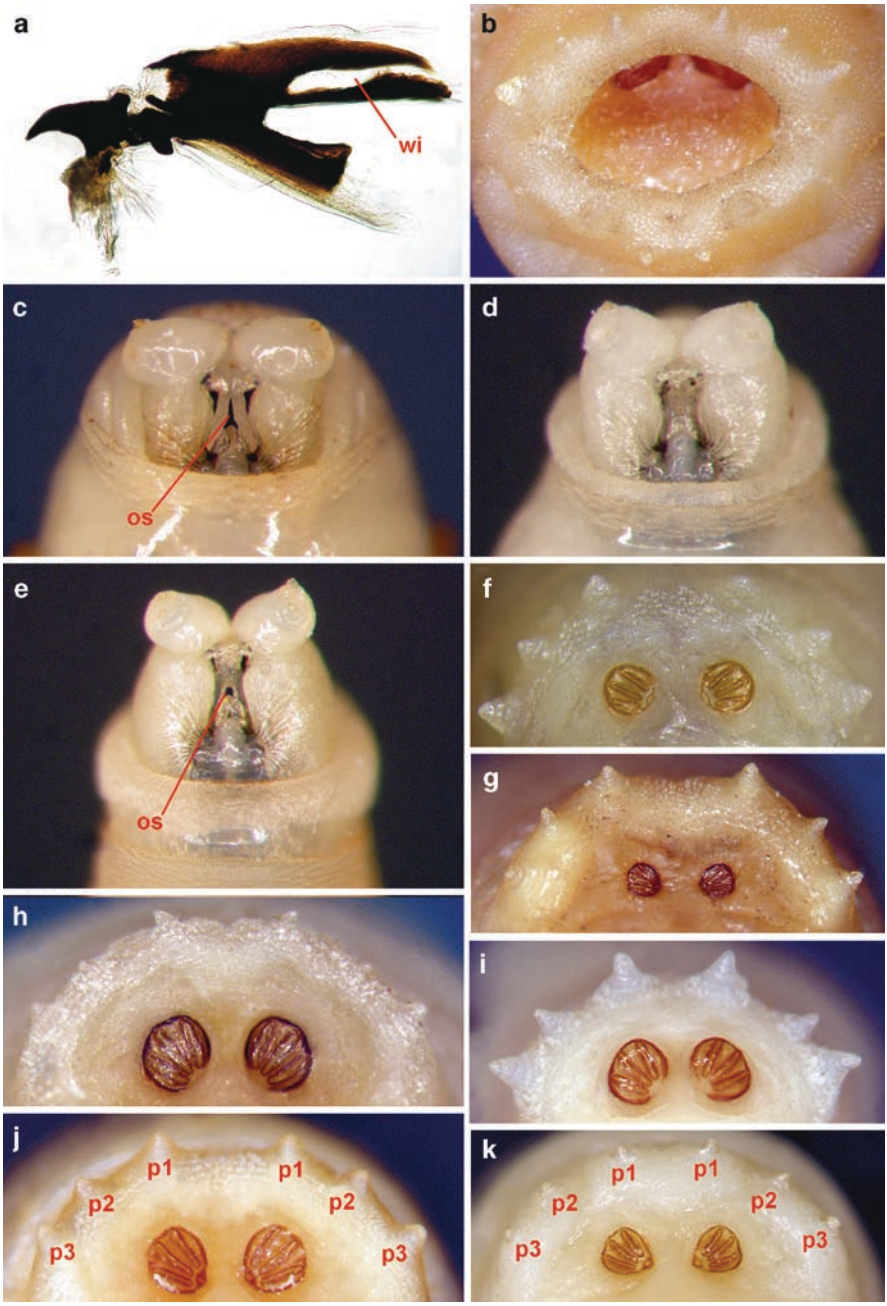

Fig. 3.3 Third instars of necrophagous Sarcophagidae and Calliphoridae: **a** – *Sarcophaga caerulescens*, cephaloskeleton, lateral view; **b** – *Sarcophaga caerulescens*, anal division, spiracular cavity; **c** – *Calliphora vomitoria*, pseudocephalon, ventral view; **d** – *Lucilia sericata*, pseudocephalon, ventral view; **e** – *L. ampullacea*, pseudocephalon, ventral view; **f** – *Calliphora vicina*, anal division, upper half of spiracular field; **g** – *Cynomya mortuorum*, anal division, upper half of spiracular field; **h** – *Phormia regina*, anal division, upper half of spiracular field; **i** – *Protophormia terraenovae*, anal division, upper half of spiracular field; **j** – *Lucilia illustris*, anal division, upper half of spiracular field; **k** – *Lucilia sericata*, anal division, upper half of spiracular field. Abbreviations: os – oral sclerite, p1-3 – papillae 1-3, wi – window

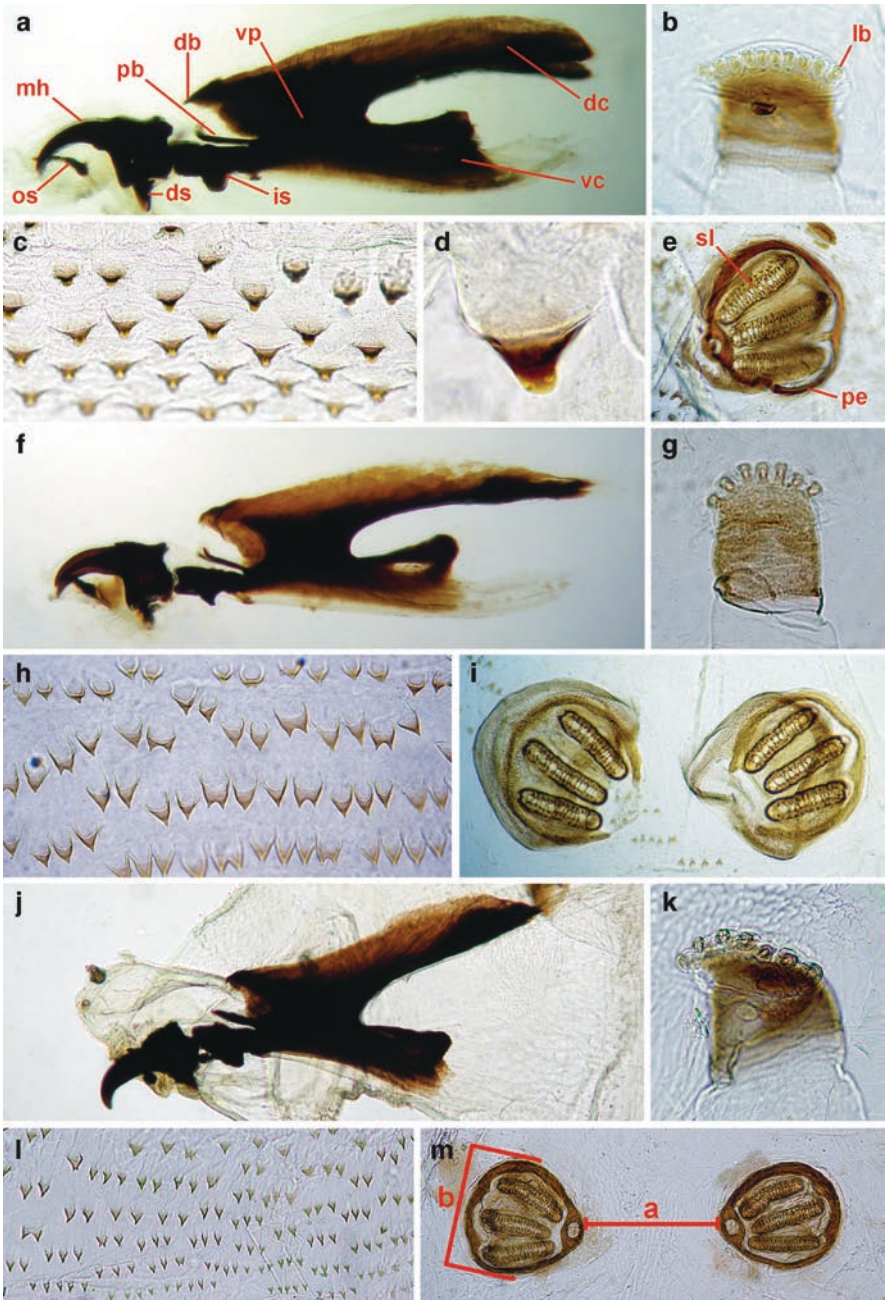

Fig. 3.4 Third instars of *Calliphora* and *Cynomya*: **a** – *Calliphora vomitoria*, cephaloskeleton, lateral view; **b** – *C. vomitoria*, anterior spiracle; **c** – *C. vomitoria*, thoracic segment III, spines; **d** – *C. vomitoria*, thoracic segment III, spine; **e** – *C. vomitoria*, posterior spiracle, **f** – *Calliphora vicina*, cephaloskeleton, lateral view; **g** – *C. vicina*, anterior spiracle; **h** – *C. vicina*, thoracic segment III, spines; **i** – *C. vicina*, posterior spiracles; **j** – *Cynomya mortuorum*, cephaloskeleton, lateral view; **k** – *C. mortuorum*, anterior spiracle; **l** – *C. mortuorum*, thoracic segment III, spines; **m** – *C. mortuorum*, posterior spiracles. Abbreviations: a – distance between posterior spiracles, b – diameter of posterior spiracle, db – dorsal bridge, dc – dorsal cornu, ds – dental sclerite, is – intermediate sclerite, lb – lobe of anterior spiracle, mh – mouthhook, os – oral sclerite, pb – parastomal bar, pe – peritreme, sl – slit of posterior spiracle, vc – ventral cornu, vp – vertical plate

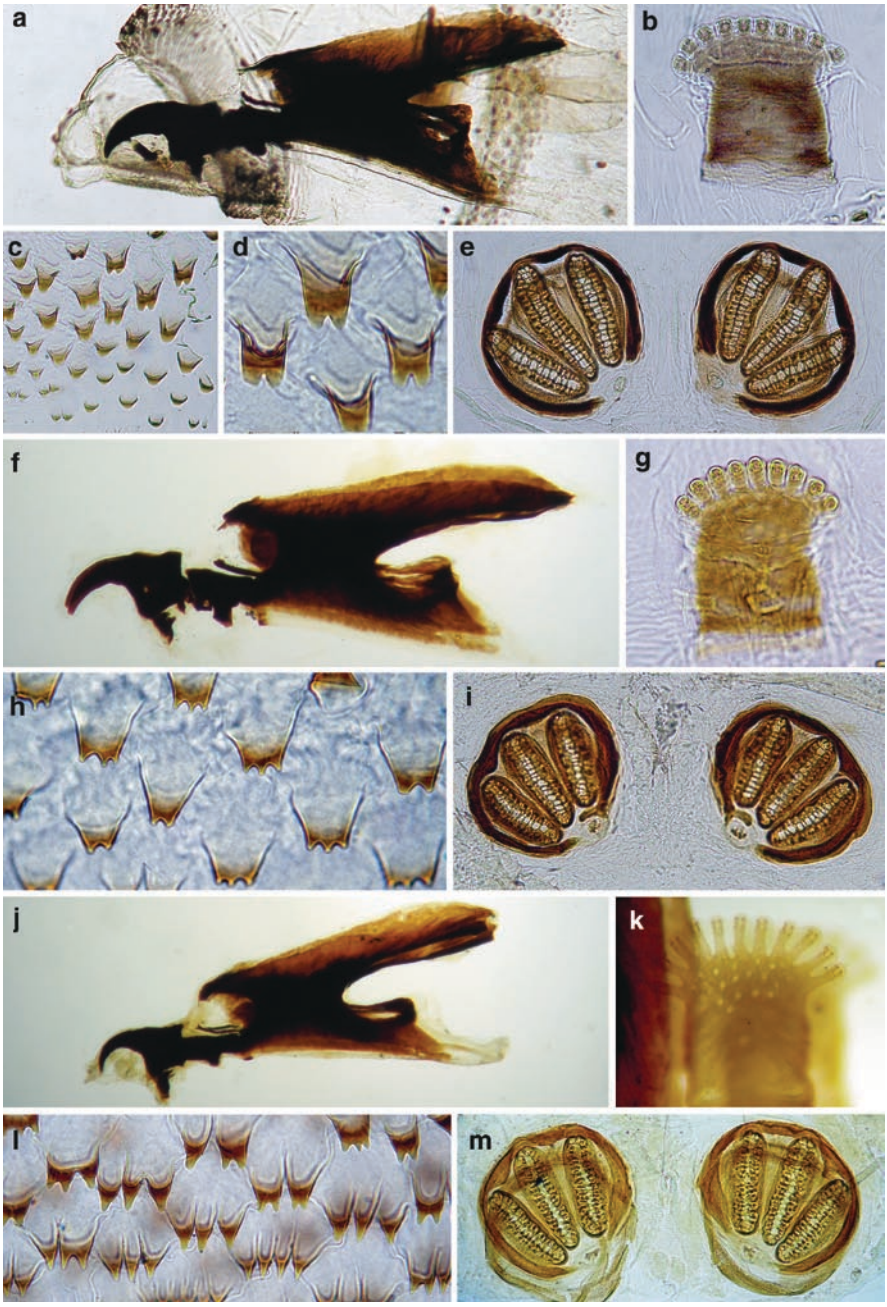

Fig. 3.5 Third instars of Chrysomyinae: **a** – *Chrysomya megacephala*, cephaloskeleton, lateral view; **b** – *C. megacephala*, anterior spiracle; **c** – *C. megacephala*, thoracic segment III, spines; **d** – *C. megacephala*, thoracic segment III, spines; **e** – *C. megacephala*, posterior spiracles, **f** – *Phormia regina*, cephaloskeleton, lateral view; **g** – *P. regina*, anterior spiracle; **h** – *P. regina*, thoracic segment III, spines; **i** – *P. regina*, posterior spiracles; **j** – *Protophormia terraenovae*, cephaloskeleton, lateral view; **k** – *P. terraenovae*, anterior spiracle; **l** – *P. terraenovae*, thoracic segment III, spines; **m** – *P. terraenovae*, posterior spiracles

mouthhooks are strongly sclerotised and divided into sharp curved anterior part and broad basal part. The intermediate sclerite is located between the mouthhooks and the basal sclerite (Fig. 3.4a). The basal sclerite is the most posterior part of the cephaloskeleton. Both parts of the basal sclerite are connected dorso-anteriorly by a dorsal bridge. The parastomal bar has the form of a thin rod directed anteriorly. The vertical plate is broad. The dorsal cornu is longer than the ventral cornu. The postero-dorsal part of the ventral cornu is equipped in a small window (Fig. 3.4f). Segments TI–TIII are equipped with spinose bands only anteriorly (Fig. 3.1a). On each lateral surface of TI is the anterior spiracle with the number of lobes varying according to particular species (Figs. 3.4b, g, k, 3.5b, g, k, and 3.6g, j, m). The number of lobes also shows some intraspecific variation. Segments AI–AVII are armed with both anterior and posterior spinose bands. The width of the anterior bands decreases toward the posterior end of the body, whereas the width of the posterior spinose bands increases in this same direction. Spinose bands are often incomplete, especially on terminal segments (AV–AVII). The shape of the spines shows infraspecific variation in size, shape, and arrangement. The tip of the spines may be single (Figs. 3.4c, l and 3.6c) or serrated (Fig. 3.5d, h, l). Particular spines are arranged separately (Figs. 3.4c and 3.5h) or in irregular rows (Figs. 3.4h, l and 3.6c). Abdominal segments are followed by the terminal region of the larval body, the anal division. The most conspicuous parts of the anal division of the blowfly's third instar are the spiracular field (Fig. 3.1a) and the anal area. The spiracular field consists of seven pairs of papillae situated marginally along its outer surface and posterior spiracles situated centrally (Figs. 3.2h and 3.3f–k). The size and position of the papillae are characteristic for particular species and the dorsalmost pairs of papillae have special taxonomic importance (Figs. 3.3f–k). Each posterior spiracle possesses three linear slits (Fig. 3.4e). The peritreme of the posterior spiracle may completely surround the spiracle (Figs. 3.4a, m and 3.6f, i, l) or be interrupted at some distance (Figs. 3.4i and 3.5e, i, m). The ratio of the distance between the posterior spiracles and their diameter is also of taxonomic value (Fig. 3.4 m). In the anal area, two conical and fleshy anal pads flank the slit-like anal opening (Fig. 3.2h). The shape of the anal pads is rather conservative among the third instars of necrophagous blowflies. The arrangement of spines around the anal area shows intraspecific variation.

3.4 Key

1. – body cylindrical and tapering (Fig. 3.1a, b), cephaloskeleton without long window in dorsal cornua and with developed parastomal bar (Fig. 3.4a), anterior spinose bands on all thoracic and most abdominal segments fully developed (Fig. 3.1a, b), posterior spiracles never in deep spiracular cavity or on long stalks, around spiracular field seven pairs of papillae (Figs. 3.2h and 3.3f–k), slits of posterior spiracles linear (Fig. 3.4e) . . . . . 2 (Calliphoridae)
   – other combination of characters (Figs. 3.1c–g and 3.3 a, b) . . . . . other Diptera
2. – abdominal segments of the larva with numerous fleshy protuberances (Fig. 3.1b) . . . . . Chrysomya albiceps (Wiedemann, 1819)

– abdominal segments of the larva without such protuberances. 3

3. – oral sclerite at least partly sclerotised (Figs. 3.3c, e, 3.4a, f, j, 3.5a and 3.6a). . . 4
– oral sclerite unsclerotised (Figs. 3.3d, 3.5f, j and 3.6e, h, k). 8

4. – sclerotised part of the oral sclerite small, almost circular (Figs. 3.3e, 3.5a and 3.6a) 5
– oral sclerite well sclerotised along the whole length (Figs. 3.3c and 3.4a, f, j) 6

5. – spines big, robust, often with serrated tips (check on the thoracic segments) (Fig. 3.5c, d); posterior spiracles close to each other and with incomplete peritreme (Fig. 3.5e) ..... Chrysomya megacephala (Fabricius, 1794)
– spines small, with single tips, arranged in short rows (check on the thoracic segments) (Fig. 3.6c); posterior spiracles wide apart and with complete peritreme (Fig. 3.6d) . . . . . Lucilia ampullacea Villeneuve, 1922

6. – spines big, arranged separately (check on the thoracic segments) (Fig. 3.4c, d) . . . . . Calliphora vomitoria (Linnaeus, 1758)
– spines small, arranged in short rows (check on the thoracic segments) (Fig. 3.4h, l) 7

7. – apical part of mouthhooks gently curved (Fig. 3.4f); posterior spiracles relatively close together (SDF ≈ 1.0) (Figs. 3.3f and 3.4i) . . . . . .Calliphora vicina (Robineau-Desvoidy, 1830)
– apical part of mouthhooks abruptly curved (Fig. 3.4j); posterior spiracles very wide apart (SDF> 1.2) (Figs. 3.3g and 3.4m) . . . . . Cynomya mortuorum (Linnaeus, 1761)

8. – spines on thoracic segments predominantly with serrated tips (Fig. 3.5h, l), spines of different sizes 9
– spines with serrated tips on thoracic segments absent (Fig. 3.6c), all spines small of similar size 10

9. – papillae around the spiracular field very big (Fig. 3.3i), all posterior spinose bands incomplete . . . . . Protophormia terraenovae (Robineau-Desvoidy, 1830)
– papillae around the spiracular field small (Fig. 3.3h), at least some posterior spinose bands complete . . . . . Phormia regina (Meigen, 1826)

10. – cephaloskeleton without sclerotised area below the posterior tip of ventral cornua (Fig. 3.6k), distance between each P1 similar to distance between P1 and P2 (Fig. 3.3k) . . . . . Lucilia sericata (Meigen, 1826)
– cephaloskeleton with sclerotised area below the posterior tip of ventral cornua (Fig. 3.6e, h), distance between each P1 larger than distance between P1 and P2 (Fig. 3.3j) 11

11. – postero-dorsal angle of the basal part of the mouthhook with process directed postero-dorsally (Fig. 3.6e), posterior spinose band on abdominal segment VI interrupted dorsally (Fig. 3.6n). . . . . Lucilia caesar (Linnaeus, 1758)
– postero-dorsal angle of the basal part of the mouthhook with process directed posteriorly (Fig. 3.6h), posterior spinose band on abdominal segment VI complete (Fig. 3.6o) . . . . . Lucilia illustris (Meigen, 1826)

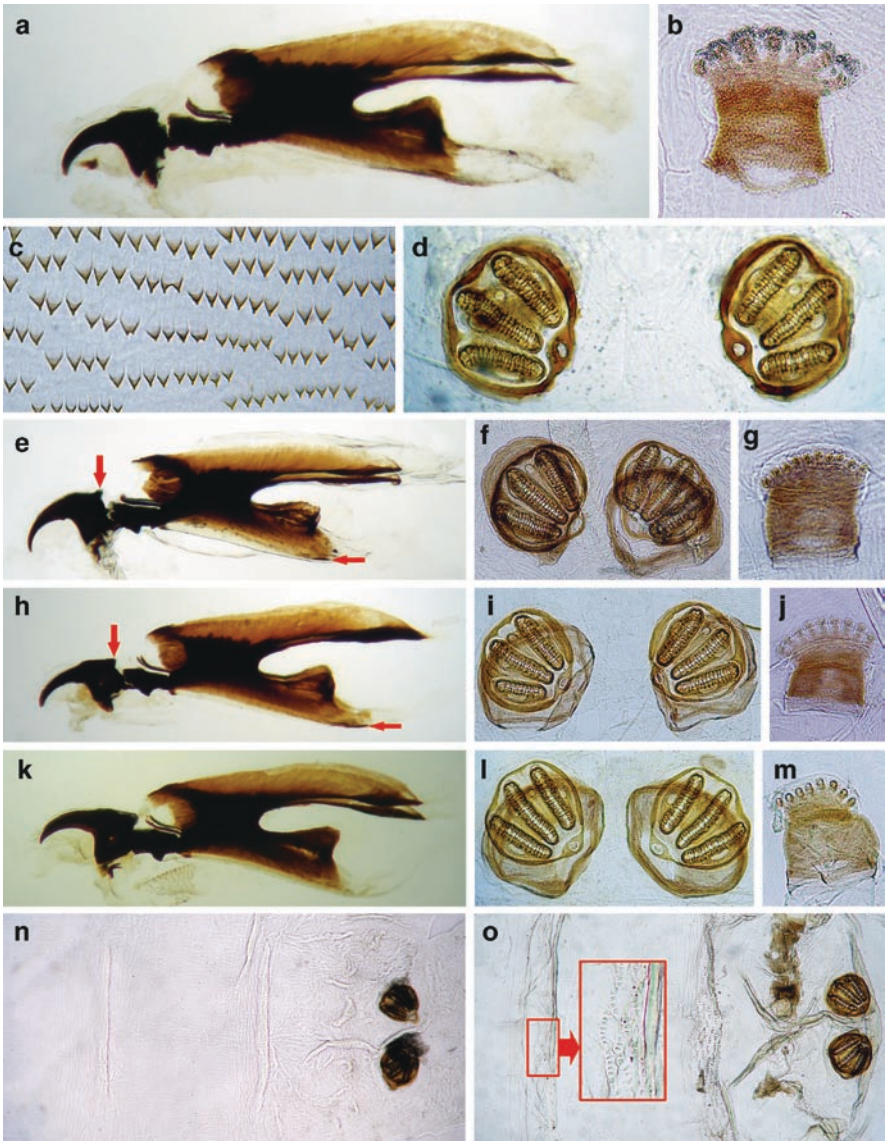

Fig. 3.6 Third instars of *Lucilia*: **a** – *Lucilia ampullacea*, cephaloskeleton, lateral view; **b** – *L. ampullacea*, anterior spiracle; **c** – *L. ampullacea*, thoracic segment III, spines; **d** – *L. ampullacea*, posterior spiracles, **e** – *L. caesar*, cephaloskeleton, lateral view; **f** – *L. caesar*, posterior spiracles; **g** – *L. caesar*, anterior spiracle; **h** – *L. illustris*, cephaloskeleton, lateral view; **i** – *L. illustris*, posterior spiracles; **j** – *L. illustris*, anterior spiracle; **k** – *L. sericata*, cephaloskeleton, lateral view; **l** – *L. sericata*, posterior spiracles; **m** – *L. sericata*, anterior spiracle; **n** – *L. caesar*, abdominal segment VII, dorsal view; **o** – *L. illustris*, abdominal segment VII, dorsal view

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