KEY DIAGNOSTIC CHARACTERISTICS OF THE DEVELOPMENTAL STAGES OF FORENSICALLY IMPORTANT CALLIPHORIDAE AND SARCOPHAGIDAE IN CENTRAL SOUTH AFRICA

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PREFACE

I declare that the thesis hereby submitted by me for the Ph.D. degree at the University of the Free State is my own independent work and has not previously been submitted by me at another university / faculty. I further more cede copyright of the thesis in favour of the University of the Free State

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S. L. Brink

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ABSTRACT

The first insects attracted to a decomposing body are usually representatives of the Diptera, in particular members of the families Calliphoridae (blow flies) and Sarcophagidae (flesh flies). These flies will deposit their eggs (or often larvae in the case of sarcophagids) on the body, within a few hours after death, depending on environmental conditions. The immature stages will complete their developmental cycle on and around the body. As a consequence, these insects are of great importance in forensic entomology; the main area of application being the determination of the postmortem interval (PMI). One of the key pieces of information needed to calculate the PMI is the correct species identification of the immature stages.

The aim of this study was to provide diagnostic descriptions for the immature stages of forensically important calliphorids and sarcophagids in central South Africa. Forensically important calliphorids prevalent in this region are *Lucilia cuprina* (Wiedemann), *Lucilia sericata* (Meigen), *Chrysomya chloropyga* (Wiedemann), *Chrysomya marginalis* (Wiedemann), *Chrysomya albiceps* (Wiedemann) and *Calliphora vicina* Robineau-Desvoidy. The sarcophagid prevalent in the region is *Sarcophaga cruentata* Meigen. A range of characteristics was evaluated by means of light and scanning electron microscopy and the most pertinent of these were identified for diagnostic purposes. The eggs were evaluated in terms of nine characteristics; six of which were of no diagnostic use, while three could be used to identify some of the species. The eggs of *C. chloropyga* and *C. albiceps* were indistinguishable from each other. Twelve features were identified for their possible diagnostic value in larvae. In first instar larvae five of these characteristics were of no diagnostic value, while six were useful to identify some of the species. All species could, however, be identified with the aid of the cephalopharyngeal skeleton. For second instar larvae, four of the characteristics were of no diagnostic use, but seven were useful to identify some of the species. All the species could be identified using the cephalopharyngeal skeleton. In the third instar larvae four characteristics were of no diagnostic use, but by using three other characteristics some of the species could be identified. All the species could be identified by means of five morphological characteristics (labrum, cephalopharyngeal skeleton, posterior spiracles, spiracular plate and anal area). Seven

characteristics were identified for their possible diagnostic values in puparia. Five of these characteristics were useful to identify some of the species, but all the species could be identified with the aid of two of the characteristics (frontal field and bubble membrane).

The ultimate aim of the study was to construct keys for use during the identification of specimens found at the crime scene. The keys that were constructed not only mapped out the pertinent diagnostic characteristics, but also considered what characteristics could be combined when a specimen was viewed from a specific angle. Since specimens often reach the forensic laboratory in less than optimal condition, it is essential that a wide range of characteristics and keys be available during identification. This will also enable the forensic entomologist to give priority to specimen preparation and the method of observation when rapid analysis is needed or where very few good specimens are available for identification.

With these tools (descriptive diagnostic characteristics and keys) the identification of immature stages of calliphorids and sarcophagids found at the crime scene should be an uncomplicated exercise.

Key terms: Calliphoridae – Sarcophagidae – forensic entomology – eggs - larvae – puparia – diagnostic characteristics – keys.

SAMEVATTING

Die insekte verteenwoordigend van die orde Diptera, spesifiek lede van die families Calliphoridae (brommers) and Sarcophagidae (vleesvlieë), is gewoonlik van die eerstes wat deur ‘n ontbindende liggaam aangelok word. Hierdie vlieë sal, afhangende van heersende omgewingstoestande, hul eiers (of in die geval van vleesvlieë, hul larwes) binne ‘n paar uur na dood op die liggaam deponeer. Die onvolwasse stadia sal dan hul ontwikkelingsfase op, of in die omgewing van die liggaam voltooi. As gevolg van hierdie gedragsaspek is insekte van groot belang in forensiese entomologie; die hooftoepassing in hierdie veld is die berekening van die nadoodse interval. Die korrekte identifisering van die spesies van die onvolwasse stadia is krities vir die berekening van die nadoodse interval.

Die doel van die studie was om ‘n diagnostiese beskrywing vir elk van die onvolwasse stadia van die forensies belangrike brommers en vleesvlieë saam te stel wat in sentraal Suid-Afrika voorkom. Die forensies belangrike brommers wat in die streek voorkom, is: *Lucilia cuprina* (Wiedemann), *Lucilia sericata* (Meigen), *Chrysomya chloropyga* (Wiedemann), *Chrysomya marginalis* (Wiedemann), *Chrysomya albiceps* (Wiedemann) and *Calliphora vicina* Robineau-Desvoidy; die vleesvlieg wat voorkom in die streek is *Sarcophaga cruentata* Meigen. ’n Reeks eienskappe is deur middel van lig- en skandeerelektronemikroskopie ondersoek en die ter saaklikstes is vir diagnostiese doeleindes geïdentifiseer. Eiers is in terme van nege eienskappe geëvalueer, ses hiervan was van geen diagnostiese waarde nie, terwyl sommige van die spesies met drie van die eienskappe geïdentifiseer kon word. Eiers van *C. chloropyga* and *C. albiceps* kon nie van mekaar onderskei word nie. Twaalf eienskappe is vir hul moontlike diagnostiese waarde in larwes geïdentifiseer. In eerste instar larwes het vyf van die einskappe geen diagnostiese waarde gehad nie, ses was van waarde om sommige van die spesies mee te identifiseer, terwyl al die spesies deur middel van die sefalofaringeaalskelet geïdentifiseer kon word. Vir tweede instar larwes was vier van die eienskappe van geen diagnostiese waarde nie, sewe was van waarde om sommige van die spesies mee te identifiseer, terwyl alle spesies deur middel van die sefalofaringeaalskelet geïdentifiseer kon word. In derde instar larwes was vier van die eienskappe van geen diagnostiese waarde nie, sommige van die spesies kon deur drie van

die eienskappe geïdentifiseer word, terwyl vyf van die eienskappe (labrum, sefalofaringeaalskelet, agterste asemhalingsstrukture, spirakulêre plaat en die anale area) gebruik kon word om al die spesies mee te identifiseer. Sewe eienskappe is vir hul moonlike diagnostiese waarde in puparia geïdentifiseer. Dit was moonlik om sommige van die spesies deur middel van vyf van die eienskappe te identifiseer, terwyl al die spesies deur middel van twee van die eienskappe (frontale gesigsveld en respiratoriese borrelmembraan) geïdentifiseer kon word.

Die hoofdoel van die studie was om sleutels wat van hulp sal wees met die identifisering van eksemplare wat op ‘n misdaadtoneel versamel word, op te stel. Die identifikasiesleutels wat opgestel was, het nie net die verloop van die mees tersaaklike diagnostiese eienskappe uitgespel nie, maar het ook in ag geneem watter eienskappe saam gebruik kon word as ‘n eksemplaar vanaf ‘n spesifieke hoek geëvalueer word. Omdat eksemplare die forensiese laboratorium in baie gevalle in ‘n swak toestand bereik, is dit belangrik dat ‘n wye verskeidenheid eienskappe en sleutels vir identifisering beskikbaar is. Dit stel ook die forensiese entomoloog in staat om die voorbereiding en metode van analise van eksemplare te optimaliseer, sou spoedige analise nodig wees of waar min eksemplare van goeie gehalte vir identifisering beskikbaar is.

Hierdie stel gereedskap (beskrywende diagnostiese kenmerke en identifikasiesleutels) behoort die identifisering van onvolwasse brommers en vleesvlieë, wat op ‘n misdaadtoneel versamel word, te vergemaklik.

Sleutelterme: Calliphoridae – Sarcophagidae –forensiese entomologie – eiers – larwes – papies – diagnostiese eienskappe – identifikasiesleutels.

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CHAPTER 1

INTRODUCTION

1. INTRODUCTION

1.1. THE ROLE OF CALLIPHORIDS AND SARCOPHAGIDS AS FORENSIC INDICATORS

At the scene of a homicide where the body has been undiscovered for some time, one of the more grisly sights is of the masses of maggots found on the body. Despite the repugnant nature of this scene, the information gained from these maggots can help with the ensuing forensic investigation. Utilising insects to solve a crime dates back as far as first century China (Cheng 1890). The first reported training manual for death scene investigation, *The washing away of wrongs*, published in the 13th century, related to a case in which insect evidence was used to solve a crime (McKnight 1981). The study of insects in the decomposition process and the use of insects in crime scene investigations have grown into what is today a vast field of research and where its application is increasing in strength. The application of the study of insects and other Arthropoda in legal issues is known as forensic entomology (Catts & Goff 1992). The category defined by Hall (1990) and Catts & Goff (1992) as medicocriminal entomology (i.e. the involvement of insects in events surrounding crimes, such as murder and suicide) utilise this type of information that will be presented in the current study as a key component.

The primary application of forensic entomology is to determine the postmortem interval (PMI). Conventional methods employed by a pathologist to determine the PMI is the superior estimate for the first 48 hours (Greenberg & Kunich 2002). After this period, the first generation of calliphorid (blow fly) and sarcophagid (flesh fly) progeny is a more precise measure of the PMI (Greenberg & Kunich 2002). The identification of the species involved and the stage of the calliphorid or sarcophagid progeny, combined with knowledge of their developmental rates form the basis for the estimation of the PMI (Smith 1986). Should the discovery of the body be delayed for more than the first generation of the calliphorid and sarcophagid progeny, i.e. basing the PMI estimate on the succeeding colonisers, a less precise, but still useful, PMI estimate can be provided (Greenberg & Kunich 2002). Where the discovery of a

body is delayed for more than a month, the concept of insect succession is utilised to determine the PMI (Catts & Goff 1992). In these latter stages of decomposition Coleoptera activity is a marker for PMI estimations (Lord 1990). At these stages, calliphorid and sarcophagid evidence in the form of their remnant pupal cases can still be utilised for PMI estimates. In these instances the PMI estimate is for a broader timeframe; i.e. determining the season a homicide occurred in (Gilbert & Bass 1967). This is based on the fact that certain species are more prevalent or occur only during certain seasons (Gilbert & Bass 1967).

1.2. BACKGROUND INFORMATION ON THE SPECIES INVESTIGATED

The forensically important blow flies prevalent in this region were determined by Louw & van der Linde (1993) and Kelly (2006) to be: *Lucilia cuprina* (Wiedemann), *Lucilia sericata* (Meigen), *Chrysomya chloropyga* (Wiedemann), *Chrysomya marginalis* (Wiedemann), *Chrysomya albiceps* (Wiedemann) and *Calliphora vicina* Robineau-Desvoidy. The same authors determined that *Sarcophaga cruentata* Meigen was the forensically important flesh fly prevalent in the region.

The two species of *Lucilia* are very similar to each other. These two species are sometimes referred to as the genus *Phaenicia* (Greenberg & Kunich 2002). *Lucilia cuprina* is commonly known as the sheep green bottle. It breeds in carrion, almost continuously throughout the year (Zumpt 1965). *Lucilia sericata* is commonly known as the common green bottle. This fly is diurnal; prevalent in urban and sub-urban environments and absent from rural environments (Zumpt 1965). Morphological descriptions of the immature stages of these two species are well documented. Because of this, one of the aspects that will be looked into is the possible existence of intraspecific variations.

Chrysomya chloropyga is commonly known as the green-tailed blowfly (Zumpt 1965). The species' niche occupation is described as follow: loves sunlight and generally does not go into the shade except in response due to strong olfactory stimulus and shelter from bad weather (Zumpt 1965). Furthermore, *C. chloropyga*

generally also do not enter buildings, except for the same reasons as entering shade (Zumpt 1965). The species is prevalent in winter and spring (Kelly 2006). *Chrysomya chloropyga* is not a cosmopolitan fly, and is restricted to parts of Africa (Zumpt 1965). The diagnostic characteristics of the immature stages of *C. chloropyga* will be compared with published descriptions of the species and also to the published descriptions given for the morphological similar *C. putoria*. Zumpt (1956) tentatively regarded *C. putoria* as a variation of *C. chloropyga* due to the similarity in the structure of the hypopygia of the adults. Preliminary crossing experiments indicated the two species to be partially genetically isolated (Zumpt 1965). Subsequent genetic evidence suggested a recent divergence of *C. putoria* from *C. chloropyga* (Wells *et al.* 2004). Further references to the divergence of the two species, based on genetic analysis, are contained in the work of Parise-Maltempi & Avancini (2001). Examination of the chromosomes of *C. chloropyga / putoria*, concluded that the species introduced to Brazil was *C. putoria* and not *C. chloropyga* (Parise-Maltempi & Avancini 2001). Based on this information, the diagnostic descriptions given for *C. chloropyga* by various authors (Greenberg & Szyska 1984, Liu & Greenberg 1989, Greenberg & Singh 1995, Wells *et al.* 1999) are possibly all diagnostic descriptions for *C. putoria*. In the current study, the species examined by these authors will be referred to as *C. putoria*. Comparing *C. chloropyga* to *C. putoria* is relevant in the South African context, due to the reported co-occurrence, albeit it for different periods of the year, of these two species in at least two localities in South Africa (Braack & de Vos 1987 and Wells *et al*. 2004). It should be noted, that no records of co-occurrence were found for the geographical area this study was conducted in, namely the central Free State Region.

Chrysomya marginalis is commonly known as the large blue blowfly. The species is a common carrion breeder in south Saharan Africa (Zumpt 1965). Prins (1982) refer to this species as *Chrysomya regalis*. This species is prevalent in the warmer summer months (Prins 1982, Braack 1986 and Kelly 2006). Very little is known about the immature stages of this species, except for the work by Prins (1982) for some of the immature stages (third instar larvae and puparia).

Chrysomya albiceps is commonly known as the western banded blowfly (Zumpt 1965). It has predatory tendencies on the larvae of other species and cannibalistic

tendencies towards its own kind (Zumpt 1965, Prins 1982, Kelly 2006). The diagnostic characteristics of the immature stages of *C. albiceps* will be compared to the published work on the species and to published work on the morphologically similar *Chrysomya rufifacies* (Macquart). The morphological separation of the two species, based on characteristics of the adults by Holdaway (1933), was in doubt due to the separation being based on what was considered to be a variable characteristic, namely the presence / absence of the prostigmatic bristle (Zumpt 1956). *Chrysomya rufifacies* was thus tentatively considered to be a sub-species of *C. albiceps* (Zumpt 1956). Consequent genetic evidence presented by Wells & Sperling (1999) validated the two species as separate monophyletic lineages. The larvae of the two species are very similar and were considered to be inseparable (Zumpt 1965). Consequent diagnostic comparisons (Erzinclioglu 1987, Tantawi & Greenberg 1993, Wells *et al.* 1999) of the third-instar larvae of the two species presented evidence to distinguish the larvae of the two species from each other. Although no records of *C. rufifacies* occurring in South Africa could be found, this work adds to the base of information where distinction between the two species is required. References to the dispersals and co-occurrences of the two species are contained in the work of Tantawi & Greenberg (1993) and Wells & Sperling (1999).

Calliphora vicina is commonly known as the European blue bottle. It was thought not to occur in Africa south of the Sahara. The identification of the species occurring in this region being *C. vicina* and not *C. croceipalpis*, was based on the adult characteristics, i.e. orange buccae, typical of *C. vicina*, opposed to the black buccae, typical of *C. croceipalpis*. *Calliphora vicina* commonly invades houses and is prevalent in the winter months (Kelly 2006). The immature stages of *C. vicina* were described by Northern hemisphere scientists. Since this species was not thought to occur in Africa south of the Sahara, any instances of intraspecific variation in the morphological features for this population of the species is of interest.

Sarcophaga cruentata is one of the synonyms listed for *S. haemorrhoidalis* by Zumpt (1965). Therefore, any comparisons made with *S. haemorrhoidalis* in the ensuing portions of this document are of the same species. *Sarcophaga cruentata* is a cosmopolitan species with a large distribution area. In warmer regions the species can be found within / around houses. Aspoas (1991) described certain aspects of third

instar larvae of the species and Zumpt (1965) offered a shortened description of some characteristics of all three larval stages. Very few descriptions of the sarcophagid eggs are available since first instar larvae are deposited as a rule by these species and not eggs. The current study will describe all immature stages of *S. cruentata* in full.

1.3. SCOPE OF THE PRESENT STUDY

The identification of calliphorid eggs presents a challenge due to similarities on genus level and variances on species level (Greenberg & Singh 1995). Various authors have previously described the eggs of most of the species evaluated as part of the current study. In some instances, these descriptions showed variances in diagnostic characteristics in different populations of the same species. The cause of this variance is still unclear. This aspect is open to further investigation, since variability in egg diagnostics of vastly separate populations was not evaluated as part of the current study. This thesis thus has a dual function regarding egg diagnostics, namely (i) to evaluate the relevance of previously identified diagnostic characteristics and (ii) to add to the base of information regarding calliphorid eggs in general from a regional perspective. Scanning electron microscopy was employed to assess the eggs for possible diagnostic characteristics to base identification on.

Generally the morphological features for third instar larvae are well documented, also for the species forming part of the current study. The current study adds to the existing body of work by evaluating the larvae for all possible diagnostic characteristics and commenting on the presence or absence of intraspecific variation for the features examined. Whereas a good base of information exists for third instar larvae, the morphological descriptions of the first and second instar larvae were documented to a lesser extent. It is important in forensic entomology to be able to identify all instar larvae to the species it belongs to since larvae cannot always be reared to the more easily identifiable adult or third instar larval stage. The approach of this study was to evaluate all possible external morphological features of larvae for their diagnostic value by means of scanning electron microscopy. Additionally, the sclerotised elements of larvae were evaluated for their diagnostic value with the aid of

light microscopy. Using various aspects of the larvae, as well as employing various methodologies allowed for a measure of flexibility when attempting identification of the larvae.

The puparium occupies a large chunk (up to 50%, according to Greenberg & Kunich 2002) of the temporal window in the life-cycle of the immature stages. Since this stage and its ecloded pupal cases is the longest available stage, it can be utilised to estimate the PMI for weeks to years (Gilbert & Bass 1967 and Greenberg & Kunich 2002). Greenberg & Kunich (2002) thus consider this stage of being of significant importance. Since puparia are merely the hardened skin of the third instar larvae, identification of this stage could be based on the external morphological features of third instar larvae. The cephalopharyngeal skeleton deposited within the wall of the empty puparium was still available in this stage to aid identification. However, this study evaluated puparia for those characteristics unique to puparia. Scanning electron microscopy as well as light microscopy was employed to assess the puparium for diagnostic characteristics.

Ultimately the information on the most relevant diagnostic characteristics of all immature stages will be assembled and keys will be constructed for all immature stages. These keys should aid species identification of eggs, larvae and puparia found at the scene of a homicide.

CHAPTER 2

MATERIALS AND METHODS

2. MATERIALS AND METHODS

2.1. COLLECTION AREA AND REARING ROOM CONDITIONS

Flies were predominantly collected from a site at the University of the Free State, Bloemfontein, South Africa at 29° 06' S, 26° 11' E and also from various locations in the surrounding municipal district. The collection area is located within a summer rainfall region, with an average summer maximum temperature range of 29°C to 31°C and an average winter maximum temperature range of 17°C to 20°C.

Collected flies were separated into cages according to the species. The steel frame cages were 30 x 30 x 30 cm in diameter and were covered in a fine mesh cloth. All colonies, except *C. vicina* colonies, were kept in a rearing room at a temperature of 25 ± 2°C and at a relative humidity of 70%. *Calliphora vicina* were kept in a rearing room at a lower temperature of 20 ± 2°C and at a relative humidity of 45%. A light / dark photoperiod of 12:12 h was maintained in the rearing rooms. Water and sugar was provided at all times. Chicken liver was introduced to initiate ovipositing in the calliphorids or larvipositing in the sarcophagid. This protein meal was successful in initiating ovipositing in most of the species. However, *C. marginalis* females did not always produce eggs with the introduction of this protein meal. Various other protein meals (pork, beef, beef liver and mutton) were experimented with, with similar dismal outcomes. Upon ovipositing (or larvipositing) the eggs (or larvae), together with the oviposition (larvipositing) medium was placed on paper towel and transferred from the cages to vented plastic buckets, half-way filled with wood shaving. Additional chicken liver was supplemented as larvae hatched and matured into larger larvae. The protein meal was lightly covered with a piece of aluminium foil to prevent desiccation. The wood shavings were placed in the buckets to absorb excess juices from the larval food source and to provide a medium for pupation.

2.2. SAMPLE PREPARATION

To facilitate the cleaning of the various immature stages, a 1% solution of household detergent was prepared.

Eggs were placed in a vial of warm water to which a few drops of the diluted cleaning solution were added. Separation of the egg batches was attempted by intermittent shaking of the vial. This process was not continued for more than a minute. Care was taken not to shake the vial with its contents too much to prevent damage to the eggs. Should eggs still adhere to each other, an attempt was made to mechanically teased the eggs apart with fine dissecting needles. Slight pressure applied to the egg mass with a small blunt object (back of a dissecting pin) helped this process along. Additional shaking, to achieve more separation, followed this process. The cleaning solution was rinsed from the eggs with numerous batches of distilled water until no soapiness was observed. It was stored in either 70% alcohol of 10% buffered neutral formaldehyde before preparing it further for scanning electron microscopy.

Larvae were killed by immersion in hot water (65°C - 75°C), which killed the larvae instantaneously and also aided in straightening out the larvae. The container in which the process took place needed to be wide enough to provide adequate room for the larvae to extend fully. Various methods of cleaning the larvae were experimented with. This included the brushing of larvae and sonication for various time periods. However, the most time effective and hassle-free method was to place the specimens in a vial of lukewarm water to which a few drops of the 1% household detergent was added and then to intermittently shake the vial for not more than a minute. The cleaning solution was removed by washing the specimens in a few batches of distilled water until no soapiness was observed anymore. After cleaning, the smaller larvae (i.e. first instar - and small second instar larvae) were kept intact and transferred to the storage solution of either 70% ethanol or 10% buffered neutral formaldehyde. The larger second - and the third instar larvae were severed at various locations before transferring to the same storage solution used for the smaller larvae. This allowed for adequate penetration of all solutions these larvae would be subjected to.

Puparia were placed in hot water (65°C - 75°C) to arrest development. Puparia were cleaned by placing them in a vial with warm soapy water and shaking the vial in an attempt to get rid of the debris adhering to the puparium surface. The wood shavings were further loosened from the puparium surface by brushing it with a series of small paintbrushes of various softness / stiffness grades. Care was taken not to use a paint brush with too unyielding bristles or to apply too much pressure during this brushing down process. Puparia were stored in 70% alcohol, instead of 10% buffered neutral formaldehyde. The 10% buffered neutral formaldehyde solution covered the puparium surface with a whitish residue, which limited some observations. Some puparia were severed at its midline and the pupae were removed from the pupal cases with a dissecting needle or fine tweezers. The pupal cases were air-dried in a closed container on filter paper before mounting for stereo or scanning electron microscopy.

For SEM studies both eggs and larvae were dehydrated in a graded alcohol series of increasing concentrations of ethanol (i.e. 30%, 50%, 70%, 80%, 95% and twice in absolute ethanol) for at least 12-hours per solution. The specimens were critical point dried using liquid CO_2 to complete the dehydration process. The emptied pupal cases were not subjected to any chemical dehydration processes. Eggs were mounted on aluminium stubs with double-sided tape. Due to the size and delicate nature of the eggs, picking them up with tweezers for placement on the stubs was not a viable option. An attempt was made to mount the eggs by scattering them over the sticky surface of the double-sided tape that was adhered to the stubs. However, this methodology resulted in almost all the eggs landing of that part of its anatomy that was vital for its diagnostics, namely in the median area. It was therefore apparent that a controlled placement of the eggs would be needed. An instrument was concocted whereby a few hairs were attached to a glass pipette. Hair of various stiffness levels were experimented with, until a type was found that yielded the best results. Eggs were picked up individually with this instrument and placed on the stubs in the desired position. The hair of the paint brush was made sticky by brushing it against a strip of sticky tape. This provided some adherence material for the eggs to cling to as it was picked up and placed on the stubs. Larvae and pupae were robust enough to be picked up with tweezers to be mounted on the stubs. The mounting media utilised was double sided tape, clear nail polish or epoxy glue. Nail vanish and epoxy glue was left

to dry somewhat, before the specimens were placed on it. The mounted stubs were sputter-coated with gold. The majority of the specimens were viewed with a JEOL 6400 scanning electron microscope. A few of the observations was done with a Shimadzu SSX550 scanning electron microscope. Scanning electron microscopy observation took place at 5 kV. For egg measurements, the scanning electron micrographs of eggs were imported into ImageJ, a public domain Java image processing program.

For compound light microscope preparation of the larval instars, the first two segments and the terminal segment were severed from the main larval body. The severed first two segments were placed in 10% KOH to facilitate adequate clearing of the segments to view the cephalopharyngeal skeleton located within it. The posterior spiracle was located on the posterior aspect of the terminal segment. This segment was also placed in a 10% KOH solution. However, this was not done to clear the terminal segment, but to soften it, which aided its positioning under the cover slip. The clearing solution with its contents was checked on a 24-hour basis and replaced when milky until the specimens were adequately cleared or softened. The KOH solution was rinsed from the specimens with a few batches of distilled water and the specimens were kept in distilled water for at least a 24 hour period to completely rinse the KOH solution from it. The specimens were dehydrated in an increasing concentration of a graded alcohol series (i.e. 50%, 70%, 80%, 95% and twice in absolute ethanol) for at least 15 minutes each to remove the water from the cleared specimens. Since the mounting medium was xylene based, the dehydrated specimens were placed in xylene for a further 15 minutes to replace the ethanol. The specimens were mounted with Eukitt on a microscope slide and cover-slipped. It should be noted that since xylene is also a clearing medium, the more delicate structures of first - and second instar larvae faded over time due to the clearing capability of the xylene in the mounting medium.

For stereo electron microscopy preparation of the pupal cases, the whole, unopened specimens were aid-dried on filter paper in a close container. It was attached to a white card with double-sided tape.

CHAPTER 3

RESULTS AND DISCUSSION

3. RESULTS AND DISCUSSION

3.1. MORPHOLOGY OF THE EGGS

Calliphorids produce eggs as a first immature stage, whereas sarcophagids generally produce newly hatched first instar larvae as a first immature stage. However, *Sarcophaga cruentata* occasionally produced eggs during the course of the current trial. This phenomenon was also observed by Knipling (1936), Zumpt (1965), Aspoas (1991) and Sukontason *et al.* (2005) for the sarcophagids examined by them.

Greenberg & Singh (1995) evaluated the eggs of 11 species of calliphorids, belonging to six genera and found the identification of calliphorid eggs to be complicated. While some congeneric species were easily distinguishable from each other, Greenberg & Singh (1995) found the identification to be difficult for some groupings at species level. Furthermore, Greenberg & Singh (1995) found similarities among certain sympatric species of different genera. These authors offered as examples to this the similarities found between *Chrysomya rufifacies* and *Cochliomyia macellaria* or *Phormia regina* and *Phaenicia* (=*Lucilia*) species. To further complicate the identification of calliphorid eggs, Greenberg & Singh (1995) observed variation in characteristics among different populations of the same species and also intraspecific variations within the same populations. Due to these idiosyncrasies, they cautioned against species identification for calliphorids based on the characteristics of the eggs alone.

3.1.1. SHAPE AND CHORIONIC STRUCTURE OF EGGS

Shape of the eggs

The eggs of the calliphorids examined were generally oblong-shaped, tapering at the ends, with the posterior end more rounded and broad than the pointed, narrower anterior end (Figs. 1A to 1F). The sarcophagid eggs (Fig. 1G) examined were barrel-shaped, tapering at both ends, with the one end more pointed than the other end.

However, a few specimens deviated somewhat from the uniform barrel-shape with a rounded distortion seen at varying locations on the eggs (Fig. 1H).

Chorionic structure

The outer layer of the egg, the chorion, generally appeared smooth, but careful examination revealed a surface sculptured in a polygonal pattern (Figs. 2A to 2F; Figs. 3B and 3C). It was difficult to grade the distinctness of the chorionic sculpturing in the different species; therefore it was not used as a diagnostic feature. The chorionic sculpturing was clearly visible in the two *Lucilia* species examined during the current study (Figs. 2A and 2B), however, conflicting reports regarding the chorionic sculpturing of the two *Lucilia* species are given by other researchers. Meskin (1991) reported a distinct reticulation pattern for *L. sericata* and described the reticulation pattern of *L. cuprina* as indistinct. The reticulation pattern was distinctly visible in the Australian specimen of *L. cuprina* presented by O'Flynn & Moorhouse (1980). Prins (1982) reported that the reticulation was not clearly visible for the specimens of *L. sericata* examined by him. These conflicting reports regarding the chorionic sculpturing might be indicative of how subjective assessing this structure can be. An alternative explanation is that the polygonal pattern of the chorion was not equally visible to all observers due to varying cleaning techniques.

Details of the eggshell structure were largely obscured from view in *C. marginalis*. The very faint polygonal sculpturing of the chorion was hidden beneath a layer of debris (Fig. 2D). This layer of debris is most probably the sticky substance that adheres the eggs to each other as it was deposited. *Chrysomya marginalis* eggs clumped together after these were separated and put through a cleaning regime. The eggs of the other species examined were not affected to the same extent as the eggs of *C. marginalis* were.

The chorionic sculpturing (Figs. 3B and 3C) was not observed during the initial observations of *S. cruentata* eggs. Sukontason *et al.* (2005) presented evidence of a multilayered eggshell for *Liosarcophaga dux*, describing the outer layer as thin, with a polygonal pattern. Thus, overlooking the polygonal pattern during the initial observations might have been due to these eggs being stripped of their thin outer layer. Furthermore, the faint polygonal pattern was not equally visible at all locations

and was easily overlooked. Eggs with partially detached eggshells (Fig. 3A) revealed the multiple layers of the eggshell. In these eggs the polygonal pattern, characteristic of the outer layer of the eggshell, was first observed.

Median area structure

The chorionic structure of the median area (the area between the two flanges) was different in structure from the rest of the chorion. The chorion here was an interconnected network of vertical struts, which flared into distinct apices. The characteristics of the apices were considered for their diagnostic value. The first aspect considered was whether the apices were individualised or whether these were merged with neighbouring apices. Furthermore the surface of the apices were evaluated for whether it contained one or a few deep-set indentations or whether numerous, small, superficial indentations adorned its surface.

In *L. cuprina*, the apices were predominantly individualised and these were broadly truncated (Figs. 6B and 6C). Numerous, small, superficial indentations were noted on the surface of the apices (Fig. 6C) of *L. cuprina* specimens.

The apices were predominantly individualised in *L. sericata* specimens examined (Figs. 6E and 6F). Furthermore, the surface of the apices of *L. sericata* specimens appeared to be concave, with some apices having a single deep-set indentation on the surface (Fig. 6F). Some apices contained two or three smaller indentations on its surface. The description and the micrograph presented by Greenberg & Singh (1995) for *L. sericata* was more in line with the results found for *L. cuprina* in the current study. The micrograph presented by Erzinclioglu (1989a) of the apices of the median area of *L. sericata* was similar to the findings of the current study.

The apices of *C. chloropyga* (Figs. 6H and 6I) and *C. albiceps* (Figs. 6N and 6O) were very similar to each other. The apices were individualised, but a few apices were merged. As with *L. sericata* specimens, one to three deep-set indentations were noted in the apices of *C. chloropyga* (Fig. 6I) and *C. albiceps* (Fig. 6O) specimens. The micrographs and descriptions offered by Greenberg & Singh (1995) for *C. chloropyga* and *C. albiceps* were similar to that found during the current investigation for the two species.

A mix of individualised and merged apices was found in the median area of *C. marginalis* specimens examined (Figs. 6K and 6L). Deep-set indentations were noted on the surface of the apices of *C. marginalis* specimens examined (Figs. 6K and 6L).

The apices of *C. vicina* (Figs. 6Q and 6R) were similar to that of *L. cuprina*. The apices were predominantly individualised in the median area of *C. vicina* specimens examined (Figs. 6Q and 6R). Generally, the apices in *C. vicina* specimens were broadly truncated, containing numerous small, superficial indentations on the surface (Fig. 6R). Contrary to the findings of the current study, Greenberg & Singh (1995) described the apices to have the tendency to coalesce. However, the micrograph of the median area structure presented by Greenberg & Singh (1995) was similar to that found for *C. vicina* in the current study.

3.1.2. MORPHOMETRICS

Egg length and width were recorded for each of the species as well as the dimensions of the median area. The median area spanned longitudinally across the length of the egg (Fig. 4A) and its relation in terms of the egg length was considered for its diagnostic value. Eggs with interrupted median areas (Fig. 5A) or abnormally short median areas (Figs. 5B and 5C) were not included in the sample presented for the descriptive statistics. The median area was considered to be abnormally short if it stretched across 70% or less of the egg length. Bordering the centrally located median area were two parallel ridges, the flanges (Figs. 2A to 2F; 6D, 6G, 6J, 6M and 6P). The width of the median area in relation to the egg width was evaluated for its diagnostic value. Initially, the median area was valued as narrow or wide / broad, based on a visual assessment alone. Where the median area was easily observed when the entire egg was viewed, it was considered to be wide. Where the median area was not easily observed and appeared as a groove, it was considered to be narrow. However, to eliminate any subjectivity, it was decided to qualify this measurement. A median area with a width of 3% or less of the egg width were considered as narrow; a value of 6% or more were considered broad and a value of 4 and 5% was considered to be intermediate. Lastly, the relationship between the median area and the flanges were evaluated for its diagnostic value.

The micropyle (Figs. 4B and 4C) was located at the anterior pole of the egg. An elevated, textured area (i.e. the micropylar area) surrounded the micropyle (Figs. 4B and 4C). The anterior pole of the egg was concave and the chorion in this area was unmarked by the polygonal pattern seen on the rest of the chorion. This region was called the micropylar region (Figs. 4B and 4C). The median area either bifurcated around the micropylar region or ended bluntly at the micropylar region without any pronounced splitting. Where the median area bifurcated, the reach of the "arms" of the median area towards the level of the micropyle was considered for its diagnostic value. Initially, the description of where the arms of the median area ended in relation to the level of the micropyle given by Greenberg & Singh (1995) caused some confusion. This was because the view from which this assessment was made was not specified. However, the micrographs presented by Greenberg & Singh (1995) served to elucidate the matter. These authors made their observation viewed from the anterior end of the egg. Where the median area did not split at the anterior pole of the egg, the shape (convex or concave) of the termination pattern was evaluated for its diagnostic value.

Lucilia cuprina (Table 1 and Figs. 1A, 2A, 6A and 7A)

Table 1: Descriptive statistics for *Lucilia cuprina* eggs

Dimension	Mean	SE	Median	SD	Min.	Max.	CL	N
EL (µm)	1085	13	1083	60	940	1180	27	21
EW (µm)	270	8	263	34	229	352	18	16
MAL of EL (%)	82	1	80	5	73	92	3	20
MAW of EW (%)	8	0.8	7	3	5	12	2	10
F of MAW (%)	53	7	50	23	27	93	16	10

CL: 95% confidence level; EL: egg length; EW: egg width; F: flange; MAL: median area length; MAW: median area width; Max: maximum; Min: minimum; N: number of specimens examined; SD: standard deviation; SE: standard error.

The average egg length of the eggs analysed (Table 1) was comparable (1.2 mm) to that of the Australian specimens collected by O'Flynn & Moorhouse (1980).

The median area did not extend across the full egg length in the specimens of *L. cuprina* examined (Table 1, Fig. 1A), contradictory to other accounts regarding the median area extent (stretching across the full length of the eggs) as reported by Meskin (1991) for specimens of *L. cuprina* collected in the Highveld of South Africa and by O'Flynn & Moorhouse (1980) in Australia. The median area was broad (Table 1, Fig. 2A), but not to the same extent, i.e. 0.14 (14%) of the egg width, as reported by

Meskin (1991). O'Flynn & Moorhouse (1980) described the median area as being narrow; contradicting the assessment made during the present study. This highlights the inherent danger of making a comparison based on descriptions alone and not based on measurements or visual assessments. The observation made by O'Flynn & Moorhouse (1980) was possibly made based on a comparative measurement to the other species forming part of that study. On average, the flange width, in relation to the median area width, (Table 1, Figs. 2A and 6A) was not comparable to the value of 0.26 (26%) reported by Meskin (1991) for *L. cuprina*. However, the wide ranging values obtained regarding the flange width in relation to the median area width for the specimens examined, and the degree of overlap observed in the ranges of the different species, rendered this aspect unsuitable for diagnostic purposes.

The median area bifurcated at the micropylar region. The lip of the chorion between the arms of the median area was pointed (Fig. 7A). The arms of the median area reached the level of the micropylar area (Fig. 7A). This was in contrast to the slight bifurcation, resulting in an almost blunt ending, observed in the median area illustrated by Meskin (1991). The lip of the chorion was bluntly rounded in the illustration of Meskin (1991); in contrast to the pointed form noted in the specimens examined during the present study. The specimens examined by O'Flynn & Moorhouse (1980) and Spradbery (1991) had a comparable anterior termination pattern of the median area to that observed in the present study. From the wide area of bifurcation of the median area at the anterior end of the egg, the median area continued with very little narrowing along its course to the posterior end of the egg.

Lucilia sericata (Table 2 and Figs. 1B, 2B, 6D and 7B to 7D)

Table 2: Descriptive statistics for *Lucilia sericata* eggs

	Mean	SE	Median	SD	Min.	Max.	CL	N
EL (µm)	913	11	921	58	805	998	23	27
EW (µm)	239	3	239	16	209	261	6	26
MAL of EL (%)	93	0.2	93	1	92	95	0.5	16
MAW of EW (%)	14	0.5	14	2	12	16	1	8
F of MAW (%)	29	3	29	9	19	50	6	11

CL: 95% confidence level; EL: egg length; EW: egg width; F: flange; MAL: median area length; MAW: median area width; Max: maximum; Min: minimum; N: number of specimens examined; SD: standard deviation; SE: standard error.

Generally, the range of the egg length and width for *L. sericata* eggs analysed was smaller (Table 2) than that reported by Prins (1982) for the specimens of *L. sericata* collected in the Cape coastal area, South Africa (egg length: 1.2 – 1.36 mm; egg

width: 0.32 – 0.36 mm). The average egg length and width of the specimens examined (Table 2) were also smaller than that of other populations of *L. sericata* world-wide – British specimens collected by Erzinclioglu (1989a) had an egg length of 1.19 ± 0.05 mm and an egg width of 0.28 ± 0.02 mm; North American specimens collected by Greenberg & Singh (1995) had an egg length of 1.1 ± 0.2 mm and an egg width of 0.33 ± 0.05 mm and the specimens collected in Japan by Greenberg & Singh (1995) had an egg length of 1.2 ± 0.08 mm and egg width of 0.35 ± 0.02 mm.

The median area length extent (Table 2, Fig. 1B) was further posterior than that of other populations: Greenberg & Singh (1995) reported it to be 60 – 80 % of the egg length; Meskin (1991) reported it to be 0.8 (80%) of the egg length and Prins (1982) reported it to be nearly seven-eighths (88%) of the egg length. Erzinclioglu (1989a) and Greenberg & Singh (1995) noted that the median area was interrupted across the length of some eggs examined by them. None of the specimens examined during the course of the current study had an interrupted median area. The width of the median area in relation to the egg width (Table 2, Fig. 2B) was more wide-ranging than the 9% reported by Meskin (1991) and by Greenberg & Singh (1995) for the specimens examined by them. The average extent of the flange width in relation to the median area width (Table 2, Figs. 2B and 6D) was comparable to the value of 0.33 (33%) presented by Meskin (1991) for *L. sericata*. However, the situation was similar to that found for *L. cuprina*, where the wide ranging values obtained regarding the flange width in relation to the median area width, and the degree of overlap observed in the ranges of the different species, rendered this aspect unsuitable for diagnostic purposes.

There was a slight bifurcation of the median area at the micropylar region with the arms only extending slightly towards the micropylar region (Figs. 7C and 7D). In some specimens examined the median area appeared to end bluntly, without any bifurcation (Fig. 7B). The lip of the chorion was rounded (Fig. 7B). This, together with the very gradual posterior narrowing of the median area, constituted more of a U- than Y-shape. This was in contrast to the termination pattern reported by Erzinclioglu (1989a) and by Greenberg & Singh (1995) where the median area bifurcated around the micropylar area and terminated in a Y-shape. The termination pattern of the median area at the anterior pole of the eggs of the Highveld, South

African specimens examined by Meskin (1991) was, in this regard, comparable to that of the specimens examined during the present study.

Chrysomya chloropyga (Table 3, Figs. 1C, 2C, 6G and 7E)

Table 3: Descriptive statistics for *Chrysomya chloropyga* eggs

	Mean	SE	Median	SD	Min.	Max.	CL	N
EL (µm)	1196	8	1183	63	1057	1350	16	61
EW (µm)	292	3	290	21	254	360	5	61
MAL of EL (%)	93	0.6	94	3	84	98	1	27
MAW of EW (%)	2	0.1	2	0.7	1	3	0.3	23
F of MAW (%)	112	11	100	48	67	250	22	20

CL: 95% confidence level; EL: egg length; EW: egg width; F: flange; MAL: median area length; MAW: median area width; Max: maximum; Min: minimum; N: number of specimens examined; SD: standard deviation; SE: standard error.

Chrysomya chloropyga eggs (Table 3) were smaller (1.6 x 0.36 mm) than *C. chloropyga* eggs collected in the Cape coastal area (South Africa) by Prins (1982). The specimens put forward by Greenberg & Singh (1995) as *C. chloropyga* were possibly *C. putoria* specimens. Greenberg & Singh (1995) sourced their specimens from Nigeria and according to Zumpt (1965) the distribution area of *C. putoria*, and not *C. chloropyga*, is West Africa. Specimens were also collected in Peru. The species introduced to Brazil, which spread to the rest of South America, was identified as *C. putoria* by Parise-Maltempi & Avancini (2001), based on chromosome analyses. On average, these *C. putoria* eggs were larger (1.4 ± 0.2 x 0.38 ± 0.02 mm) than *C. chloropyga* eggs examined during the current study (Table 3).

A few specimens in our sample had an interrupted median area, but the median area was uninterrupted in the majority of the specimens examined. The median area stretched over almost the entire length of the egg (Table 3, Fig. 1C). This was a bit more extensive than the 0.88 (88%) reported by Meskin (1991). The median area was narrow (Table 3, Fig. 2C) and the plastron area not visible when the entire egg was viewed; an aspect confirmed by Meskin (1991) who described the median area as narrow, appearing as a groove. On average, the extent of the flange, in relation to the median area, (Table 3, Figs. 2C and 6G) was less than the 1.34 (134%) obtained for *C. chloropyga* specimens examined by Meskin (1991). This aspect was similar to the situation found for the other species examined, where the wide ranging values obtained regarding the flange width in relation to the median area width, and the degree of overlap observed in the ranges of the different species, rendered this aspect unsuitable for diagnostic purposes.

The median area bifurcated into a Y-form at the micropylar area (Fig. 7E). The arms of the Y were generally symmetrical and terminated at the level of the micropyle in a few specimens, however, in the majority of the specimens examined it ended level or just below the level of the micropylar area (Fig. 7E). The lip of the chorion was rounded. The specimens of *C. chloropyga* examined by Meskin (1991) were similar in this respect to the eggs evaluated during the current study, however, the arms of the median area appeared to be shorter in the drawing presented by Meskin (1991). The reach of the median area arms as described by Greenberg & Singh (1995) for the congeneric *C. putoria* was similar to *C. chloropyga* specimens examined during the current study. Subsequent to the broad area of bifurcation of the median area at the anterior end of the egg, the median area narrowed severely and continued as a narrow strip along its path to the posterior end of the egg.

Chrysomya marginalis (Table 4, Figs. 1D, 2D, 5A, 5B, 6J, 7F and 7G)

Table 4: Descriptive statistics for *Chrysomya marginalis* eggs

	Mean	SE	Median	SD	Min.	Max.	CL	N
EL (µm)	1117	6	1128	46	1021	1211	13	52
EW (µm)	286	3	285	18	252	328	5	44
MAL of EL (%)	88	0.6	88	4	80	94	1	34
MAW of EW (%)	3	0.2	3	0.9	2	5	0.4	21
F of MAW (%)	69	4	67	18	40	122	8	21

CL: 95% confidence level; EL: egg length; EW: egg width; F: flange; MAL: median area length; MAW: median area width; Max: maximum; Min: minimum; N: number of specimens examined; SD: standard deviation; SE: standard error.

No other accounts on the eggs of *C. marginalis* were found. A general description of *C. marginalis* eggs is as follow: *Chrysomya marginalis* eggs occupied a similar egg length range (Table 4) as the majority of the other calliphorids (Tables 1, 3, 5 and 6) forming part of the current study; the exception being *L. sericata* (Table 2). The median area stretched across the greatest path of the egg length (Table 4, Fig. 1D). A few specimens had interrupted median areas (Fig. 5A), whereas one of the specimens examined had an unusually short median area (Fig. 5B). Although the median strip was narrow (Table 4, Fig. 2D), the median area was visible when the entire egg was viewed (Fig. 1D). The width of the flanges in relation to the median area was varied (Table 4, Figs. 2D and 6J). Similar to the situation found for the other species examined, the wide ranging values obtained regarding the flange width in relation to the median area width, and the degree of overlap observed in the ranges of the different species, rendered this aspect unsuitable as a diagnostic characteristic.

The median area bifurcated at the micropylar area into a Y-shape (Figs. 7F and 7G). The lip of the chorion was more pointed than rounded in the area of bifurcation. The Y-shape was accentuated due to the distinct narrowing of the median strip after the point of bifurcation. The arms of the median area were not symmetrical in some of the specimens (Fig. 7F). In these instances one of the arms of the median area reached the level of the micropylar area, while the other arm was below the level of the micropylar area. Where the arms of the median area were symmetrical (Fig. 7G), these ended well below the level of the micropylar area.

Chrysomya albiceps (Table 5, Figs. 1E, 2E, 6M and 7H)

Table 5: Descriptive statistics for *Chrysomya albiceps* eggs

	Mean	SE	Median	SD	Min.	Max.	CL	N
EL (µm)	1048	14	1090	132	665	1271	27	91
EW (µm)	252	5	250	37	171	343	11	49
MAL of EL (%)	91	0.9	93	4	82	97	2	18
MAW of EW (%)	2	0.1	2	0.5	1	3	0.2	18
F of MAW (%)	140	7	141	29	98	195	14	18

CL: 95% confidence level; EL: egg length; EW: egg width; F: flange; MAL: median area length; MAW: median area width; Max: maximum; Min: minimum; N: number of specimens examined; SD: standard deviation; SE: standard error.

The eggs examined (Table 5) were generally smaller than that reported for other populations worldwide: Zumpt (1965) reported an egg size of 1.5 mm; Prins (1982) reported an egg size of 1.4 to 1.5 mm x 0.3 to 0.34 mm; Greenberg & Singh (1995) reported an egg size for *C. albiceps* and the congeneric *C. rufifacies*, measuring 1.4 ± 0.2 x 0.39 ± 0.02 mm. Greenberg & Singh (1995) found no difference in egg size between the two congeneric species, *C. albiceps* and *C. rufifacies*.

Meskin (1991) and Greenberg & Singh (1995) illustrated a median area extending over the full egg length, comparable to the findings in the current study (Table 5, Fig. 1E). It was also similar to the full length the median area extended across the length of *C. rufifacies* eggs as reported by Greenberg & Singh (1995). Kitching (1976a) reported a shorter hatching line extent for *C. rufifacies* of three-quarters (75%) of the egg length. The median area was narrow (Table 5, Figs. 1E and 2E), appearing as a groove, occupying about 2 % of the egg width, similar to the account Meskin (1991) had regarding *C. albiceps* eggs. Meskin (1991) defined two groupings based on the width of the median area in relation to the egg width; i.e. those species falling within a range of 0.06 (6%) to 0.14 (14%) and those species with a much narrower medium

strip, appearing as a groove. Thus, the value obtained for *C. albiceps* eggs during the current study (Table 5) was well below the minimum value of 0.06 (6%) specified by Meskin (1991) for those species with a "broad" median area. The median area width extent was narrower that the ≈4 % Greenberg & Singh (1995) reported for *C. rufifacies.* On average, the flange was broader than the median area (Table 5, Figs. 2E and 6M), contradicting the finding of Meskin (1991) for the specimens of *C. albiceps* examined by him. Similar to the situation found for the other species examined, the wide ranging values obtained regarding the flange width in relation to the median area width, and the degree of overlap observed in the ranges of the different species, rendered this aspect unsuitable as a diagnostic characteristic.

The median area bifurcated into a Y-form around the micropylar area reaching the level of the micropylar area (Fig. 7H). This aspect was similar to that reported by Greenberg & Singh (1995) for *C. albiceps*. In contrast, the arms of the median area appeared to be slightly shorter in the specimens of *C. albiceps* examined by Meskin (1991). A comparison with the congeneric *C. rufifacies* was complicated due to varying reports of the median area ending at the level of the micropyle (Liu & Greenberg 1989) and being shorter, ending above the micropyle (Greenberg & Singh 1995).

Calliphora vicina (Table 6; Figs. 1F, 2F, 5C, 6P and 7I)

Table 6: Descriptive statistics for *Calliphora vicina* eggs

	Mean	SE	Median	SD	Min.	Max.	CL	N
EL (µm)	1136	12	1134	74	979	1261	24	38
EW (µm)	297	5	302	24	242	330	10	24
MAL of EL (%)	88	0.5	88	2	82	92	1	25
MAW of EW (%)	7	0.4	7	1.6	4	11	0.9	16
F of MAW (%)	50	3	52	13	27	76	7	16

CL: 95% confidence level; EL: egg length; EW: egg width; F: flange; MAL: median area length; MAW: median area width; Max: maximum; Min: minimum; N: number of specimens examined; SD: standard deviation; SE: standard error.

The eggs were generally smaller (Table 6) than the 1.7 mm reported by Zumpt (1965); the 1.40 ± 0.05 x 0.4 ± 0.2 mm reported by Erzincliogl (1989a) and the 1.4 ± 0.2 x 0.4 ± 0.05 mm reported by Greenberg & Singh (1995) for *C. vicina*. It was also smaller than the 1.7 x 0.44 mm to 1.8 x 0.44 mm reported by Prins (1982) for *Calliphora croceipalpis.*

The median area extent (Table 6, Fig. 1F) was on average not as far reaching as the 90% reported by Liu & Greenberg (1989) and Greenberg & Singh (1995) for *C. vicina*. However, the range of values, as obtained during the current study (Table 6), were inclusive of the 90% the median area length occupied in relation to the egg length as reported by Liu & Greenberg (1989) and Greenberg & Singh (1995). Similarly, the median area extent was similar to that of *C. croceipalpis*, where it occupied 90% or extended across the full length of the egg as reported by Meskin (1991) and Prins (1982) respectively. Liu & Greenberg (1989) noted that the median area was extremely short in some of the specimens examined by them, occupying a quarter (25%) of the egg length; a phenomenon also found in some specimens (Fig. 5C) examined during the current study. The median area width in relation to the egg width (Table 6, Fig. 2F) was similar to the 6% reported by Greenberg & Singh (1995) for *C. vicina* and the 0.06 (6%) reported by Meskin (1991) for *C. croceipalpis*. The average value of the flange width in relation to the median area width (Table 6, Figs. 2E and 6P) was similar to the 0.5 (50%) reported by Meskin (1991) for *C. croceipalpis*. However, similar to the situation found for the other species examined, where the wide ranging values obtained regarding the flange width in relation to the median area width, and the degree of overlap observed in the ranges of the different species, this aspect was considered unsuitable as a diagnostic characteristics.

The arms of the median area ended before reaching the level of the micropylar collar, as a slightly concave structure, without any bifurcation (Fig. 7I). A similar anterior termination pattern was reported by Erzincliogl (1989a), Liu & Greenberg (1989) and Greenberg & Singh (1995) for *C. vicina* and also reported as such for *C. croceipalpis* by Meskin (1991).

Sarcophaga cruentata (Table 7; Figs. 1G and 1H)

Table 7: Descriptive statistics for *Sarcophaga cruentata* eggs

	Mean	SE	Median	SD	Min.	Max.	CL	N
EL (µm)	1476	16	1479	80	1270	1588	32	26
EW (µm)	397	15	404	46	313	447	33	10

CL: 95% confidence level; EL: egg length; EW: egg width; Max: maximum; Min: minimum; N: number of specimens examined; SD: standard deviation; SE: standard error.

The egg length (Table 7) was similar (≈1.5 mm) to that of *Liosarcophaga dux* eggs as reported by Sukontason *et al.* (2005).

Similar to the situation reported for *L. dux* by Sukontason *et al.* (2005), *S. cruentata* eggs contained no median area (Figs. 1G and 1H). Sukontason *et al.* (2005) considered the function of a thick eggshell and a plastron area in calliphorid eggs to explain the thin eggshell and the lack of a median area in sarcophagid eggs. The thick eggshell of calliphorid eggs provides protection from the environment these eggs are deposited on (Sukontason *et al.* 2005). The median area in calliphorid eggs makes allowance for the respiratory needs of the developing embryo with its relatively long developmental rate into a first instar larva (Sukontason *et al.* 2005). On the contrary, should sarcophagids deposits eggs it would not need the protection of a thick eggshell and the increased respiration capability provided by a plastron network due to the short period it would be exposed to the environment outside of the female and the very rapid development rate from embryo to first instar larvae (Sukontason *et al.* 2005).

3.1.3. DIAGNOSTIC VALUE OF CHARACTERISTICS

One of the concerns Greenberg & Singh (1995) raised concerning the identification of calliphorid eggs was the variation noted in characteristics among different populations of the same species. This was also found for most of the characteristics considered during the current study where the results of this study were compared to that of other researchers (Zumpt 1965, O'Flynn & Moorhouse 1980, Prins 1982, Erzinclioglu 1989a, Liu & Greenberg 1989, Meskin 1991, Greenberg & Singh 1995). Although intraspecific variation was not an issue during the current study and a partial separation for the species forming part of the current study could be achieved, a measure of unease prevailed with the identification of eggs. This lack of confidence in using this characteristic can only be addressed by conducting research into the causes that give rise to the variation among different populations of the same species.

Characteristics with limited diagnostic value

The shape of the eggs can only be used to distinguish calliphorid (Figs. 1A to 1F) from sarcophagid eggs (Figs. 1G and 1H). In calliphorids the posterior ends of the eggs were more rounded and broad in comparison to the pointed, narrow anterior ends. In contrast to the calliphorid form, both ends of sarcophagid eggs were pointed

and narrow. The shape of calliphorid eggs offered nothing further to distinguish them from each other.

Due to the difficulty experienced to grade the intensity of the polygonal sculpturing of the chorion of eggs (Figs. 2A to F), it was not considered for its diagnostic value.

A large overlap in the ranges regarding the median area length in relation to the egg length (Fig. 8) existed, which limited the use of this aspect as a diagnostic feature. This aspect might be of some use in identifying *L. cuprina* specimens where the median area length was below 80% of the egg width. During any process of identifying calliphorid eggs cognisance should be taken of some specimens of certain species (*C. marginalis* and *C. vicina*) presenting with abnormally short median areas, i.e. less than 70% of the egg length (Figs. 5A and 5B), and interrupted median areas (Fig. 5C).

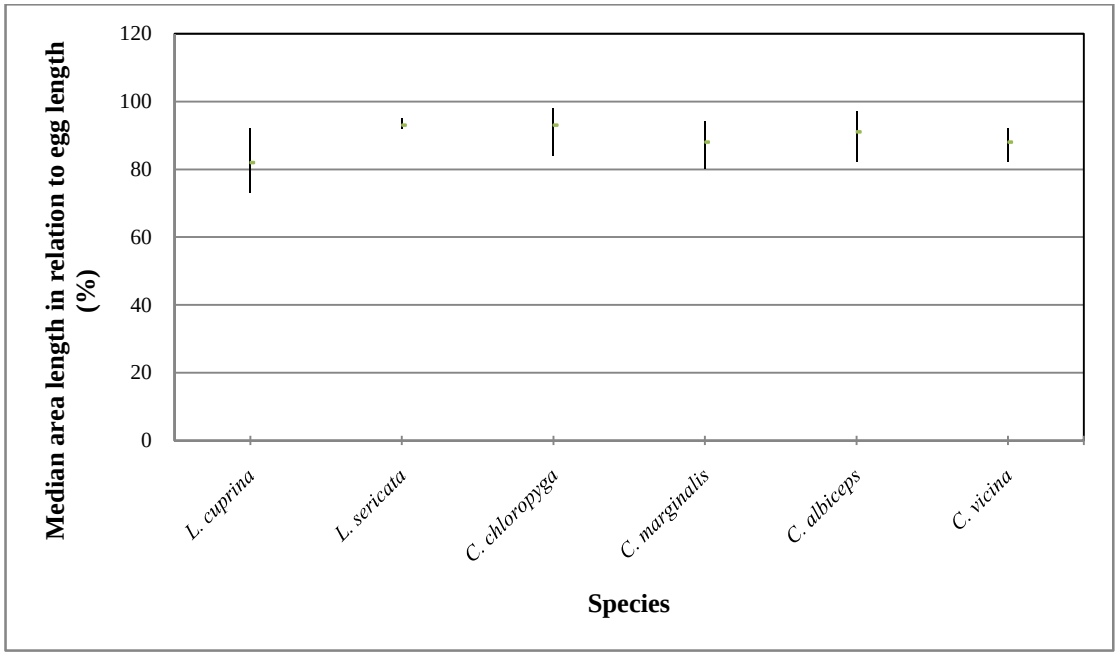


Fig. 8: Median area length in relation to egg length of the various species investigated.

A decision was made not use the flange width in relation to the median area width due to the wide ranging measurements obtained in this regard (Tables 1 to 6 and Fig. 9). Furthermore, the large overlap of this measurement between the different species (Fig. 9) also rendered it unsuitable as a diagnostic characteristic. It was noted in many instances that the median area appeared to be more suitable, in terms of it being free

of distortions or debris obscuring features, in areas away from where the measurement was taken from. The practice was to take this measurement at the midline of the eggs since this is a constant reference point. It was not investigated how much the measurement at areas away from the midline varied from the midline measurement, therefore it was decided against presenting measurements of areas away from the midline of the egg.

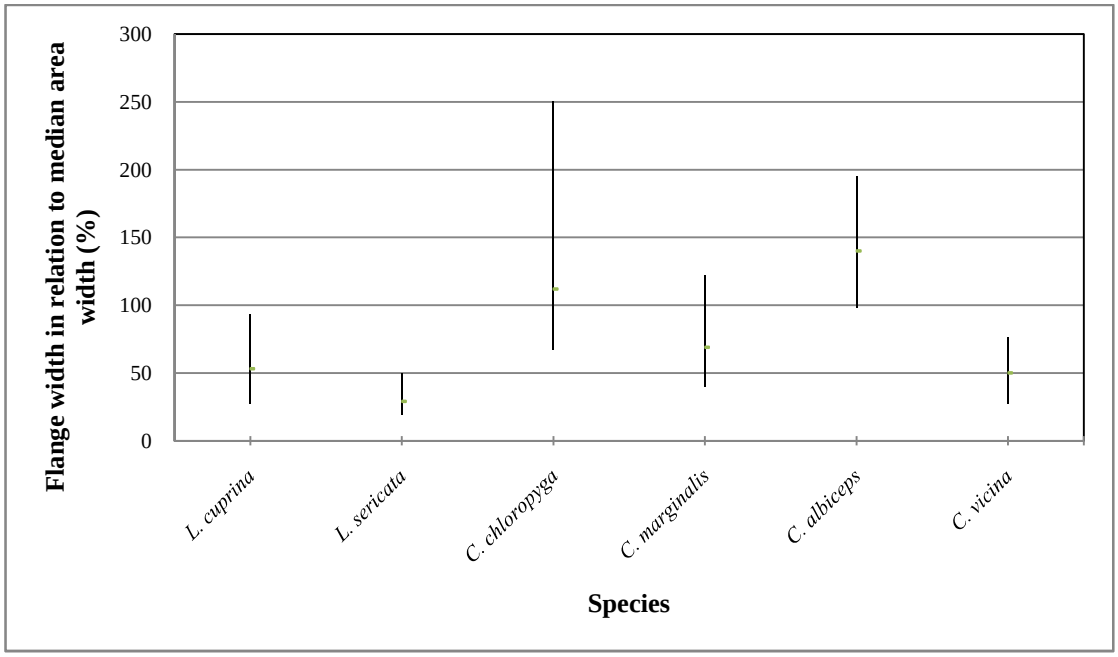


Fig. 9: Flange width in relation to median area width of the various species investigated.

Median area structure as diagnostic feature

Sarcophaga cruentata is unique and can be omitted from this diagnostic regarding the median area structure, since it does not have a median area. For the rest of the species examined (*L. cuprina*, *L. sericata*, *C. chloropyga*, *C. marginalis*, *C. albiceps* and *C. vicina*) a partial distinction can be achieved utilising the structure of the median area. *Chrysomya marginalis* was unique since a large proportion of the apices in the median area were merged (Fig. 6L), opposed to the other species examined where the apices were individualised or with only some apices merged. To distinguish the remaining species from each other, the indentations on the surface of the apices were assessed. In *L. sericata* (Fig. 6F), *C. chloropyga* (Fig. 6I) and *C. albiceps* (Fig. 6O), a single, deep-set indentation of two to three smaller deep-set indentations was noted on the surfaces of the apices, whereas the surfaces of the apices were marked with

numerous, small, superficial indentations in *L. cuprina* (Fig. 6C) and *C. vicina* (Fig. 6R) specimens examined. *Lucilia sericata* (Fig. 6F) can be separated from *C. chloropyga* (Fig. 6I) and *C. albiceps* (Fig. 6O) since it contained very little to no merged apices in its median area while the incidence of merged apices were higher in *C. chloropyga* and *C. albiceps* specimens examined. No further distinct variation was noted in the median area structure of *C. chloropyga* and *C. albiceps* to distinguish these two species. The median area structure also offered no further distinguishing characteristics between *L. cuprina* and *C. vicina*.

Egg length and width as diagnostic feature

With the exception of *S. cruentata* and *L. cuprina*, the eggs of the species examined were overall smaller than that of other populations in other locations as reported by the various researchers (Zumpt 1965, O'Flynn & Moorhouse 1980, Prins 1982, Erzinclioglu 1989a, Greenberg & Singh 1995, Sukontason *et al*. 2005). During the course of the current study, eggs were measured from scanning electron micrographs. Processing these eggs, might have been responsible for a size distortion. Many of the researchers did not specify whether their measurements were taken before preservation or from processed material, therefore, the current results could not be meaningfully compared in this regard. The possibility of egg size being influenced by the size of the ovipositing female was refuted by Erzinclioglu (1989a), who noted that the size of blowfly eggs was not dependant on the size of the female that deposited the eggs. Since this statement was not supported by a cross-reference or by empirical data in this specific study by Erzinclioglu (1989a), this aspect is worth investigating in a future research project.

The use of egg size as a diagnostic characteristic was limited due to the overlap in egg size ranges noted for the different species (Figs. 10 and 11).

Utilising egg size can be of value for the identification of certain species, but it should be used in conjunction with other characteristics. The first case in point is the identification of *S. cruentata* eggs. In the rare occasion of *S. cruentata* depositing eggs, these eggs can be tentatively identified based on the egg size. Although it shares part of its egg size range with some of the other species (Figs. 10 and 11), the unique features of these eggs (absence of a median area and its shape) would enable a

positive identification. The next case in line is the identification of *L. sericata* and *C. vicina* eggs. In a subsequent section on the diagnostic value of the anterior termination pattern of the median area, an ambiguity was identified regarding the identification of *L. sericata* and *C. vicina* eggs. Although another characteristic (i.e. the median area structure) can be utilised to clear this ambiguity; the largely separate egg size ranges that these two species occupy (Figs. 10 and 11), can be utilised for identification purposes.

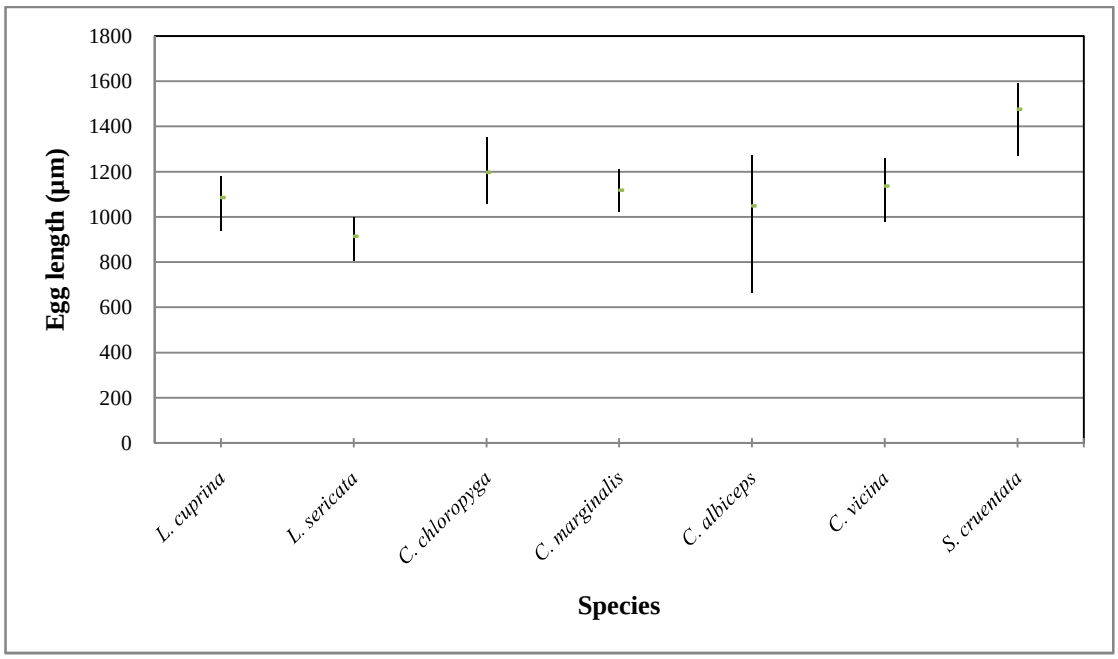


Fig. 10: Egg length ranges of the various species investigated.

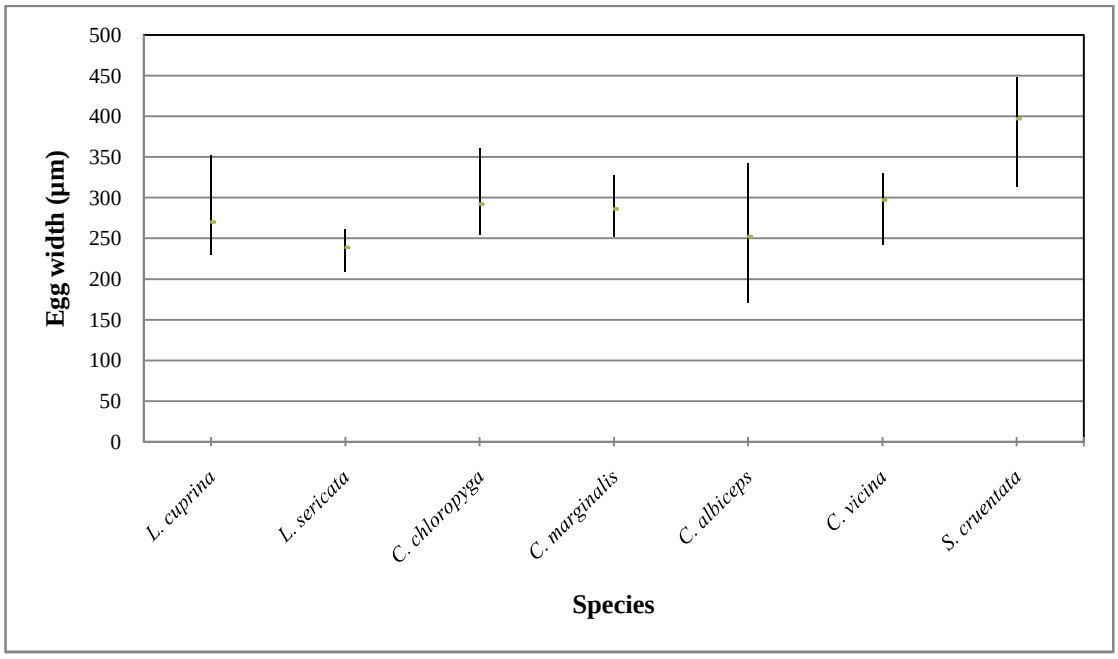


Fig. 11: Egg width ranges of the various species investigated.

Morphometrics of the median area as diagnostic feature

The absence of a median area distinguishes *S. cruentata* from the rest of the species. For those species with a median area, the width allowed for two groupings (i) those with a broad median area (*L. cuprina*, *L. sericata* and *C. vicina*) and (ii) those with a narrow median area (*C. chloropyga*, *C. marginalis* and *C. albiceps*). As discussed previously, a median area that occupied 3% or less of the egg width was considered to be narrow; it was considered to be broad when it occupied 6% or more of the egg width and to be intermediate if values of 4 and 5% occurred. Intermediate values were found for some specimens of *L. cuprina*, *C. marginalis* and *C. vicina* examined (Fig. 12). When constructing a key, an alternative path or key should be developed that make provision for specimens presenting with intermediate values.

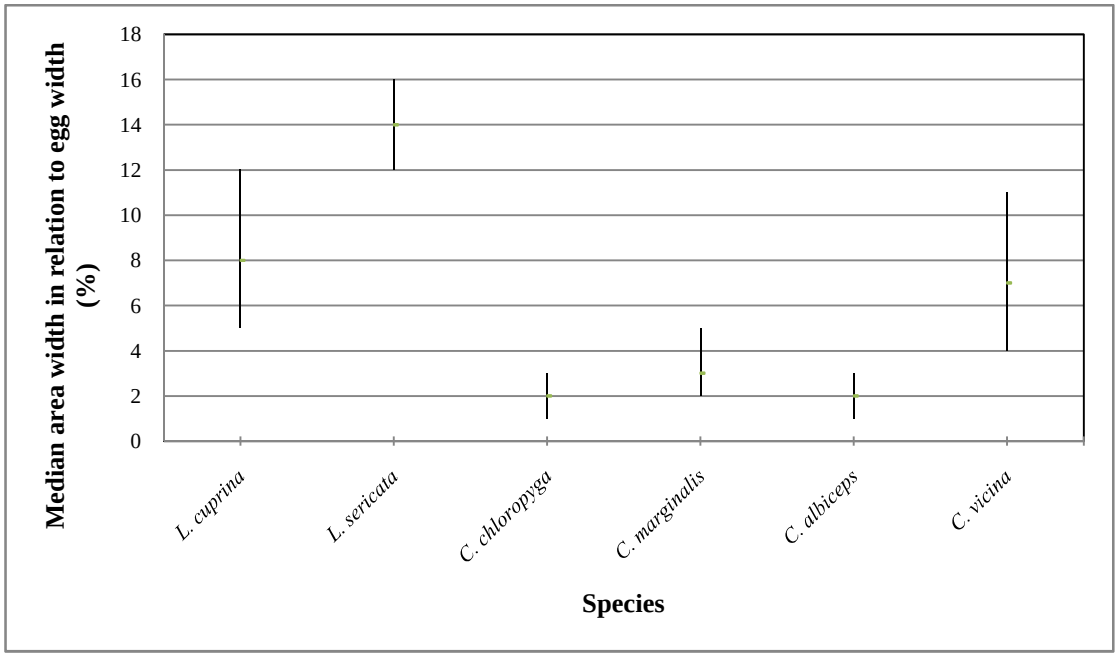


Fig. 12: Median area width in relation to egg width of the various species investigated.

Anterior termination pattern of the median area

As with many of the aspects considered previously, *S. cruentata* can be excluded from this analysis due to the absence of a median area in this species. Two groups can be identified regarding the termination pattern of the median area at the anterior end of the egg. The first group includes those species where the median area bifurcated at the anterior end of the egg (*L. cuprina*, *L. sericata*, *C. chloropyga*, *C. marginalis* and *C.*

albiceps) and the group where the median area ends without splitting at the micropylar region (*C. vicina*). However, in some specimens of *L. sericata* the anterior termination pattern was not distinctly bifurcated (Fig. 7B), appearing very similar to the anterior termination pattern of *C. vicina* (Fig. 7I). Therefore, caution should be exercised not to identify specimens with a blunt-ending anterior termination pattern as *C. vicina* without considering other characteristics to confirm the finding.

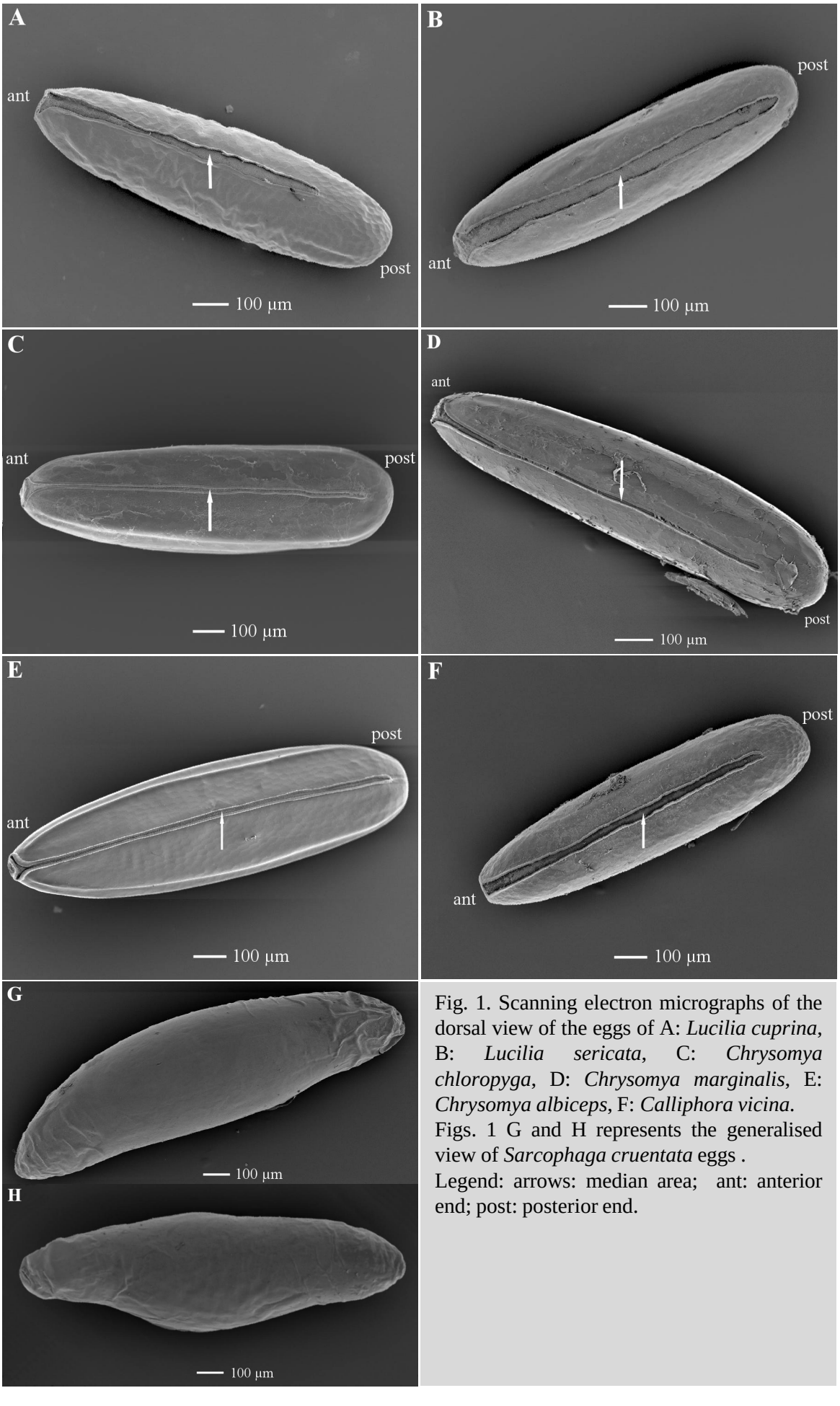


Fig. 1. Scanning electron micrographs of the dorsal view of the eggs of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina*. Figs. 1 G and H represents the generalised view of *Sarcophaga cruentata* eggs .
Legend: arrows: median area; ant: anterior end; post: posterior end.

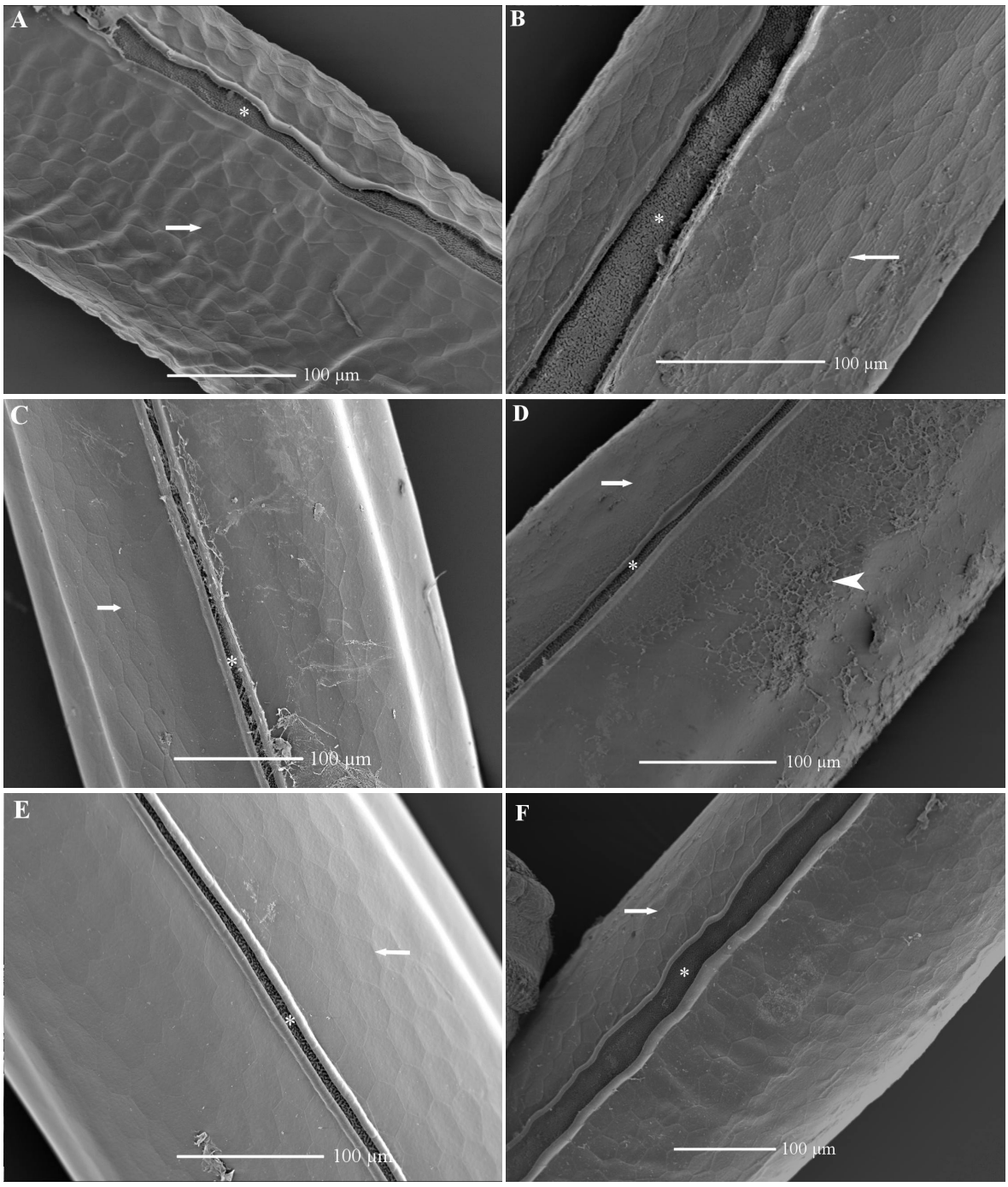


Fig. 2. Scanning electron micrographs of the dorsal view of the eggs of the various calliphorid species, detailing the midsection of the eggs, showing the relation of the median area (asterisk) to the egg width. A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina*. Legend: arrow: polygonal sculpturing on the egg surface; arrow head: debris on egg surface.

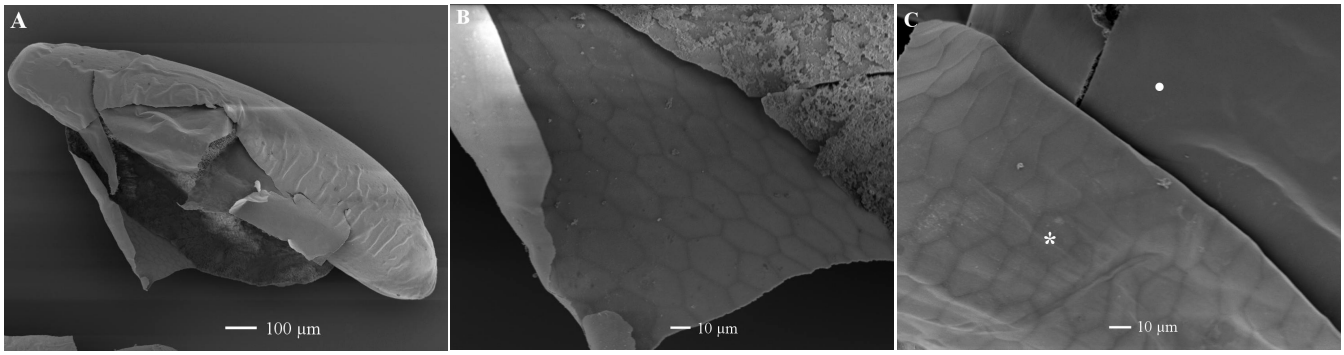


Fig. 3A: Scanning electron micrograph of egg of *Sarcophaga cruentata* with ruptured membranes.
Figs. 3B and 3C: Scanning electron micrographs detailing the egg membrane, showing thin outer membrane (asterisk) with polygonal sculpturing and inner membrane (dot) without any sculpturing.

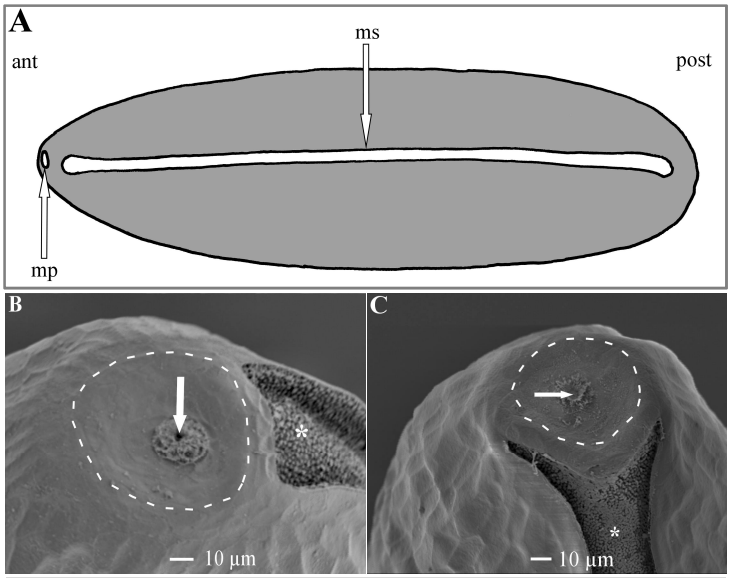


Fig. 4A: Diagrammatic representation of a calliphorid egg. Figs. 4B and 4C: Scanning electron micrographs detailing view of micropylar region (encircled area) of eggs of *C. vicina* and *L. cuprina* respectively.
Legend: arrow: micropyle; ant: anterior end; asterisk: median area; mp: micropyle, ms: median strip; post: posterior end.

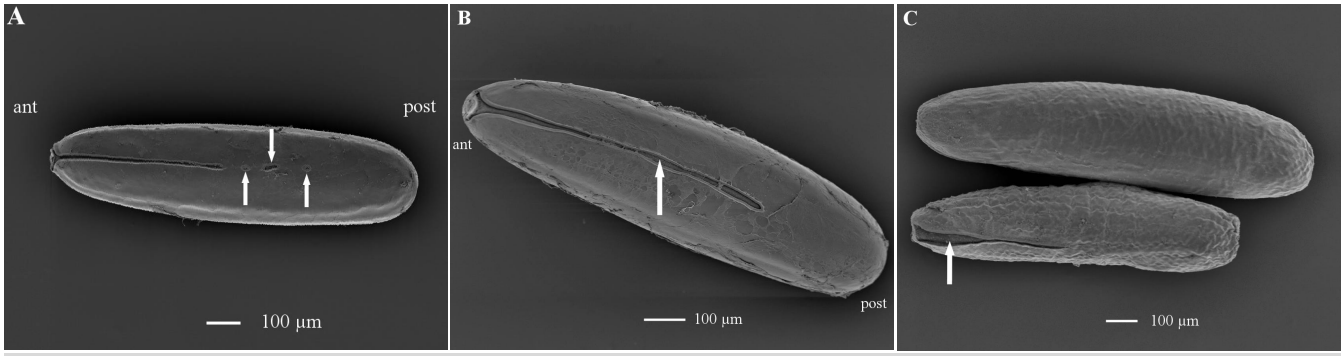


Fig. 5A: Scanning electron micrograph of egg of *C. marginalis* with an interrupted median area, indicated by arrows.
Figs. 5B and 5C: Scanning electron micrographs of eggs of *C. marginalis* and *C. vicina* respectively, showing eggs with short median areas, indicated by arrows.
Legend: ant: anterior end; post: posterior end.

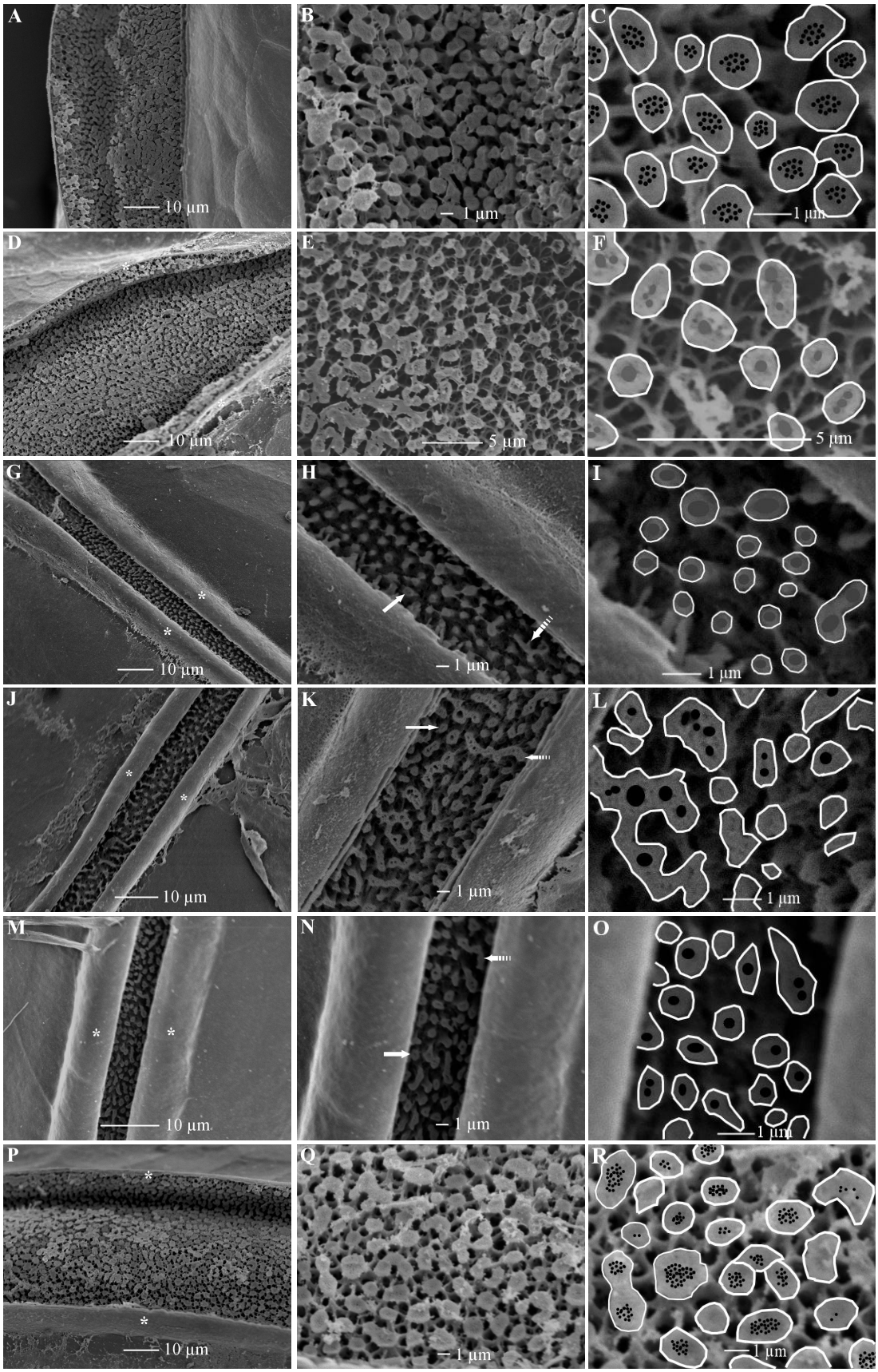


Fig. 6: Scanning electron micrographs of median areas of various calliphorid eggs. A to C: *Lucilia cuprina;* D to F: *Lucilia sericata;* G to I: *Chrysomya chloropyga;* J to L: *Chrysomya marginalis;* M to O: *Chrysomya albiceps;* P to R: *Calliphora vicina.*
Legend: simple-ended arrows: individualised struts, interrupted-ended arrows: merged struts; asterisks: flanges.

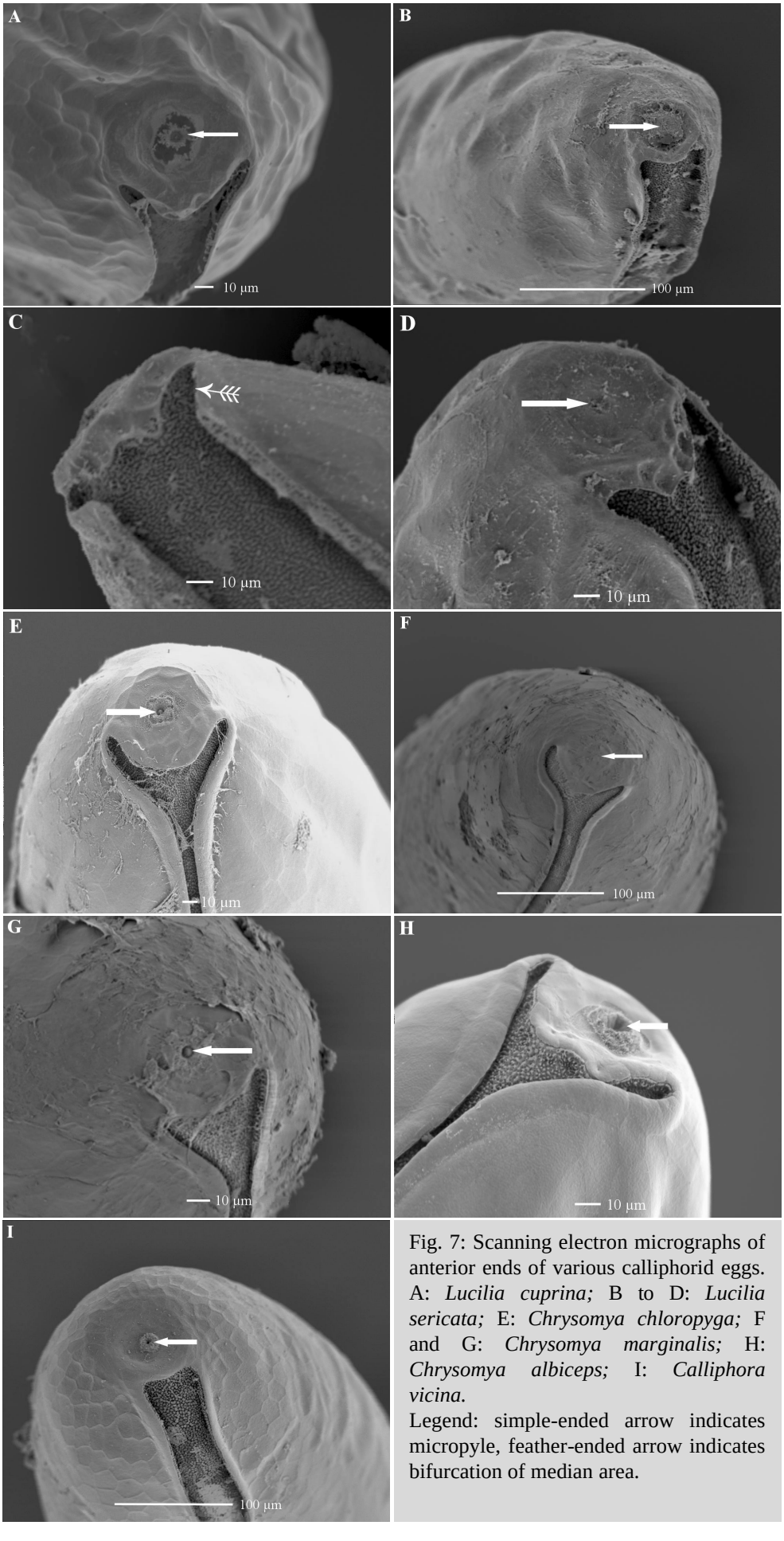


Fig. 7: Scanning electron micrographs of anterior ends of various calliphorid eggs. A: *Lucilia cuprina;* B to D: *Lucilia sericata;* E: *Chrysomya chloropyga;* F and G: *Chrysomya marginalis;* H: *Chrysomya albiceps;* I: *Calliphora vicina.*
Legend: simple-ended arrow indicates micropyle, feather-ended arrow indicates bifurcation of median area.

3.2. GENERAL MORPHOLOGICAL ASPECTS OF LARVAE

The terminology used to describe the morphological structures of the larval instars was largely a combination of terminology used by Zumpt (1965), Teskey (1981) and Erzinçlioglu (1985). The body shape (Fig. 13) of all three larval instars were typical of that described for musciform larvae, i.e. apodous with an elongated body, pointed at its anterior end and broad and truncated at its posterior end (Zumpt 1965). Musciform larvae have 12 body segments. Various authors name and number these 12 segments referring to the distinct groupings of the larval body, namely the cephalic segment, the three thoracic segments and the eight abdominal segments. However, the preference during the current study was, like Zumpt (1965), to employ a continuous numbering of the segments. This was to simplify references made to the structures on the various segments. Musciform larvae were termed acephalic due to invagination of the cephalic segment into the body (Teskey 1981). Due to this, the first segment was referred to as the pseudocephalon. Musciform larvae are generally soft bodied, poorly sclerotised organisms; the only sclerotised elements of the larvae being the cephalopharyngeal skeleton and some elements of the posterior spiracles.

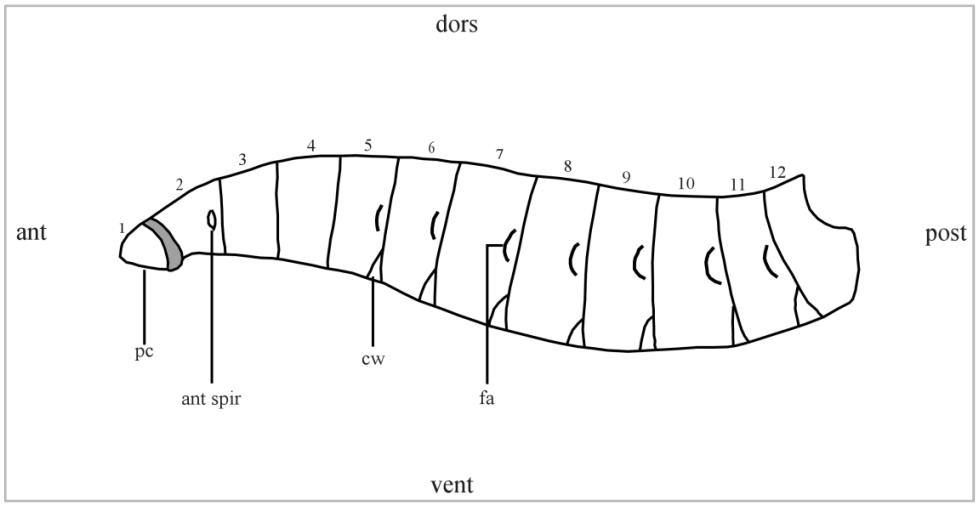


Fig. 13: Lateral view of a musciform larva. Legend: ant: anterior; ant spir: anterior spiracle; cw: creeping welt; dors: dorsal; fa: fusiform area; pc: pseudocephalon; post: posterior; vent: ventral; 1 – 12: body segments.

3.2.1. SENSILLAE (Fig. 14)

Three types of sensillae were identified on various parts of the larval body, namely trichoid {*t sens*} (Figs. 14A and E), pit {*p sens*} (Figs. 14B and E) and button sensillae {*b sens*} (Figs. 14C and 14D). Two types of button sensillae were observed, namely those encapsulated within a socket-like outgrowth of the cuticle {*encap b sens*} (Fig. 14D) and those without a socket-like outgrowth {*un-encap b sens*} (Fig. 14D). In all the larval instars, for all the species under discussion, a set of trichoid and pit sensillae (Fig. 14E) were noted on the ventral aspect of segments 2, 3 and 4, arranged around the midline of the specified segments. Pit sensillae were the most common type of sensillae and were noted at various locations on all segments. The antennomaxillary sensory complex, located on the pseudocephalon, is in essence sensillae organised as specialised structures and will be discussed in latter sections in more detail. Sensillae on the larval body were not evaluated for their diagnostic value, due to their vast numbers and small size that could lead to these structures being easily overlooked.

3.2.2. INTEGUMENT

Based on macroscopic evaluations, various authors refer to calliphorid larvae as smooth or "hairy" / "spiny" larva. Microscopic evaluation (scanning electron microscopy) revealed variations in the integument of calliphorid and sarcophagid larvae and it was therefore considered for its diagnostic value during the course of the present study. These variations are presented as setae, swellings and papillae. Previous ultrastructural evaluations of "hairy" / "spiny" larvae revealed the "hairs"/ "spines" to be elongated processes of the integument. The exact nature of the processes of the "hairy" / "spiny" larvae was evaluated during the course of the current study.

3.2.3. STRUCTURES OF THE PSEUDOCEPHALON (Fig. 15)

The **antennomaxillary sensory complex** is located terminally on the pseudocephalon (Fig. 15) in all the larval instars for all the species under discussion. It is made up of two portions, namely the antennal sensory complex {*asc*} and the maxillary sensory complex {*mxsc*}.

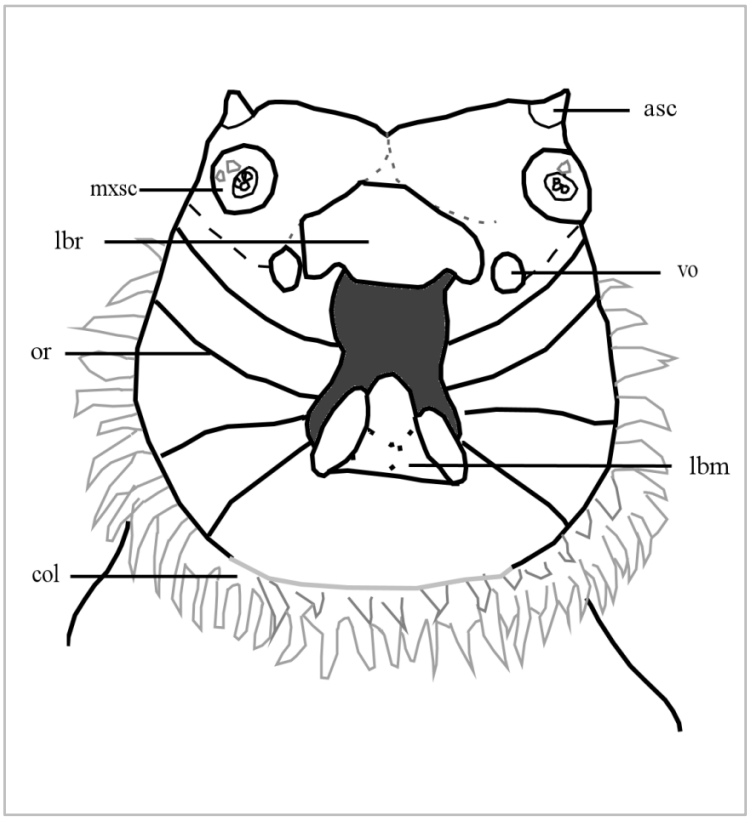


Fig. 15: Ventral view of the first larval segment, i.e. the pseudocephalon. Legend: asc: antennal sensory complex; col: collar; lbm: labium; lbr: labrum; mxsc: maxillary sensory complex; or: oral ridge; vo: ventral organ.

The **ventral organ** {*vo*}, also known as the mandibular sensory complex, was located on the ventral surface of the pseudocephalon (Fig. 15), posterior-medially from the antennomaxillary complex.

The **oral ridges** {*or*} were located on the ventral surface of the pseudocephalon (Fig. 15), radiating away from the preoral cavity.

The **labium** {*lbm*} was located on the ventral surface of the pseudocephalon (Fig. 15) on the posterior margin of the preoral cavity.

A lobe-like structure was located on the anterior margin of the preoral cavity (Fig. 15). Naming this structure was difficult. The location of the structure was similar to the **labrum** {*lbr*} of more primitive Diptera larvae, or to the labrum of the imago, and thus named so. In the chapter on the organs of ingestion of insects, Snodgrass (1935) warned against this comparison. He theorised that due to the invagination of the cephalic segment into the body in this grouping of higher Diptera larvae, it could not be compared to the labrum of more primitive Diptera larvae. Snodgrass (1935) further noted that it could also not be compared to the labrum of the imago due to the drastic changes occurring during pupation. Despite all this, this structure was referred to as the labrum in the current study, based purely on the position the structure occupied. The labrum as a diagnostic feature was introduced for the first time in this study.

3.2.4. CEPHALOPHARYNGEAL SKELETON

The sclerotised cephalopharyngeal skeleton is located within the anterior two segments of the larva. Teskey (1981) gave a thorough account of the cephalopharyngeal skeleton of a third instar larva and this reference was used as a guideline in the present study. Second instar larvae were similar to third instar larvae and the same terminology used for third instar larvae could be utilised for second instar larvae. Only some of the elements of a first instar larval cephalopharyngeal skeleton were similar to that of second - or third instar larvae. Erzinçlioglu (1985) gave a thorough account of the cephalopharyngeal skeleton of first instar larvae. Some of the terminology employed to describe the elements of the first instar cephalopharyngeal skeletons by Erzinçlioglu (1985) were different from that used to describe second - and third instar larvae. In such instances it was changed to the synonyms suggested by Teskey (1981) in a bid to ensure uniformity.

3.2.5. RESPIRATORY STRUCTURES

The **anterior spiracles** were located on the lateral aspect of segment 2 (Fig. 13). First instar larvae of the species forming part of the present study were thought to be metapneustic, lacking functional anterior spiracles. This was in contrast to the amphipneustic respiratory system of second and third instar larvae, where both anterior and posterior spiracles were present. Kitching

(1976b) revealed the presence of a slit in the integument of first instar larvae with the aid of scanning electron microscopy, while the anterior spiracles are located at the same position in second - and third instar larvae. Further analysis presented strong circumstantial evidence that this was the location of open spiracles opposed to closed pits (Kitching 1976b). From this evidence it was concluded that first instar larvae, like second and third instar larvae, have an amphipneustic respiratory system.

The two **posterior spiracles** (Fig. 16) are located on the spiracular disc (spiracular field / spiracular cavity). The spiracular disc is the posterior aspect of the last (12^{th}) larval segment.

3.2.6. SPINES

The spines showed variation in (i) being single- or multi-pointed, (ii) being sharp- or blunt-tipped and (iii) their location on segments. Spine bands were found on the periphery of segments. Regarding location, spine bands were described as being located on the anterior or posterior aspect of the segment and whether it completely surrounded the segment or not. In addition to the spines found in the spine band area between segments, two other distinct groupings of spines were found intersegmentally (Fig. 13). The first grouping was the creeping welt, found on the ventral aspects of the larval body and the second specialised grouping was the lateral fusiform area. Both of these two spine groupings first appeared in the area between segments 5 and 6 and were found up to the area between segments 11 and 12. The spine band was split ventrally, to form an island of no spines centrally to the structure, surrounded by spines, i.e. the creeping welt. The lateral fusiform area was a thickened area of the cuticle, furnished with spines. The spine band on the anterior margin of segment 2 was conspicuously better developed than the other spine bands and was referred to as the collar {*col*} (Fig. 15) by various other researchers.

3.2.7. CAUDAL SEGMENT (Fig. 16)

The rim of the spiracular field is covered with fine hairs. Six major pairs of tubercles, the perispiracular tubercles, are located on the rim of the spiracular field (Fig. 16). The inner dorsal tubercles {*idt*} and the inner ventral tubercles {*ivt*} are located on the dorsal and ventral aspect

of the spiracular field respectively, off-centre from the midline of the spiracular field. The outer dorsal tubercles {*odt*} and the outer ventral tubercles {*ovt*} are located on the dorsal and ventral aspect of the spiracular field respectively, in a lateral position on the spiracular field. The middle dorsal tubercles {*mdt*} and the middle ventral tubercles {*mvt*} are located on the dorsal and ventral aspect of the spiracular field respectively, between the inner and outer tubercles of their respective hemispheres.

The anal area is located on the ventral aspect of the last segment (Fig. 16). It consists of anal pads on either side of the anal opening. The anal opening is obscured from view by the anal pads. Projecting from each anal pad is a lateral horn-like structure, i.e. the anal horns. The presence of spines on and around the anal area spines are varied in the different species forming part of the current study.

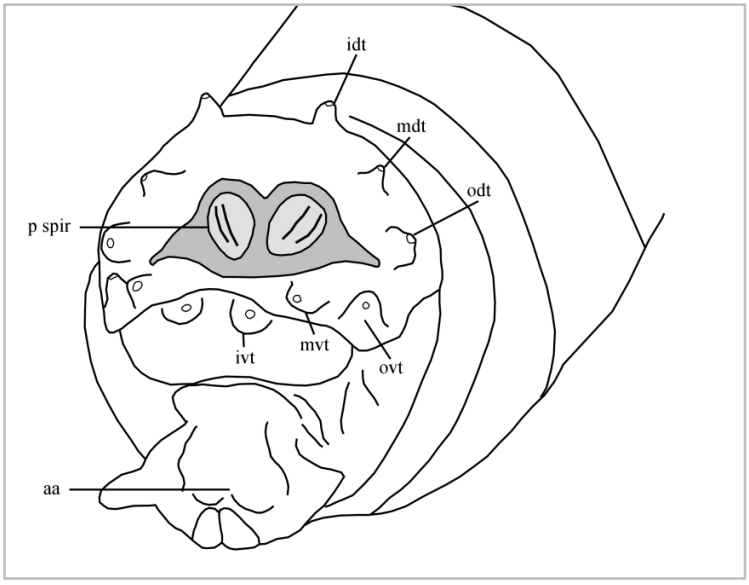


Fig. 16: Posterior view of the caudal segment of a larva. Legend: aa: anal area; idt: inner dorsal tubercle, ivt: inner dorsal tubercle; mdt: middle dorsal tubercle; mvt: middle ventral tubercle; odt: outer dorsal tubercle; ovt: outer ventral tubercle; p spir: posterior spiracle.

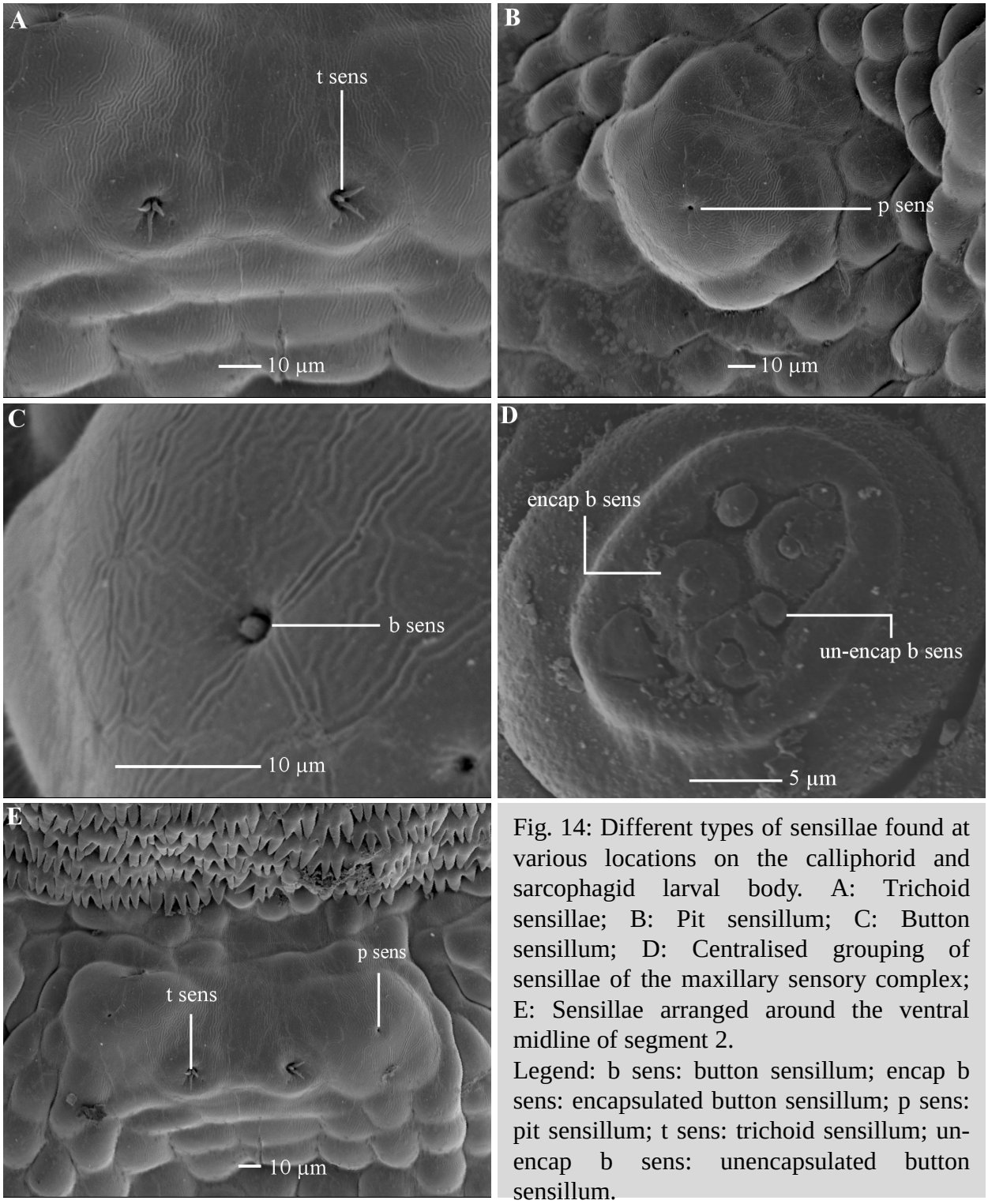


Fig. 14: Different types of sensillae found at various locations on the calliphorid and sarcophagid larval body. A: Trichoid sensillae; B: Pit sensillum; C: Button sensillum; D: Centralised grouping of sensillae of the maxillary sensory complex; E: Sensillae arranged around the ventral midline of segment 2.

Legend: b sens: button sensillum; encap b sens: encapsulated button sensillum; p sens: pit sensillum; t sens: trichoid sensillum; un-encap b sens: unencapsulated button sensillum.

3.3. MORPHOLOGY OF FIRST INSTAR LARVAE

3.3.1. INTEGUMENT (Figs. 17A to 17G)

The integuments of all the first instar larvae examined were smooth (Figs. 17A to 17G). Due to the lack of variation observed for this feature, it was considered unsuitable as a diagnostic feature to distinguish among the various species examined.

3.3.2. ANTENNOMAXILLARY COMPLEX (Figs. 18A to 18G and 19A to 19G)

The antennomaxillary complex {*aml*} (Figs. 18A to 18G) is located in an anterior position on the pseudocephalon.

The antennal sensory complex {*asc*} (Figs. 19A to 19G) is a pointed structure, encircled within a socket-like outgrowth of the cuticle. It was morphologically similar in the different species examined and was thus of no diagnostic value.

The maxillary sensory complex was morphologically similar in the different calliphorids examined (Figs. 19A to 19F). The maxillary sensory complex was slightly raised from the overall surface of the pseudocephalon. A cluster of encapsulated and un-encapsulated button sensillae occupied a centralised position in the maxillary sensory complex {*cent sens*}. This cluster of button sensillae was contained or semi-contained within a single row of half moon-shaped cuticular ridges. Two encapsulated button sensillae were located anteriorly from the cluster of centralised button sensillae {*out sens*}. Due to the morphological similarity of this feature in the different calliphorid species examined, it was considered an unsuitable diagnostic feature to distinguish them from each other. In *S. cruentata* larvae (Fig. 19G) all aspects regarding the sensillae was similar to that found for the calliphorids examined. *Sarcophaga cruentata* was different from the calliphorids examined in that a series of folds enclosed both the centralised and the outlying sensillae, whereas a single row of cuticular folds encircled or semi-encircled the centralised grouping of sensillae in calliphorids.

3.3.3. ORAL RIDGES (Figs. 18A to 18G)

The oral ridges {*or*} (Figs. 18A to 18G) are located on the ventral surface of the pseudocephalon. In first instar larvae, two oral ridges were present. The oral ridges radiated away from the preoral cavity. The oral ridges revealed no further significant variations in the various species examined to be of diagnostic value.

3.3.4. LABIUM (Figs. 18A to 18G)

The labium {*lbm*} is located on the ventral surface of the pseudocephalon on the posterior margin of the preoral cavity (Figs. 18A to 18G). The labium was made up of a centralised lobe with two lobes arranged bilaterally from the centralised lobe. Visibility of the complete labium was dependant on how much the pseudocephalon was retracted within the larval body. The morphology of the labium was consistent in the different species under discussion and was therefore not considered to be a suitable diagnostic characteristic.

3.3.5. LABRUM (Figs. 18A to 18G and 20A to 20G)

The structure of the labrum {*lbr*} was similar in all calliphorid species examined (Figs. 18A to 18F and 20A to 20G). In calliphorids the labrum extended from the anterior margin of the preoral cavity as two tube-like structures adorned with spine-like extensions to its distal ends. Szpila *et al*. (2008) named these spine-like extensions cirri. In the sarcophagid examined (Figs. 18G and 20G) the edges around the preoral cavity was simplified, without being extended significantly and without spine-like extensions to its distal end. The labrum was a useful characteristic to distinguish *S. cruentata* from the calliphorids examined.

3.3.6. CEPHALOPHARYNGEAL SKELETON (Figs. 21 to 29)

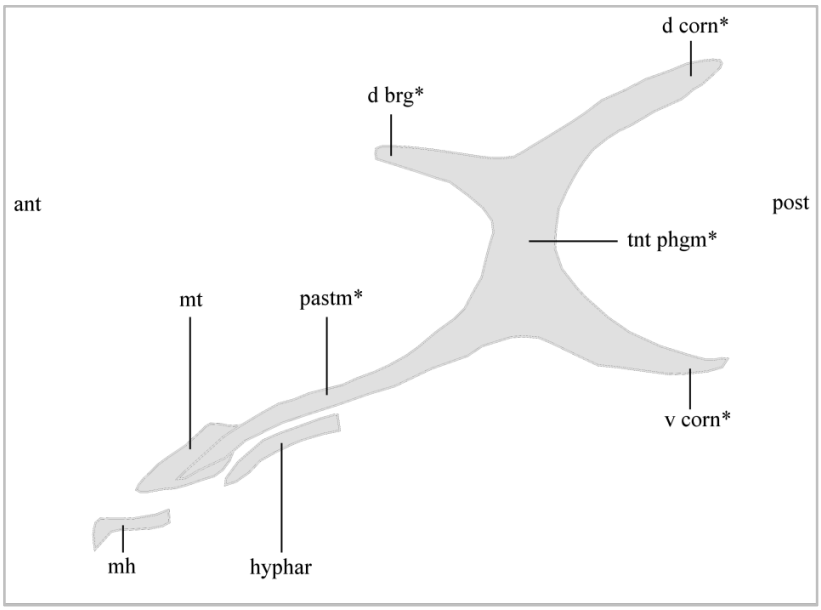


Fig. 21: Representation of the lateral aspect of a generalised first instar larva cephalopharyngeal skeleton. Abbreviations: ant: anterior end; d. brg*: dorsal bridge; d corn*: dorsal cornu; hyphar: hypopharyngeal sclerite; mh: mouth hook; mt: median tooth; pastm*: parastomal sclerite; post: posterior end; tnt phgm*: tentorial phragma; v corn*: ventral cornu. * = Elements of the tentoropharyngeal sclerite.

The terminology and diagrammatic representation of a first instar cephalopharyngeal skeleton (Fig. 21) was based on that presented by Erzinçlioglu (1985). Some of the terminology was replaced by synonyms sourced from the work of Teskey (1981) on larvae. The description of the cephalopharyngeal skeleton was given in terms of its lateral aspect. The anterio-ventral aspect of the tentoropharyngeal sclerite was extended to the anterior (Fig. 21). This extension is equivalent to the parastomal bar of the second and third instar larvae. This structure was, however, more robust than in second and third instar larvae. Because of its extent and size it is referred to as the parastomal sclerite in first instar larvae. Erzinçlioglu (1985) and Liu & Greenberg (1989) illustrated the parastomal sclerite {*pastm*} terminating in a tooth-like structure, the median tooth {*mt*}. In the current study, this transition could not be confirmed. The median tooth appeared to be a separate structure, although it was closely associated with the parastomal sclerite. The

median tooth was also less sclerotised than the parastomal sclerite (Fig. 21). The hypopharyngeal sclerite {*hyphar*} was located ventrally from the parastomal sclerite. This structure was not the typical T-shape as observed in second and third instar larvae. Smaller sclerites can occur anterior from the hypopharyngeal sclerite and ventral from the medial tooth. Erzinçlioglu (1985) referred to these structures as chitinised teeth, but the preference here was to refer to it as dental sclerites like Liu & Greenberg (1989) did, since it corresponds to the location of these sclerites in second and third instar larvae. These sclerites were not distinctly noticeable in most of the specimens examined; the clearing technique employed during the current study possibly faded it. The most anterior element of the cephalopharyngeal skeleton was the mouth hook {*mh*}. Erzinçlioglu (1985) indicated weakly sclerotised structures anterior to the mouth hooks which he named anterior teeth.

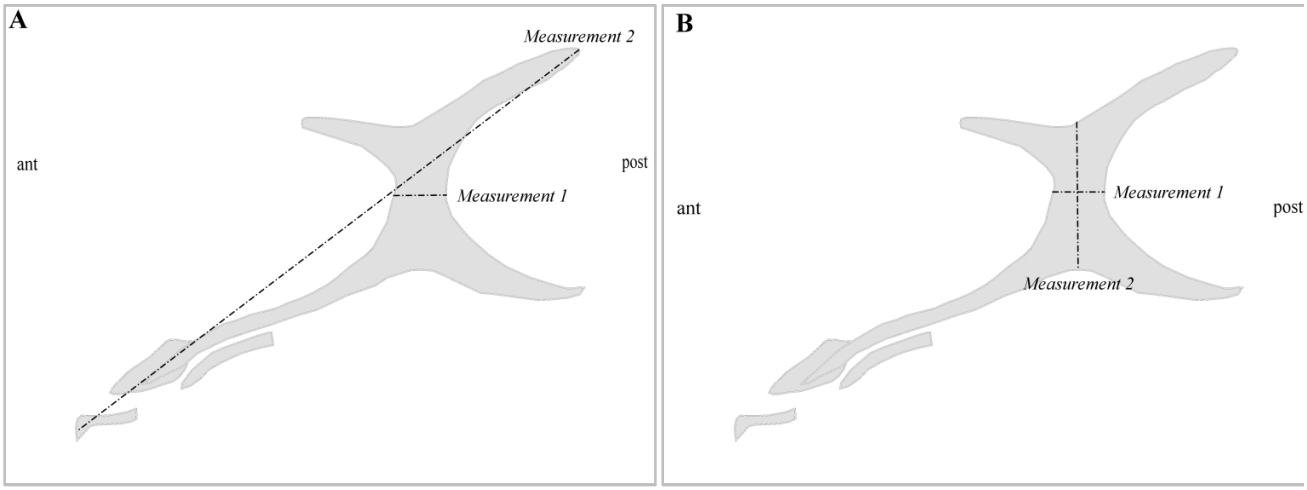


Fig. 22: A guide to the measurements taken to evaluate the different aspects of the cephalopharyngeal skeleton; Fig. 22A Measurement 1 = width of the tentorial phragma and Measurement 2 = length of the cephalopharyngeal skeleton; Fig. 22B, Measurement 1 = width of the tentorial phragma and Measurement 2 = height of the tentoropharyngeal skeleton.

Erzinçlioglu (1985) and Liu & Greenberg (1989) evaluated various aspects of the cephalopharyngeal skeleton of first instar calliphorid larvae for possible diagnostic characteristics. It was not possible to use those recommended aspects of the cephalopharyngeal skeleton as set out by Erzinçlioglu (1985) during the present study. Erzinçlioglu (1985) based his identification on various relationships between the median tooth, the hypopharyngeal sclerite and the tentorial phragma. Possibly due to the clearing technique employed during the current study,

the median tooth and the hypopharyngeal sclerite were not clearly defined; therefore measurements based on these structures were not used. Since the tentorial phragma was clearly defined, measurements based on this structure were assessed for its possible diagnostic value during the current study. The first aspect considered was the width of the tentorial phragma in relation to the length of cephalopharyngeal skeleton (Fig. 22A). A visual inspection of the cephalopharyngeal skeletons clearly showed the tentorial phragma to be narrow in some of the species investigated and being broad in others. To eliminate any subjectivity with regard to this assessment, this measurement was qualified as follows: (i) where the width of the tentorial phragma occupied less than a tenth of the length of the whole cephalopharyngeal skeleton it was considered to be narrow; (ii) values above a tenth of the length represented a broad tentorial phragma. The length of the cephalopharyngeal skeleton was taken from the most posterior tip of the dorsal cornu to the most anterior tip of the mouth hooks. Liu & Greenberg (1989) relied on the relationship between the length and the width of the tentorial phragma as a diagnostic characteristic (Fig. 22B); an aspect also considered during the current study. They used this criterion to group species as (i) those where width of the tentorial phragma was less than half the height of the tentoropharyngeal sclerite and (ii) those where the width of the tentorial phragma was more than half the height of the tentoropharyngeal sclerite (Fig. 22B). The final aspects considered for their diagnostic value were (i) the shape of the mouth-hooks and (ii) the shape of the hypopharyngeal sclerite.

Lucilia cuprina (Figs. 23A to 23C)

The tentorial phragma was narrow in relation to the length of the cephalopharyngeal skeleton (Fig. 23A) and its width was less than half of the height of the tentoropharyngeal sclerite (Fig. 23B). The median tooth (Fig. 23C) had no obvious notch to its dorsal aspect. The hypopharyngeal sclerite (Fig. 23C) appeared to narrow somewhat to its anterior end. A dental sclerite (Fig. 23C) appeared to be closely associated with the base of the mouth hooks and was also closely associated with the anterior end of the hypopharyngeal sclerite. The mouth hooks were angled L-shaped structures (Fig. 23C); similar to that indicated by Erzinclioglu (1989b) for *L. cuprina*.

Lucilia sericata (Figs. 24A to 24C)

The tentorial phragma was narrow in relation to the length of the cephalopharyngeal skeleton (Fig. 24A). The drawings presented of *L. sericata* by Erzinclioglu (1989b) and Liu & Greenberg (1989) also showed the tentorial phragma to be narrow. The width of the tentorial phragma was less than half the height of the tentoropharyngeal sclerite (Fig. 24B); a finding confirmed by Liu & Greenberg (1989) for *L. sericata*. Both Erzinclioglu (1989b) and Liu & Greenberg (1989) showed the dorsal arch to be pointed while the dorsal arch ended bluntly (Figs. 24A and 24B) in the specimens examined during the current study. In the specimens examined, the median tooth (Fig. 24C) had no obvious notches to its dorsal aspect. The hypopharyngeal sclerite appeared to be of equal thickness along its length, narrowing somewhat to its anterior end. Erzinclioglu (1989b) and Liu & Greenberg (1989) noted dental sclerites in the specimens of *L. sericata* examined by them, while these sclerites were absent from the specimens examined during the current study. Erzinçlioglu (1985 and 1989b) indicated that the dental sclerites were ill-defined. These structures were probably dissolved in the specimens examined due to the clearing techniques employed. Similar to the description given by Erzinclioglu (1989b), the mouth hook was an angled L-shaped structure (Fig. 24C).

Chrysomya chloropyga (Figs. 25A to 25C)

The tentorial phragma was broad in relation to the length of the cephalopharyngeal skeleton (Fig. 25A). This aspect was illustrated similarly in the drawing of the congeneric *C. putoria* presented by Greenberg & Szyska (1984). The width of the tentorial phragma was approximately half the height of the tentoropharyngeal sclerite (Fig. 25B). The shape of the dorsal arch was also different in the specimens of *C. putoria* as illustrated by Greenberg & Szyska (1984) to that of the specimens of *C. chloropyga* (Figs. 25A and 25B) examined during the current study. Greenberg & Szyska (1984) showed the dorsal arch as short and robust in *C. putoria*, whereas it was extended and slender in the specimens of *C. chloropyga* (Figs. 25A and 25B) examined during the current study. The ventral cornu (Figs. 25A and 25B) also appeared to be narrower in the specimens of *C. chloropyga* examined compared to the wide ventral cornu presented by Greenberg & Szyska (1984) for *C. putoria*. However, it could be that the true shape of the ventral cornu was destroyed in *C. chloropyga* specimens examined during the current study due to the clearing technique employed. The delicacy of this structure was indicated by Greenberg &

Szyska (1984) by illustrating it as a lighter area in relation to the rest of the cephalopharyngeal skeleton. The median tooth (Fig. 25C) had a well defined notch to its dorsal aspect. This aspect is possibly a further distinguishing feature between *C. chloropyga* and *C. putoria* since the noticeable notch seen in the specimens of *C. chloropyga* examined was not indicated in the drawing presented by Greenberg & Szyska (1984) for *C. putoria*. The hypopharyngeal sclerite (Figs. 25B and 25C) was closely associated with the parastomal sclerite by means of a pedicle. From this pedicle, the hypopharyngeal sclerite broadened into a rounded ventral curve from where it narrowed to the distal end of the structure. All aspects of the hypopharyngeal sclerite were similar to that of *C. putoria* as presented by Greenberg & Szyska (1984). The mouth hook (Fig. 25C) was not conspicuously angled and appeared to be made up of more than one element. The base portion was a knobbly linear structure of roughly equal thickness along its length and the distal portion consisted of a few hook-like structures.

Chrysomya marginalis (Figs. 26A to 26C)

The tentorial phragma was broad in relation to the length of the cephalopharyngeal skeleton (Fig. 26A). The width of the tentorial phragma was more than half the height of the tentoropharyngeal sclerite (Fig. 26B). No obvious notch was noted to the dorsal aspect of the median tooth (Fig. 26C). The hypopharyngeal sclerite (Fig. 26C) was slightly broader at its posterior end than the rest of the structure. The mouth hooks (Fig. 26C) were angled structures. The base portion of the mouth hook was slightly narrower at its posterior margin, but was generally of equal thickness along its length. The hook portion of the mouth hook was blunt-tipped.

Chrysomya albiceps (Figs. 27A to 27C)

The tentorial phragma was broad in relation to the length of the cephalopharyngeal skeleton (Fig. 27A). The width of the tentorial phragma was more than half the height of the tentoropharyngeal sclerite (Fig. 27B); Liu & Greenberg (1989) reported similar dimensions for the congeneric *C. rufifacies*. No obvious notch was noted to the dorsal aspect of the median tooth (Fig. 27C). The posterior end of the hypopharyngeal sclerite (Fig. 27C) was C-shaped. The mouth hook (Fig. 27C) was not conspicuously angled and it appeared to be made up of more than one element. The posterior end of the base portion of the mouth hook was large and rounded and narrowed

considerably anteriorly. The distal end of this structure was made up of numerous hook-like structures of which the most distal ends showed less sclerotisation than the basal ends.

Calliphora vicina (Figs. 28A to 28C)

The tentorial phragma was narrow in relation to the length of the cephalopharyngeal skeleton (Fig. 28A). The width of the tentorial phragma was less than half the height of the tentoropharyngeal sclerite (Fig. 28B); an aspect also reported as such by Liu & Greenberg (1989) for *C. vicina* larvae. Erzinçlioglu (1985) referred to the slender nature of the tentorial phragma, although this comparison was made in relation to the median tooth. A slight notch was noted on the dorsal aspect of the median tooth (Fig. 28C). Erzinçlioglu (1985), Liu & Greenberg (1989) and Szpila *et al.* (2008) documented similar findings for the specimens of *C. vicina* examined by them as did Prins (1982) for the congeneric *C. croceipalpis*. However, the notch on the ventral margin of the median tooth (Fig. 28C) was only indicated by Liu & Greenberg (1989) and Szpila *et al.* (2008) for *C. vicina* and not by Erzinçlioglu (1985) for *C. vicina* or by Prins (1982) for *C. croceipalpis*. The hypopharyngeal sclerite (Figs. 28A to 28C) was broad at its posterior end and it narrowed to its anterior end. The overall shape of the hypopharyngeal sclerite was not similarly indicated by Erzinçlioglu (1985), Liu & Greenberg (1989) and Szpila *et al.* (2008) for the specimens of *C. vicina* examined by them or for the specimens of *C. croceipalpis* examined by Prins (1982). Erzinçlioglu (1985), Liu & Greenberg (1989) and Szpila *et al.* (2008) all indicated a dental sclerite/s, which could not be confirmed in the specimens of *C. vicina* examined during the current study. These sclerites were probably dissolved in the specimens examined, due to clearing techniques employed. The mouth hooks (Figs. 28A and 28C) were angled, L-shaped structures and were made up of more than one element. The L-shape structure of the mouth hooks was confirmed by Erzinçlioglu (1985), Liu & Greenberg (1989) and Szpila *et al.* (2008) for the specimens of *C. vicina* examined by them and also for the specimens of *C. croceipalpis* examined by Prins (1982). The posterior end of the basal portion of the mouth-hook was narrow and pointed and it broadened slightly towards its anterior end. The distal portion was made up of a single element; a sharp-tipped tooth and was attached at a 90° angle to the posterior portion of the structure.

Sarcophaga cruentata (Figs. 29A to 29C)

The cephalopharyngeal skeleton of first instar *S. cruentata* larvae were not as distinctly different from its second and third instar forms as was the case for the calliphorid species. The tentorial phragma was broad in relation to the length of the cephalopharyngeal skeleton (Fig. 29A). The width of the tentorial phragma was equal to or slightly larger than half the height of the tentoropharyngeal sclerite (Fig. 29B). The dorsal arch of the tentoropharyngeal sclerite extended to the anterior and dipped somewhat ventrally (Figs. 29A and 29B). The dorsal cornu (Figs. 29A and 29B) was not split in this instar as was the case in second and third instar *S. cruentata* larvae. The cephalopharyngeal skeleton of *S. cruentata* larvae was clearly distinguishable from that of first instar calliphorid larvae examined during the current study due to the absence of a median tooth in the cephalopharyngeal skeleton of *S. cruentata*. The hypopharyngeal sclerite (Fig. 29C) was located below the anterior end of the parastomal sclerite and the base section of the mouth hooks. It was closely associated with these structures and only discernable as a separate structure with careful focussing and use of contrast. In some specimens a dark area (Figs. 29A and 29C) was noted anterio-ventrally from the hypopharyngeal sclerite. This structure was not what was expected of auxiliary sclerites located in this position due to its size. This dark patch might be due to excessive folding of the collar in this area. The concentration of spines in this area would appear as a dark area. The mouth hook (Figs. 29A and 29C) was made up of a basal portion and a long, slender hook at its distal end. Zumpt (1965) showed the hooks of *Sarcophaga haemorrhoidalis* as more curved and not as extended and straight as the hooks of *S. cruentata* examined during the current study.

The cephalopharyngeal skeleton as diagnostic characteristic

All species examined can be uniquely identified using the features of the cephalopharyngeal skeleton. *Sarcophaga cruentata* can be distinguished from the calliphorid species examined based on the absence of a median tooth in this species (Fig. 29A). The remaining species were grouped in two categories based on the dimensions of the tentorial phragma. The tentorial phragma was narrow in *L. cuprina, L. sericata* and *C. vicina*, whereas it was broad in *Chrysomya* species. Distinguishing between those species grouped together due to their narrow tentorial phragma was based on the shape of the median tooth. The dorsal aspect of the median tooth presented a distinct notch in *C. vicina* (Fig. 28C), whereas no distinct notches was observed in *L.*

cuprina (Fig. 23C) and *L. sericata* (Fig. 24C). *Lucilia cuprina* and *L. sericata* could be distinguished from each other based on the extent of the dorsal bridge. The dorsal bridge was short in *L. cuprina* (Fig. 23A), whereas it was more extended in *L. sericata* (Fig. 24A). Distinguishing between *Chrysomya* species was based on the uniqueness of the mouth hooks and / or the hypopharyngeal sclerite. In *C. chloropyga*: (i) the base of the mouth hook was of approximately equal thickness along its length; the hook portion was made up of a few hook-like structures (Fig. 25C) and (ii) the hypopharyngeal sclerite was closely associated with the parastomal sclerite by means of a thin pedicle, which broadened ventrally into a rounded shape and tapered significantly into a narrow distal end (Fig. 25C). In *C. marginalis*: (i) the base portion of the mouth hook was generally of equal thickness along its length (Fig. 26C); the hook portion of the mouth hook was made up of a single, blunt-tipped hook (Fig. 26C) and (ii) the hypopharyngeal sclerite was slightly broader at its posterior end and tapered slightly to its distal end (Fig. 26C). In *C. albiceps*: (i) the anterior end of the base portion of the mouth hook was broadly rounded, narrowing significantly to it distal end (Figs. 27A and 27C); the mouth hook terminated as multiple hooks (Fig. 27C) and (ii) the posterior end of the hypostomal sclerite was C-shaped (Fig. 27C).

3.3.7. ANTERIOR SPIRACLES OF THE RESPIRATORY SYSTEM (Figs. 30A to 30G)

It was generally accepted that first instar larvae of most free-living Cyclorrhapha were metapneustic (i.e. lacking functional anterior spiracles) until Kitching (1976b) presented evidence that suggested otherwise. Scanning electron microscopy, revealed the anterior spiracle to be a small, simple, open orifice (Kitching 1976b). The current study confirmed the findings of Kitching (1976b); the anterior spiracle of the first instar larvae of all the species examined presented as a simple slit in the body wall (Figs. 30A, 30B and 30E to 30G). In some of the specimens examined the orifice could not be located on the lateral aspect of segment 2 (Figs. 30C and 30D). The small opening was easily hidden from view in specimens presenting with folds in this area. These folds were due to complications of preservation and / or processing. The anterior spiracles showed no variation in the different species examined and was thus of no diagnostic value.

3.3.8. POSTERIOR SPIRACLES OF THE RESPIRATORY SYSTEM (Figs. 31 to 32)

The posterior spiracles could not be assessed by light microscopy for possible sclerotisation patterns since it was difficult to optimally mount the posterior spiracles of first instar larvae. Furthermore, posterior spiracles of first instar larvae were weakly sclerotised and presented very little by means of distinguishing diagnostic features. Assessment by scanning electron microscopy revealed more of the posterior spiracles than what light microscopy did. A posterior spiracle of a first instar larva (Fig. 31) contained two spiracular openings {*so*}. The margins of the spiracular openings, the rimae, were not well defined and consequently the attachment position of the spiracular hair clusters to the margins of the rimae could not be ascertained. The rimae were fused to each other at their inner, ventral margins (Fig. 31). The spiracular hair clusters (Fig. 31) were as follows: (i) the inner spiracular hair cluster {*i shc*} was attached to the outer margin of the inner spiracular opening; (ii) the middle-inner spiracular hair cluster {*mi shc*} was attached to a position between the two spiracular openings; (iii) the middle-outer spiracular hair cluster {*mo shc*} was attached to the upper margin of the outer spiracular opening and (iv) the outer spiracular hair cluster {*o shc*} was attached to the lower margin of the outer spiracular opening.

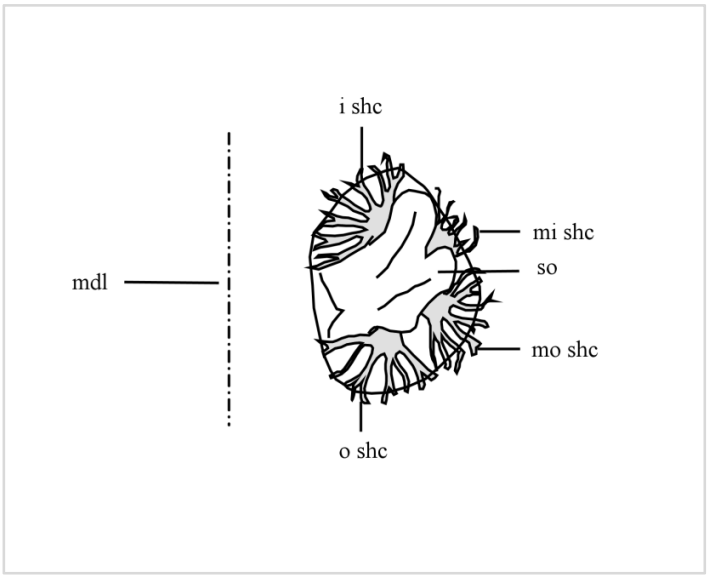


Fig. 31: Representation of a generalised posterior spiracle of a first instar larva. Abbreviations: i shc: inner spiracular hair cluster; mdl: midline of spiracular field; mi shc: middle-inner spiracular hair cluster; mo shc: middle-outer spiracular hair cluster; o shc: outer spiracular hair cluster; so: spiracular opening.

Lucilia cuprina (Fig. 32A) | ***Lucilia sericata*** (Fig. 32B)

The hair of three of the spiracular hairs clusters were branched out in a fan-like fashion. The exception being the middle-inner spiracular hair cluster being limited with no branching noted in some specimens and a tiny split in the tip of the spiracular hair noticed in other specimens of *L. cuprina* (Fig. 32A). The whole structure of the middle-inner spiracular hair cluster was not always clearly seen in *L. sericata* specimens (Fig. 32B) examined; however, where it was visible the middle-inner spiracular hair cluster was made up of a single branch with a split tip. The spiracular hair appeared to be more robust in *L. sericata* specimens (Fig. 32B) than in *L. cuprina* specimens (Fig. 32A).

Chrysomya chloropyga (Fig. 32C) | ***Chrysomya marginalis*** (Fig. 32D) | ***Chrysomya albiceps*** (Fig. 32E) | ***Calliphora vicina*** (Fig. 32F)

All four spiracular hair clusters of *Chrysomya* – (Figs. 32C to 32E) and *Calliphora* (Fig. 32F) species examined were branched in a fan-like pattern. The spiracular hairs of these species were, similar to that of *L. cuprina* (Fig. 32A), i.e. finely textured compared to the more robust spiracular hair seen in *L. sericata* specimens (Fig. 32B).

Sarcophaga cruentata (Fig. 32G)

A description of the posterior spiracles was not possible for *S. cruentata* due to the location of these structures within a cavity. This cavity was structured in such a way that the posterior spiracles within it were largely obscured from view (Fig. 32G). Zumpt (1965) described the posterior spiracles of *S. haemorrhoidalis* as two oval openings without a peritreme.

The posterior spiracles as diagnostic feature

Lucilia cuprina, *L. sericata* and *S. cruentata* could be distinguished from the other species examined, based on the features of the posterior spiracles or features associated with the posterior spiracles. The posterior spiracles in *S. cruentata* (Fig. 32G) was not available for assessment due to the deep spiracular cavity it were located within, opposed to the situation found in the other species examined where the posterior spiracles was located on an open

spiracular plate. The lack of branching noted for the middle-inner spiracular hair cluster of *Lucilia* species, distinguished it from the other species where the hair of this spiracular hair cluster was branched in a fan-like pattern. The spiracular hair was finely-textured in *L. cuprina* (Fig. 32A), whereas it was more robust in *L. sericata* (Fig. 32B). The posterior spiracles were morphologically similar in *Chrysomya* (Figs. 32C to 32E) and *Calliphora* (Fig. 32F) species examined, therefore, this feature was of no value to further distinguish these species from each other.

3.3.9. SPINES (Figs. 33 to 35)

Spines were generally single-pointed in the first instar larvae of the various species examined. One of the aspects considered regarding spines was whether the spines were located on the anterior or posterior margins of the segments. Furthermore, the completeness of the spine band around the circumference of segments was assessed. A creeping welt was located on the ventral aspect of the larvae between segments 5 and 6 up to segments 11 and 12. The creeping welts (Figs. 35A to 35G) were morphologically similar in the different species examined. A fusiform area of spines was located on the lateral aspect of the larvae between segments, in a corresponding position as the creeping welt. The prominence of the fusiform area of spines was noted.

Lucilia cuprina (Figs. 33A and 34A)

Spines were single-pointed (Fig. 33A). The base of the spines was broad, with a narrow, sharp tip (Fig. 33A). Spines were located on the anterior margins of segments. The spine bands were complete on the anterior margins of segments 2 to 7. In some specimens examined, a small number of spines were located on the dorsum of segment 8, but generally spines were absent from the anterior dorsal aspects of segments 8 to 12. The fusiform areas were not clearly defined at the position it was expected to occur at. Various aspects about the spines were confirmed by other authors: O'Flynn & Moorhouse (1980) referred to the single-pointed nature of the spines; Zumpt (1965) and Erzinclioglu (1989b) described complete spine bands from segments 2 to 7. Zumpt (1965) did not specify whether spines were located on the anterior or posterior margins of segments, but specified the position of the complete spine bands on segments 10 and 11, as being

posterior. O'Flynn & Moorhouse (1980) were also not specific regarding the position of the spines on segments 2 to 7, but specifically mentioned the posterior position of the spine band of segment 11. This implies that the position of the spine bands of segments 2 to 7 as reported by Zumpt (1965) and O'Flynn & Moorhouse (1980) was anterior. The presence of the spines on the posterior margins on the last few segments could not be confirmed in the specimens of *L. cuprina* examined during the present study. The fusiform area was indistinct on the lateral aspect of the larva (Fig. 34A).

Lucilia sericata (Figs. 33B and 34B)

Spines were single-pointed. The base of the spines was broad, with a narrow, sharp tip (Fig. 33B). Spines were located on the anterior margins of segments. The spine bands were complete on the anterior margins of segments 2 to 7. A small number of spines, and in some specimens no spines were seen on the anterior dorsal margins of segments 8 and 9. No spines were observed on the anterior dorsal margins of segments 10 to 12. The fusiform areas contained very little spines and the fusiform areas were indistinct on the lateral aspects of larvae. Erzincliogl u (1989b) indicated complete anterior spine bands for segments 2 to 6 and Liu & Greenberg (1989) reported complete anterior spine bands for segments 2 to 7. Zumpt (1965) did not specify whether the spine bands were anteriorly or posteriorly located, but reported complete spine bands for segments 2 to 7. The fusiform area was indistinct on the lateral aspect of the larva (Fig. 34B).

Chrysomya chloropyga (Figs. 33C and 34C)

Spines were single-pointed. The base of the spines was broad, with a narrow, sharp tip (Fig. 33C). Spines were only positioned on the anterior margins of segments, with no spines noted on the posterior margins of segments. The spine bands were complete on the anterior margins of segments 2 to 9. Zumpt (1965) reported the spine bands to be also complete on segment 10. Few spines were present on the lateral aspect of segments 8 to 12, as well as on the lateral fusiform area. The fusiform areas were not clearly demarcated structures. The fusiform area was indistinct on the lateral aspect of the larva (Fig. 34C).

Chrysomya marginalis (Figs. 33D and 34D)

Spines were single-pointed. The base of the spines was broad, with a narrow, sharp tip (Fig. 33D). The spine bands were located on the anterior margins of segment, with no posterior located spines noted. The spine bands were complete on the anterior margins of segments 2 to 10. The spines on the anterior dorsal aspect of segment 10 were few and in some specimens no spines were found in this position. The lateral fusiform areas were weakly defined structures containing no spines to very few spines on this structure. The fusiform area was indistinct on the lateral aspect of the larva (Fig. 34D).

Chrysomya albiceps (Figs. 33E and 34E)

Only single-pointed spines were present. These spines had a broad base, with narrow, sharp tips (Fig. 33E). This was similar to that reported by Liu & Greenberg (1989) and Sukontason *et al*. (2003) for the congeneric *C. rufifacies*. O'Flynn & Moorhouse (1980) reported mostly single-pointed spines for *C. rufifacies*, but also noted spines with 2 or 3 tips in some specimens. Spines were located on the anterior margins of the segments, contrary to the situation reported by Sukontason *et al*. (2003) for *C. rufifacies* where spines were located on both the anterior and the posterior margins of segments. This is possibly a distinguishing characteristic between the two species. Spine bands were complete on segments 2 to 6, similar to the situation reported by Zumpt (1965) for *C. albiceps* and by O'Flynn & Moorhouse (1980) and Liu & Greenberg (1989) for *C. rufifacies*. Clearly defined fusiform areas of spines were noted from segments 6 to 10. The fusiform area was distinctly visible on the lateral aspect of the larva (Fig. 34E).

Calliphora vicina (Figs. 33F and 34F)

Spines were single-pointed with a relatively broad base and a narrow, sharp tip (Fig. 33F). Spines were located on the anterior and posterior margins of segments. The spine bands were complete from segments 2 to 11. This was largely due to the complete anterior spine bands from segments 2 to 10 and complete posterior spine bands from segments 6 to 11. It was noted that as the anterior located spines became less on the dorsum between segments, spines located on the posterior margins of segments replaced these. The posterior spine bands were not always visible, and could be hidden in the folds between segments in larvae that were not optimally stretched out. Zumpt (1965), Erzinçlioglu (1985) and Liu & Greenberg (1989) generally all reported

complete spine bands from segments 2 to 11. It is where the anterior spine bands were complete and where the posterior bands began that did not only differed from the account given for the specimens examined as part of the current study, but also among the accounts given by the various authors. The fusiform areas were clearly defined on segments 10 and 11 and less so on the other segments. The fusiform area was indistinct on the lateral aspect of the larva (Fig. 34F).

Sarcophaga cruentata (Figs. 33G, 33H and 34G)

Spines on segment 2 were single-pointed and had a broad base with a sharp tip (Fig. 33G). The tips of the single-pointed spines on segments 6 to 12 were extended and these spines had a hair-like appearance (Fig. 33H). The spine bands were complete from segments 2 to 12, through anterior located spine bands on segments 2 to 12 and complete posteriorly located spine bands on segments 6 to 11. The fusiform areas were well defined. Zumpt (1965) reported complete anterior spine bands from segments 2 to 10 and complete posterior spine bands from segments 6 to 12. The fusiform area was distinctly visible on the lateral aspect of the larva (Fig. 34G).

Spines as a diagnostic characteristic

Only a partial identification can be achieved using the various aspects of the spines. *Sarcophaga cruentata* could be uniquely identified due to the hair-like appearance of spines (Figs. 33G and 33H) of segments 6 to 12, whereas the spines were typical, i.e. a broad base with a sharp tip, in the other species examined (Figs. 33A to 33F). *Calliphora vicina* was the only other species with spines on both the anterior and posterior margins of segments, whereas spines were located on only the anterior margin of segments in the remainder of the species. The completeness of the spine bands on specific segments can be used to distinguish the rest of the species examined. This feature, however, should be used with caution since some variation regarding this aspect was noted when comparing the results of the current study with other studies. Furthermore, some specimens examined contained spines, albeit in very limited numbers, on some of the subsequent segments to what will be reported below. The cautionary attempt to distinguish the remaining species was as follow: complete anterior spine bands were located from segments 2 to 7 for *L. cuprina* and *L. sericata*; from segments 2 to 9 for *C. chloropyga* and *C. marginalis* and for segments 2 to 6 for *C. albiceps*. No further distinguishing aspects regarding spines were found to differentiate *L. cuprina* from *L. sericata*. However, the scarcity of spines on the lateral aspect of

C. chloropyga larvae separated it from *C. marginalis* larvae where plenty of spines were present in this region. As stated previously, basing identification on the completeness of spine bands for certain segments can be problematic; therefore the separation achieved above by means of this aspect was not ideal. When discarding this aspect, only *C. chloropyga* and *C. albiceps* can be distinguished from the other species by means of more stable characteristics. Spines on the lateral aspect of segments 8 to 12 were scant in *C. chloropyga*, whereas it was present in more significant numbers in the other species examined. The fusiform area was weakly defined in all species examined, except in *C. albiceps* where it was well defined from segments 6 to 10.

3.3.10. CAUDAL SEGMENT (Figs. 36 to 37)

The posterior spiracles {*p spir*} and anal area {*aa*} are contained on the caudal segment of the larvae. The posterior spiracles are located on a spiracular plate or within a spiracular cavity. Hair was present on the rim of the spiracular field. This hair was assessed for their density and whether it was present around the entire rim of the spiracular field or not. The perispiracular tubercles located on the rim of the spiracular field were small in some specimens examined and inconspicuous in others. Due to this, the distances between the various groupings of perispiracular tubercles were not assessed for its diagnostic value for first instar larvae. The anal area is located on the ventral aspect of the caudal segment. It consists of anal pads {*ap*} on either side of the anal opening, with a single, lateral horn-like element projecting from it. The relative size relation between the anal pads and anal horns {*ah*} was assessed. Also evaluated for its diagnostic value was the extent of, or the lack of, spines in the vicinity of the anal area.

Lucilia cuprina (Figs. 36A and 37A)

The posterior spiracles were located on a spiracular plate. The hair on the rim of the spiracular field was sparse (Fig. 36A). Hair was present on the entire circumference of the spiracular field (Fig. 36A). The anal pads and anal horns were similar in size (Fig. 37A). A few spines were noted on the inner margins of the anal pads.

Lucilia sericata (Figs. 36B and 37B)

The posterior spiracles were located on a spiracular plate. Hair on the rim of the spiracular field was similar to that of *L. cuprina*, i.e. sparsely distributed hair on the entire circumference of the spiracular field (Fig. 36B). The anal horns were larger than the anal pads (Fig. 37B). A few spines were noted posterior from the anal pads (Fig. 37B).

Chrysomya chloropyga (Figs. 36C and 37C)

The posterior spiracles were located on a spiracular plate. The coverage of hair on the rim of the spiracular field was sparse, with no hair noted on the dorsal aspect of the spiracular field (Fig. 36C). The anal horns were slightly larger than the anal pads (Fig. 37C). A few spines were noticed posterior from the anal area (Fig. 37C).

Chrysomya marginalis (Figs. 36D and 37D)

The posterior spiracles were located on a spiracular plate. Sparsely distributed hair was noted on the ventral and lateral aspects of the rim of the spiracular field, with no hair noted on the dorsal aspect of the spiracular field (Fig. 36D). The anal pads were smaller than the anal horns (Fig. 37D). A few spines were noticed posterior from the anal area (Fig. 37D).

Chrysomya albiceps (Figs. 36E and 37E)

The posterior spiracles were located on a spiracular plate. The hair on the rim of the spiracular field was sparsely distributed, with almost no hair on the dorsal aspect of the spiracular field (Fig. 36E). The anal pads and horns were similar in size (Fig. 37E). Stubby, single-pointed spines were found around the anal area (Fig. 37E).

Calliphora vicina (Figs 36F and 37F)

The posterior spiracles were located on a spiracular plate. The hair on the rim was distributed around the entire circumference of the spiracular field (Fig. 36F). The coverage of this hair was intermediate, i.e. more than the sparse covering seen in the other calliphorids examined (Figs. 36A to 36E), but considerably less dense than the thick covering seen in the sarcophagid examined (Fig. 36G). The anal pads were smaller than the anal horns (Fig. 37F). A few spines were noted on the internal margins of the anal pads.

Sarcophaga cruentata (Figs. 36G and 37G)

The posterior spiracles were located in a spiracular atrium. The rim of the spiracular field was covered with a thick covering of hair (Fig. 36G). The anal pads were much smaller than the anal horns (Fig. 37G). A few robust spines were noted posterior to the anal pads (Fig. 37G).

The caudal segment as a diagnostic characteristic

Spiracular field

A partial separation was achieved using the features of the spiracular field. *Sarcophaga cruentata* was unique due to the posterior spiracles being located within a spiracular cavity (Fig. 36G), whereas it was located on a spiracular plate in the rest of the species examined (Figs. 36A to 36F). Furthermore, the hair on the rim of the spiracular field was dense for *S. cruentata* (Fig. 36G), whereas less hair was found on the rim of the spiracular field in the rest of the species examined (Figs. 36A to 36F). Sparsely distributed hair was found on the rim of the spiracular field in *Lucilia* (Figs 36A and 36B) and *Chrysomya* (Figs. 36C to 36E) species, while more hair, albeit not to the extent as in *S. cruentata*, were found on the rim of the spiracular field of *C. vicina* (Fig. 36F). In *L. cuprina* and *L. sericata*, the fine covering of hairs were found on the entire rim of the spiracular field, whereas the dorsal aspect of the spiracular field contained no hair in *Chrysomya* species (Figs. 36C to 36E) examined. The spiracular field presented with no further unique features to distinguish between the two *Lucilia* species or among the three *Chrysomya* species examined.

Anal area

Two categories were defined based on the dimensions of the elements of the anal area. In the first category were those species where the anal pads were approximately equal in size to the anal horns (*L. cuprina*, *C. chloropyga* and *C. albiceps*). In the second category were those species where the anal horns were larger than the anal pads (*L. sericata*, *C. marginalis*, *C. vicina* and *S. cruentata*). Spines or the lack of spines associated with the anal area can be used in an attempt to distinguish the various species. It should be noted that the spines found on the inner margins of the anal pads in *L. cuprina* and *C. vicina* were not always observed in all specimens examined. The use of this feature to uniquely identify these species was therefore not

recommended. In that category where the anal pads and horns were similar in size: a few spines were found on the inner margins of the anal pads (not seen in all specimens examined) and posterior to the anal area in *L. cuprina*; a few spines were found posterior to the anal area in *C. chloropyga* (Fig. 37C) and robust spines were found in the area surrounding the anal area in *C. albiceps* (Fig. 37E). Because the spines on the inner margins of the anal pads were not observed in all specimens of *L. cuprina* examined, *C. albiceps* was the only species uniquely described through the arrangement of spines in this category. In the category where the anal horns were larger than the anal pads: a few spines were noted in the area posterior to the anal area of *L. sericata* (Fig. 37B), *C. marginalis* (Fig. 37D) and *S. cruentata* (Fig. 37G); a few spines were found on the inner margins of the anal pads in *C. vicina*. No further unique features regarding the anal areas were found to distinguish *L. sericata, C marginalis* and *S. cruentata* from each other. Similar to the concern raised about the spines on the inner margins of the anal pads for *L. cuprina,* uniquely identifying *C. vicina* through this feature was considered problematic. Taking this reservation into consideration, none of the species in this category could be uniquely identified.

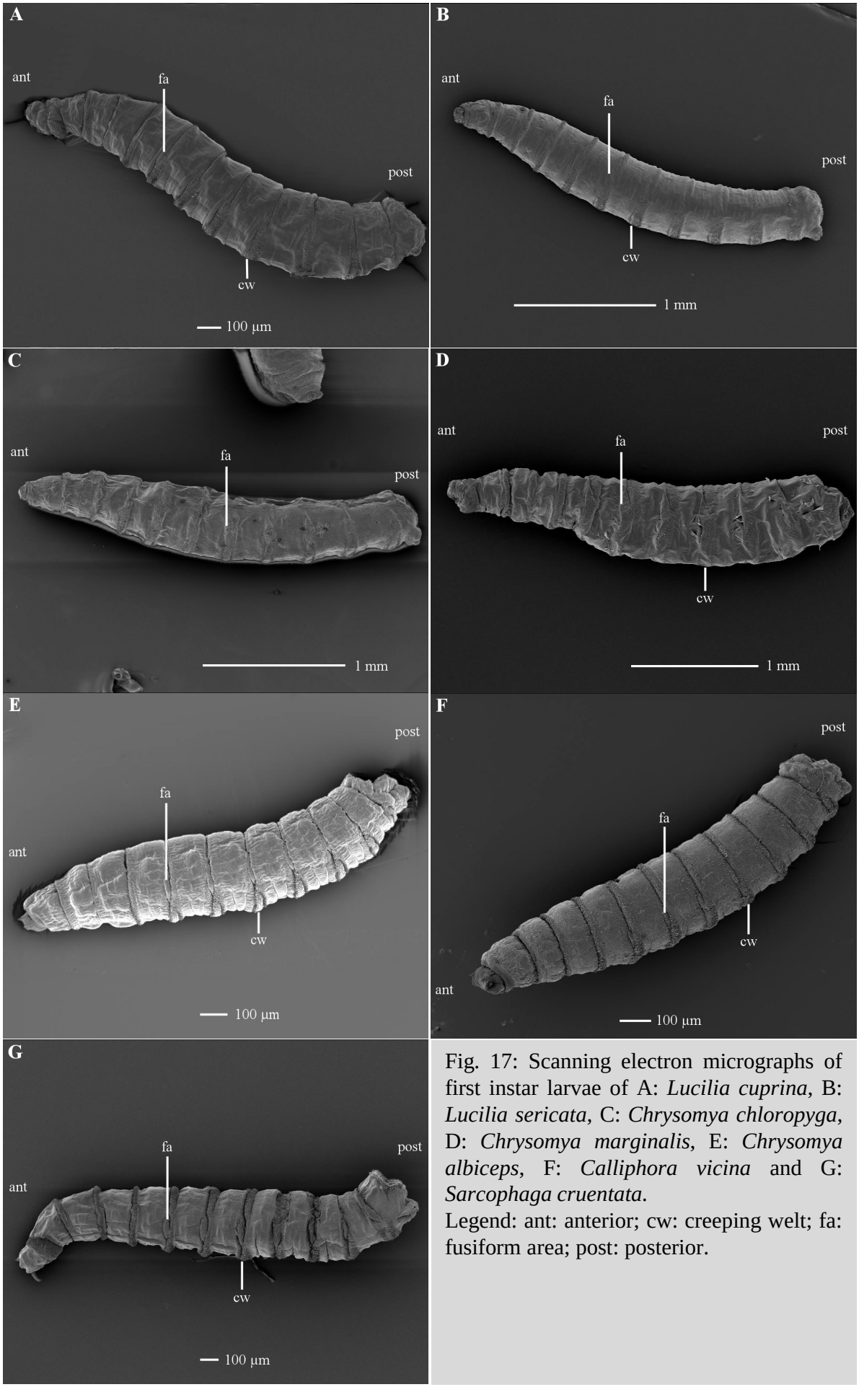


Fig. 17: Scanning electron micrographs of first instar larvae of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina* and G: *Sarcophaga cruentata*.
Legend: ant: anterior; cw: creeping welt; fa: fusiform area; post: posterior.

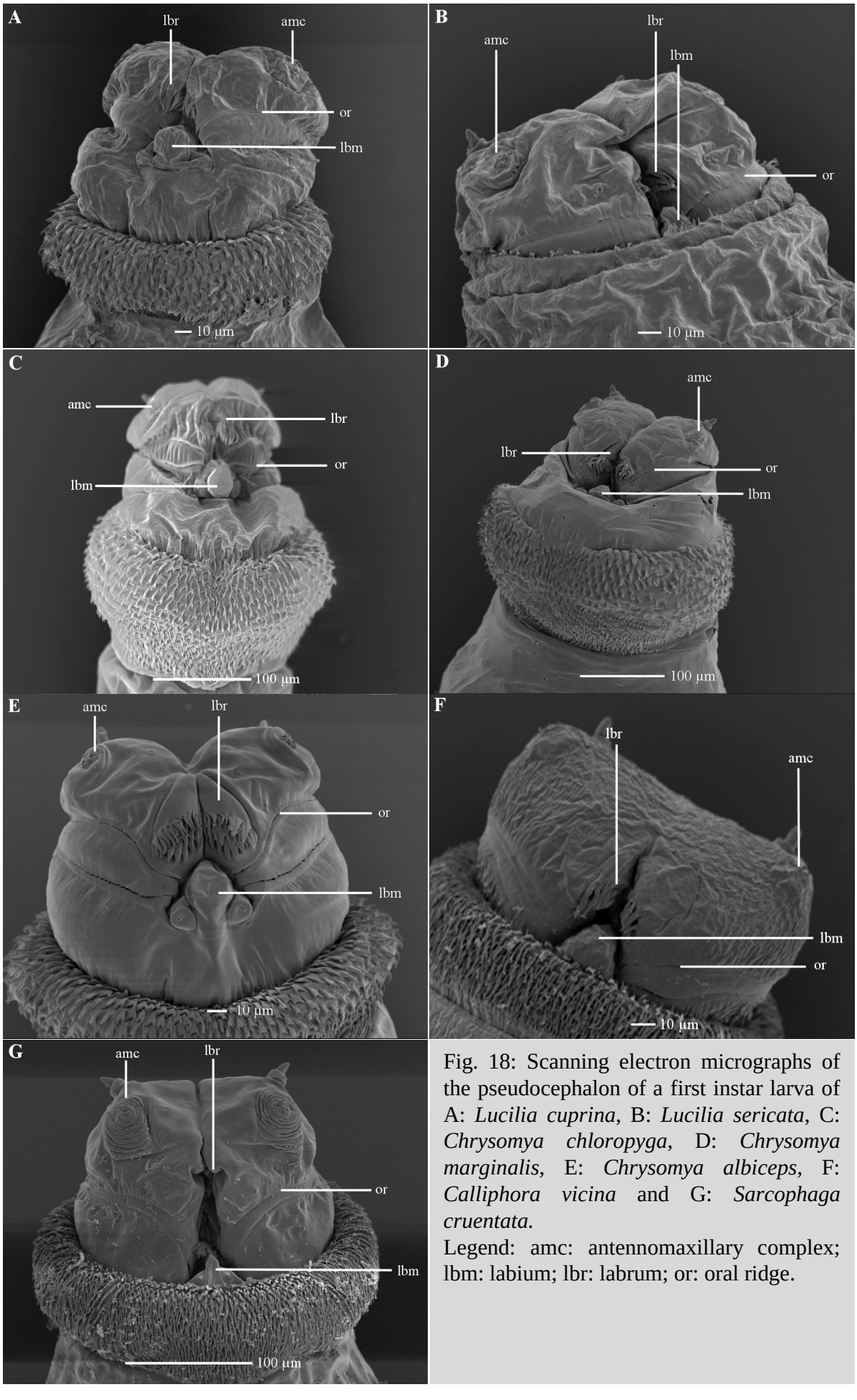


Fig. 18: Scanning electron micrographs of the pseudocephalon of a first instar larva of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina* and G: *Sarcophaga cruentata.*

Legend: amc: antennomaxillary complex; lbm: labium; lbr: labrum; or: oral ridge.

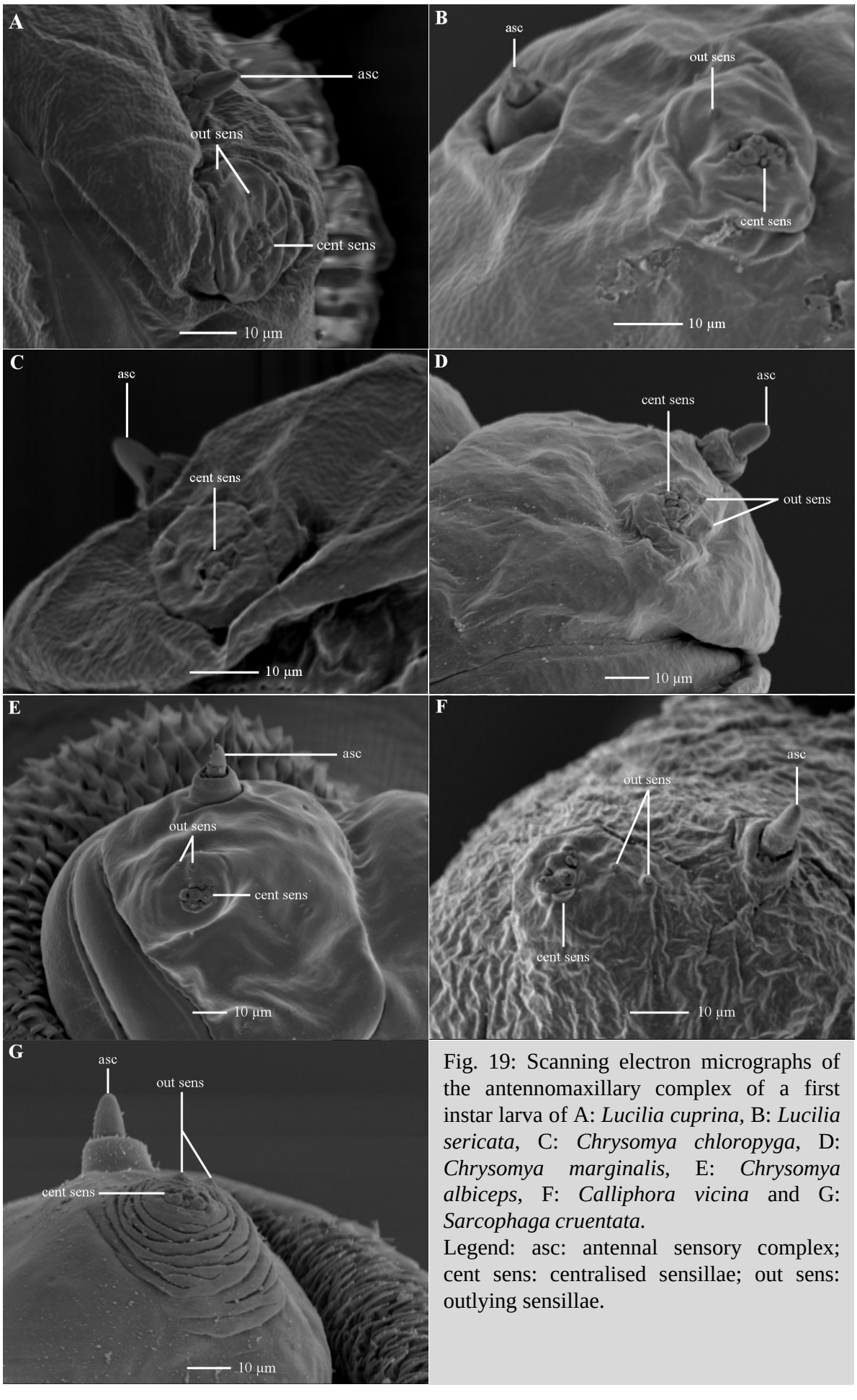


Fig. 19: Scanning electron micrographs of the antennomaxillary complex of a first instar larva of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina* and G: *Sarcophaga cruentata*.
Legend: asc: antennal sensory complex; cent sens: centralised sensillae; out sens: outlying sensillae.

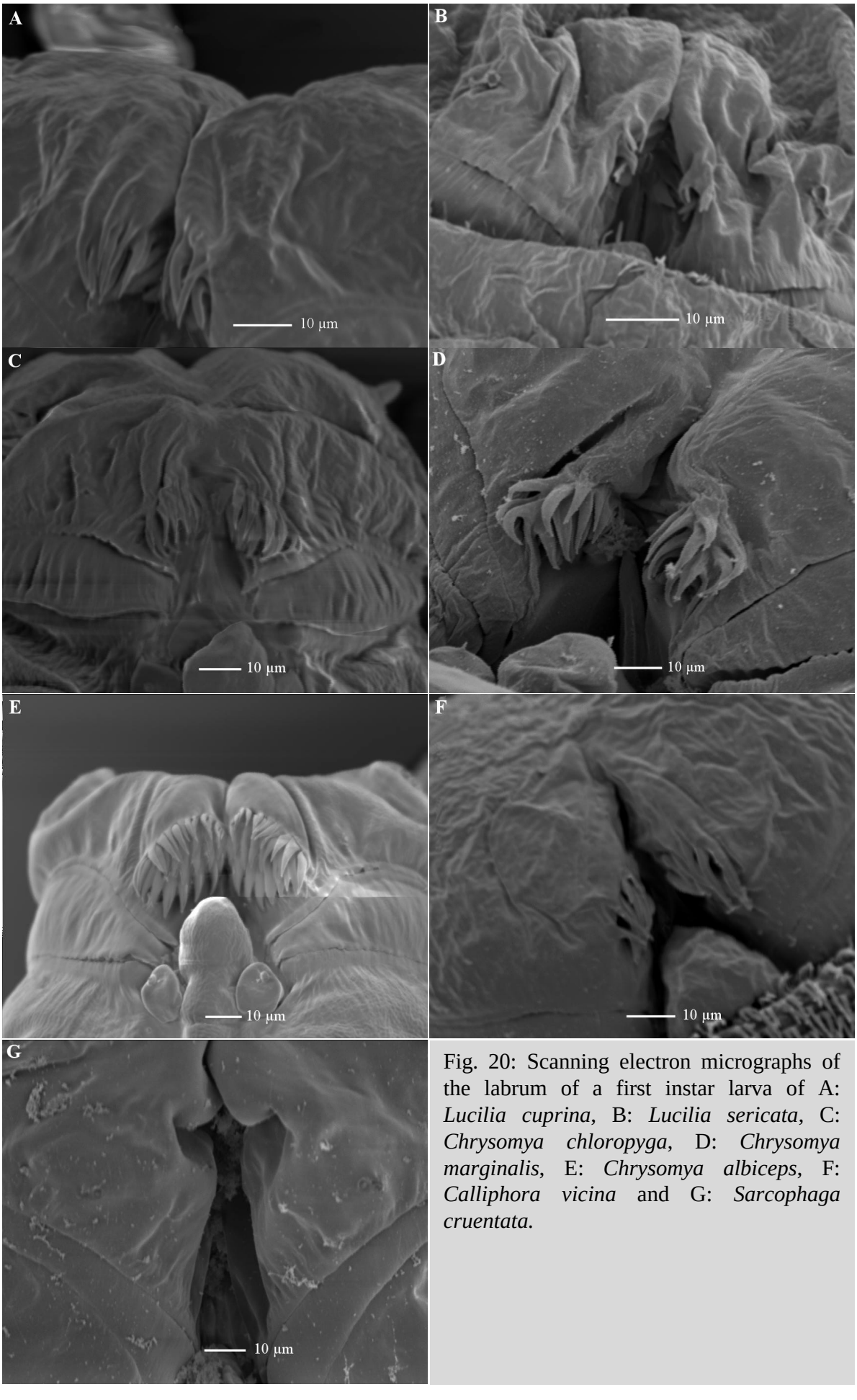


Fig. 20: Scanning electron micrographs of the labrum of a first instar larva of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina* and G: *Sarcophaga cruentata.*

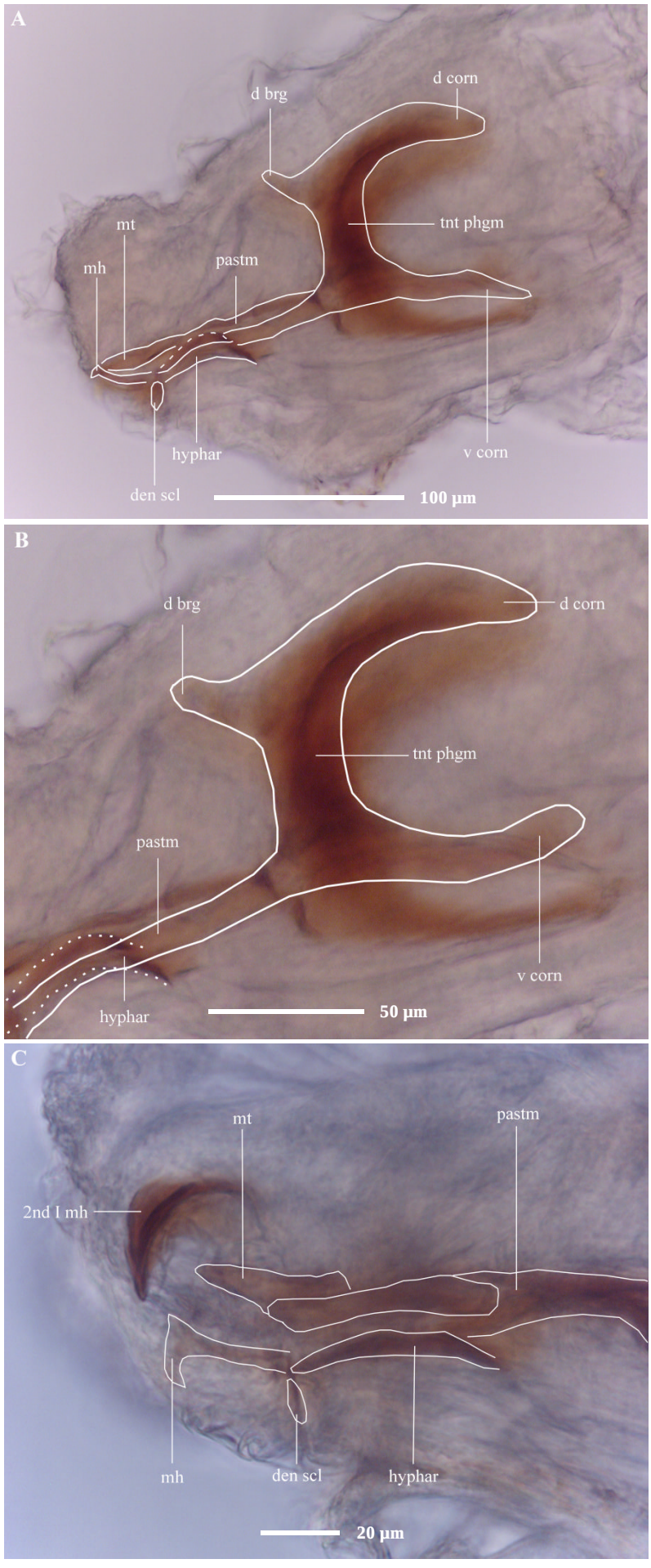
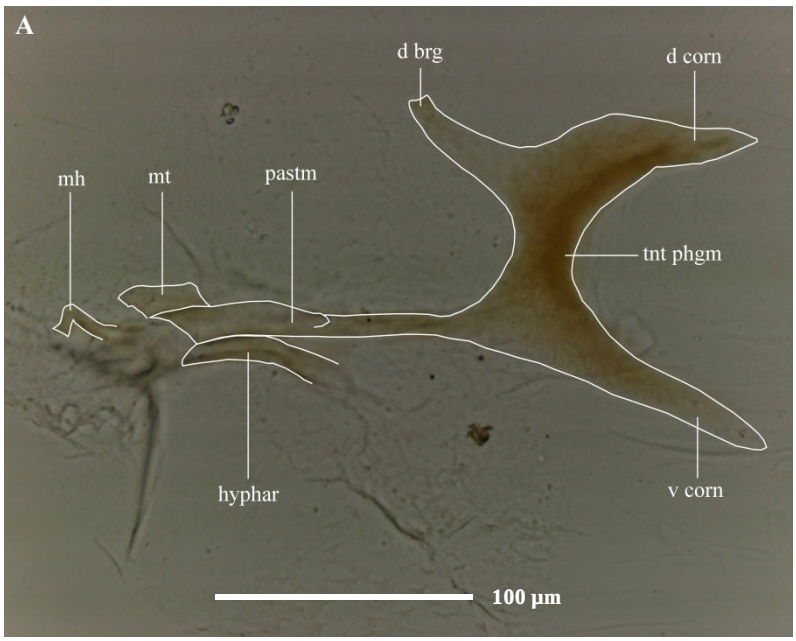
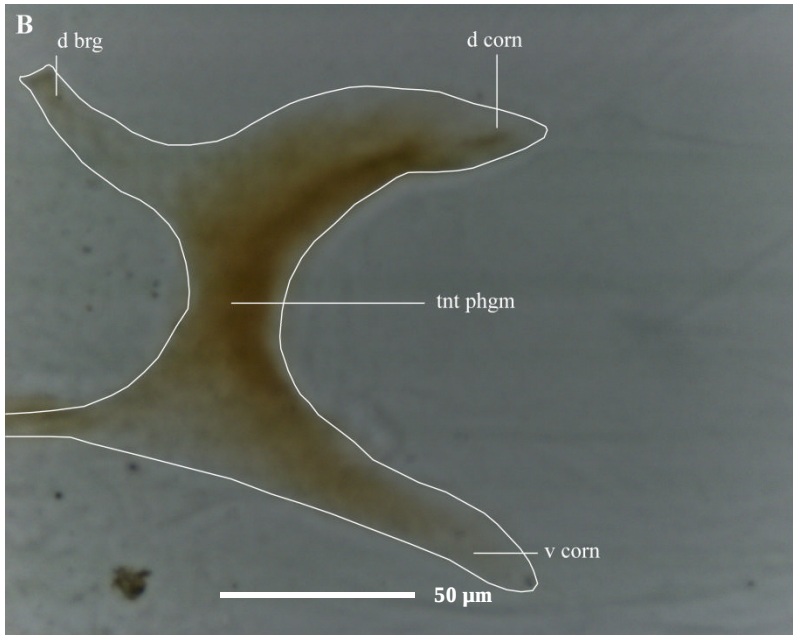


Fig. 23: Light micrographs of the lateral aspect of the cephalopharyngeal skeleton (CPS) of a first instar larva of *Lucilia cuprina*.
23A: Whole CPS.
23B: Posterior elements of the CPS.
23C: Anterior elements of the CPS.
Legend: interrupted lines: less sclerotised elements; 2nd I mh: emerging second instar mouth hook; d brg: dorsal bridge; d corn: dorsal cornu; den scl: dental sclerite; hyphar: hypopharyngeal sclerite; mh: mouth hook; mt: median tooth; pastm: parastomal sclerite; tnt phgm: tentorial phragma; v corn: ventral cornu.





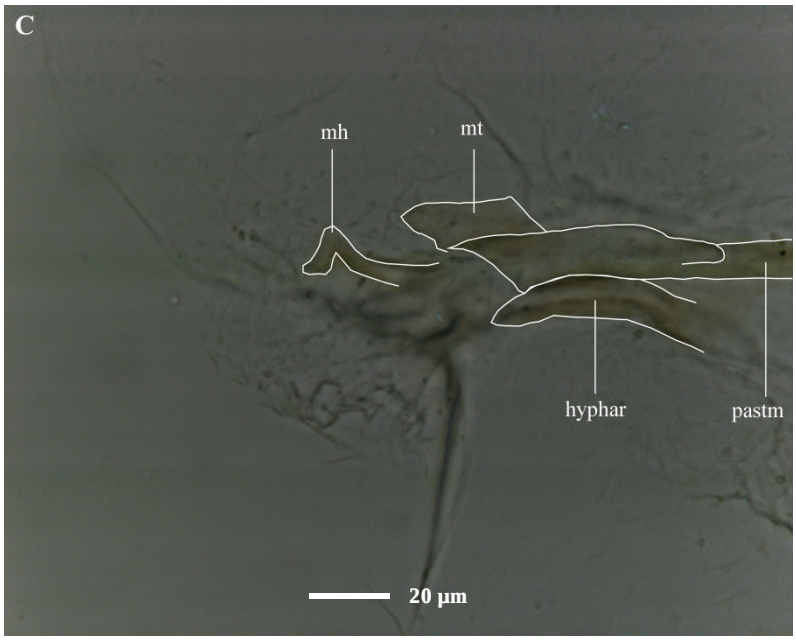
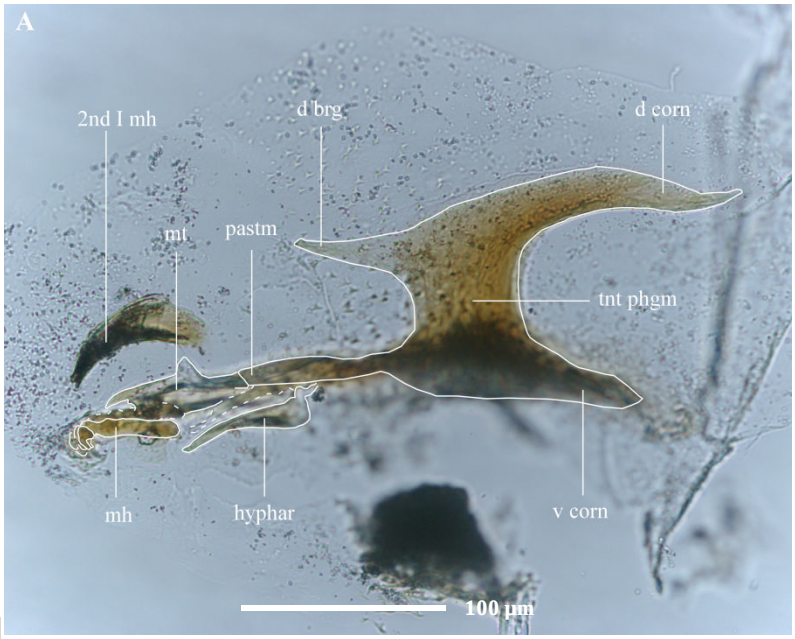
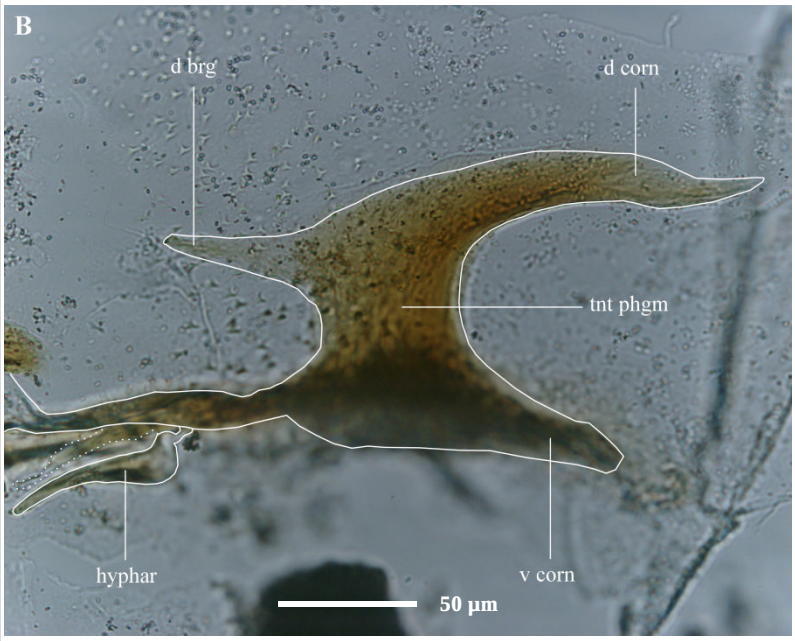


Fig. 24: Light micrographs of the lateral aspect of the cephalopharyngeal skeleton (CPS) of a first instar larva of *Lucilia sericata*.
24A: Whole CPS.
24B: Posterior elements of the CPS.
24C: Anterior elements of the CPS.
Legend: d brg: dorsal bridge; d corn: dorsal cornu; hyphar: hypopharyngeal sclerite; mh: mouth hook; mt: median tooth; pastm: parastomal sclerite; tnt phgm: tentorial phragma; v corn: ventral cornu.





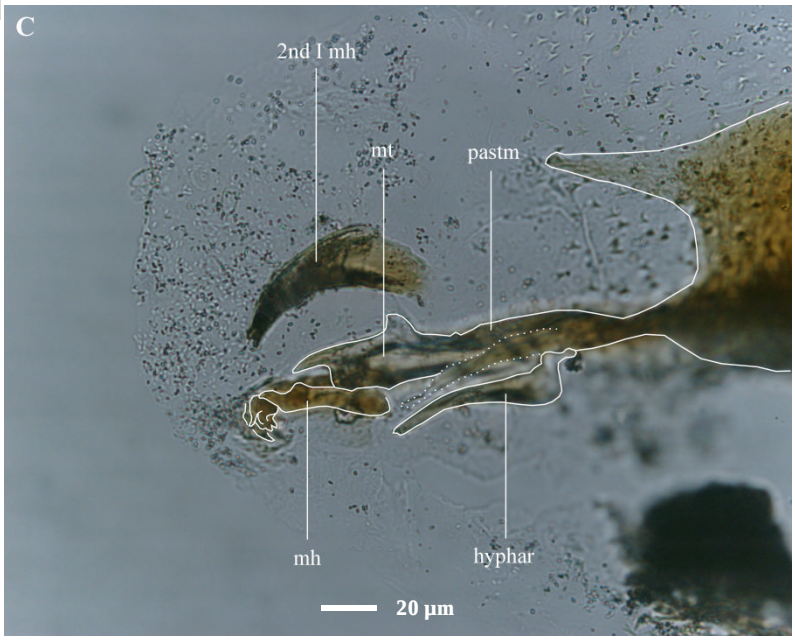


Fig. 25: Light micrographs of the lateral aspect of the cephalopharyngeal skeleton (CPS) of a first instar larva of *Chrysomya chloropyga*.
25A: Whole CPS.
25B: Posterior elements of the CPS.
25C: Anterior elements of the CPS.
Legend: interrupted lines: less sclerotised elements; 2nd I mh: emerging second instar mouth hook; d brg: dorsal bridge; d corn: dorsal cornu; hyphar: hypopharyngeal sclerite; mh: mouth hook; mt: median tooth; pastm: parastomal sclerite; tnt phgm: tentorial phragma; v corn: ventral cornu.

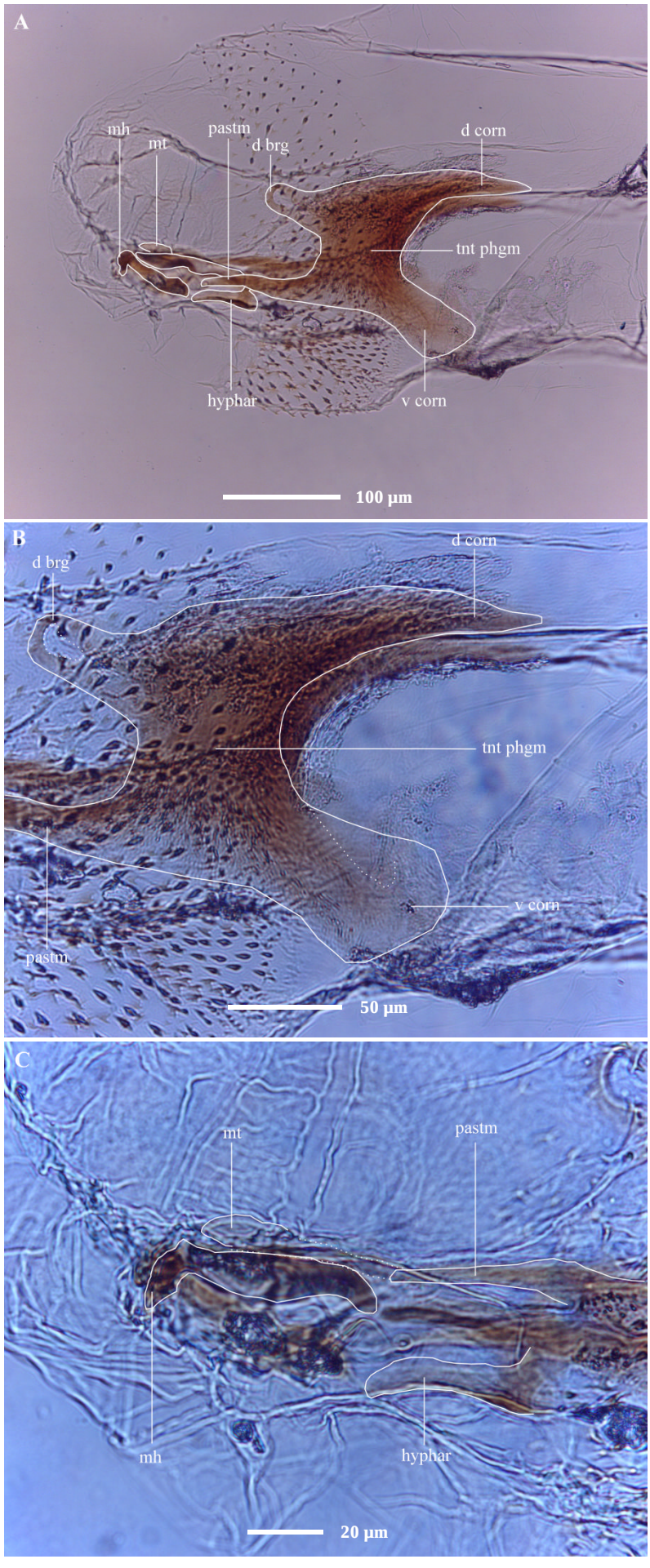
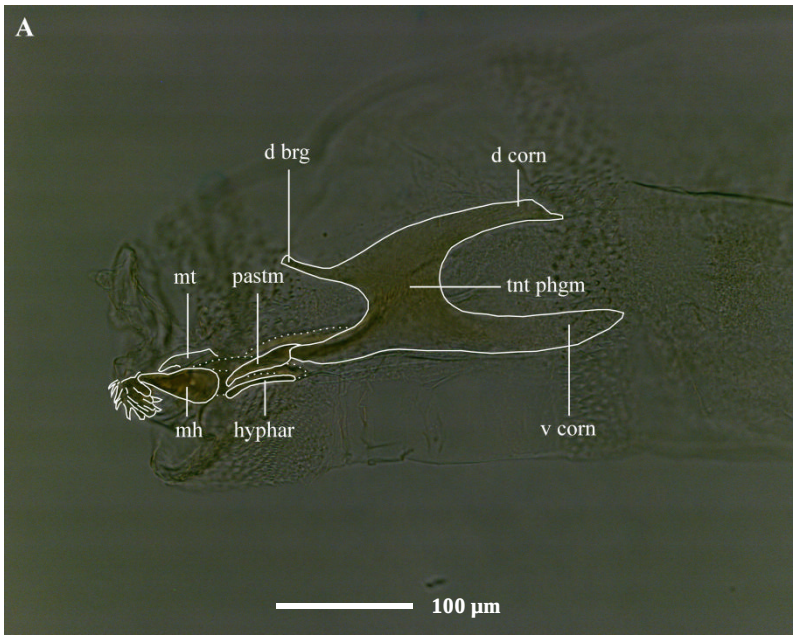
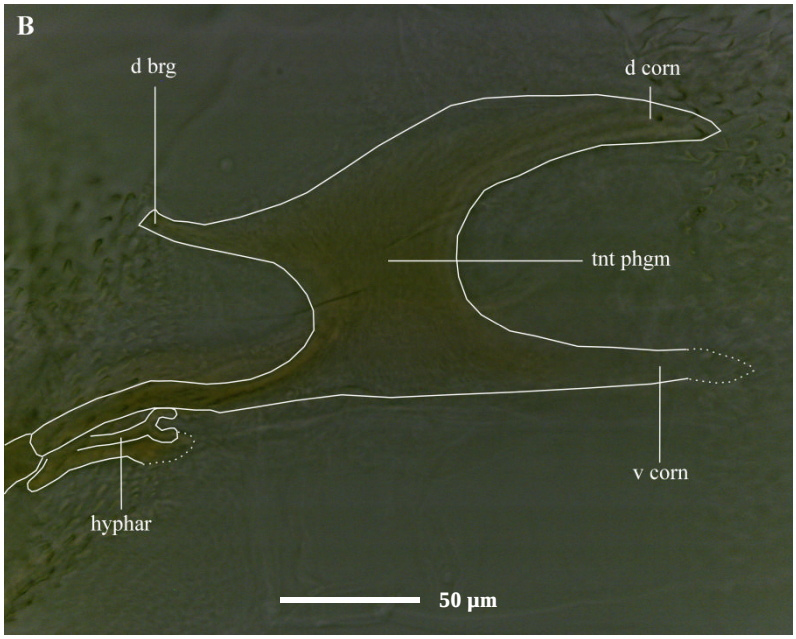


Fig. 26: Light micrographs of the lateral aspect of the cephalopharyngeal skeleton (CPS) of a first instar larva of *Chrysomya marginalis*.
26A: Whole CPS.
26B: Posterior elements of the CPS.
26C: Anterior elements of the CPS.
Legend: d brg: dorsal bridge; d corn: dorsal cornu; hyphar: hypopharyngeal sclerite; mh: mouth hook; mt: median tooth; pastm: parastomal sclerite; tnt phgm: tentorial phragma; v corn: ventral cornu.





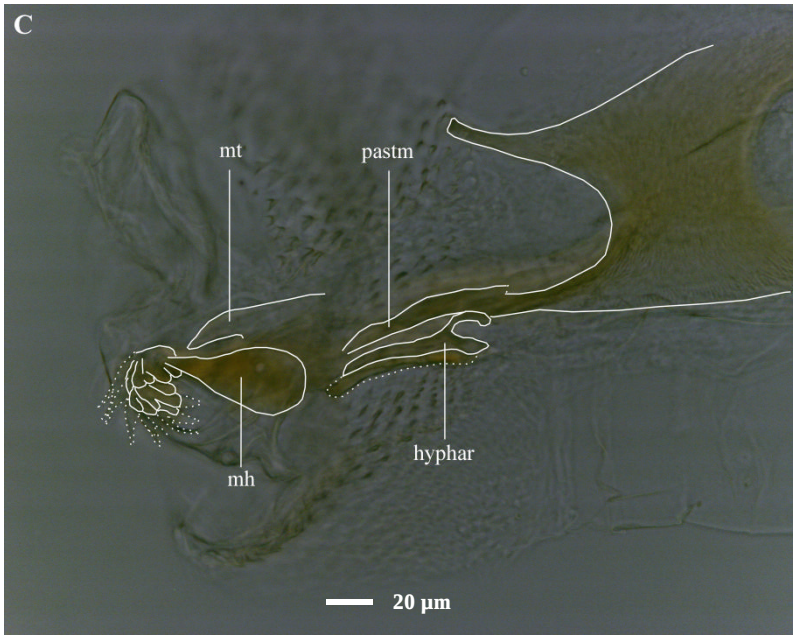


Fig. 27: Light micrographs of the lateral aspect of the cephalopharyngeal skeleton (CPS) of a first instar larva of *Chrysomya albiceps*.
27A: Whole CPS.
27B: Posterior elements of the CPS.
27C: Anterior elements of the CPS.
Legend: interrupted lines: less sclerotised elements; d brg: dorsal bridge; d corn: dorsal cornu; hyphar: hypopharyngeal sclerite; mh: mouth hook; mt: median tooth; pastm: parastomal sclerite; tnt phgm: tentorial phragma; v corn: ventral cornu.

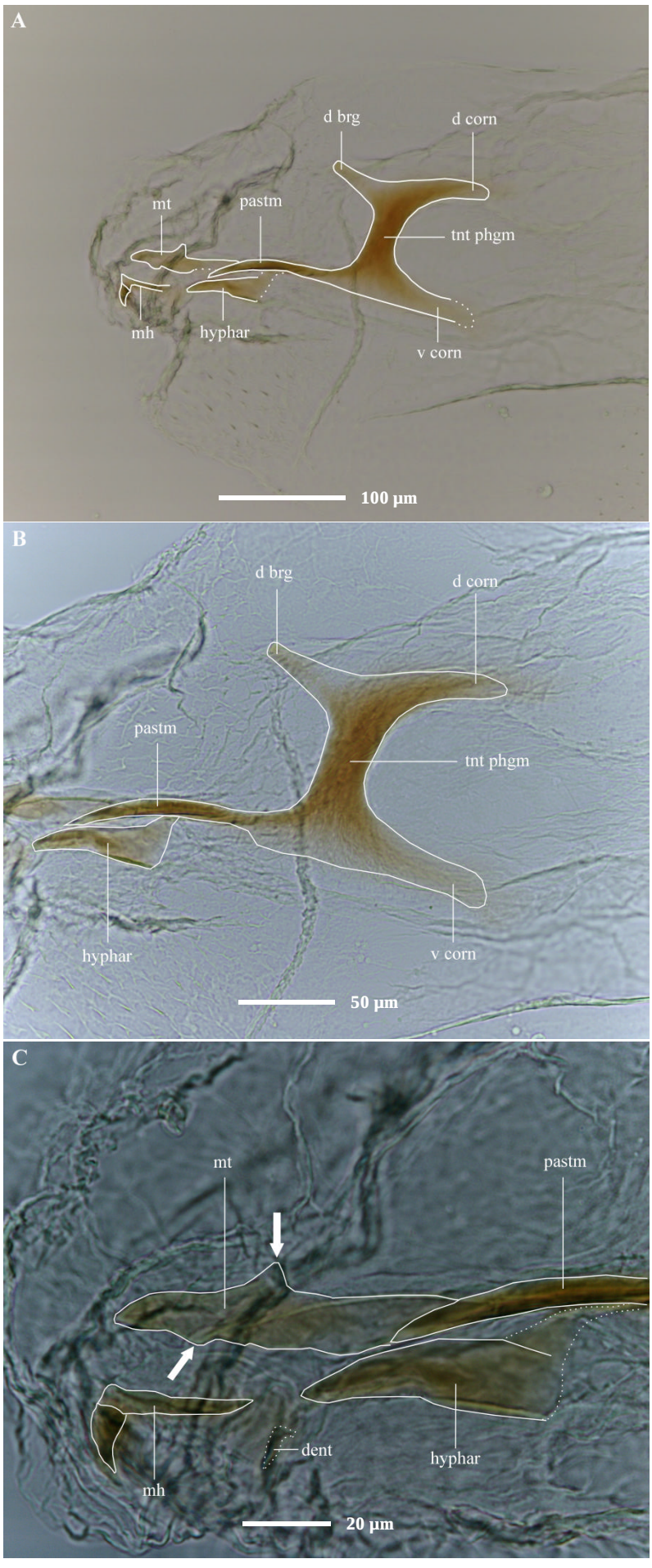
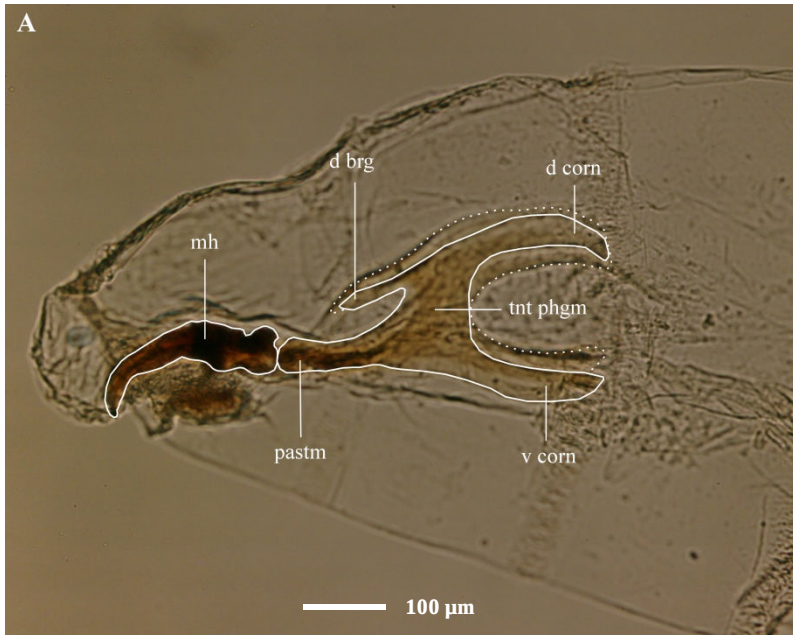
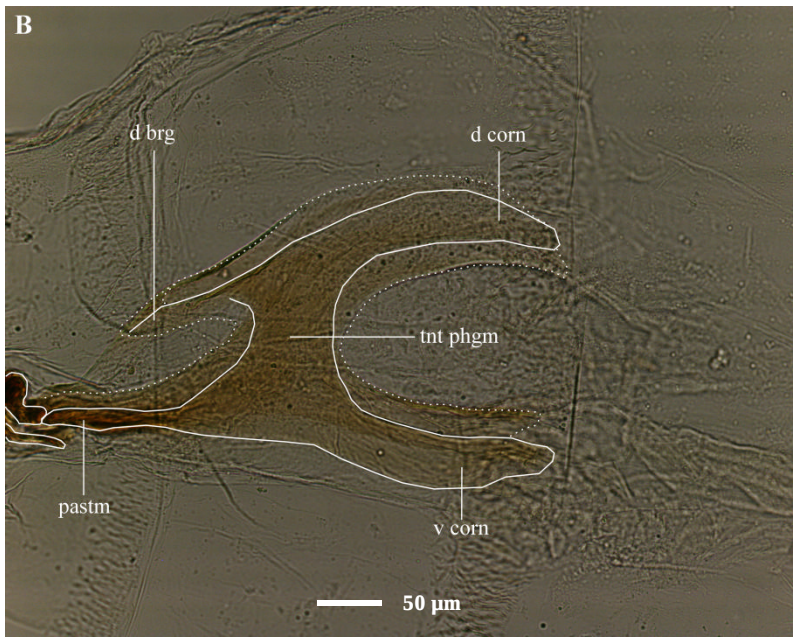


Fig. 28: Light micrographs of the lateral aspect of the cephalopharyngeal skeleton (CPS) of a first instar larva of *Calliphora vicina*.
28A: Whole CPS.
28B: Posterior elements of the CPS.
28C: Anterior elements of the CPS.
Legend: arrow: notches on median tooth; interrupted lines: less sclerotised elements; d brg: dorsal bridge; d corn: dorsal cornu; dent: dental sclerite; hyphar: hypopharyngeal sclerite; mh: mouth hook; mt: median tooth; pastm: parastomal sclerite; tnt phgm: tentorial phragma; v corn: ventral cornu.





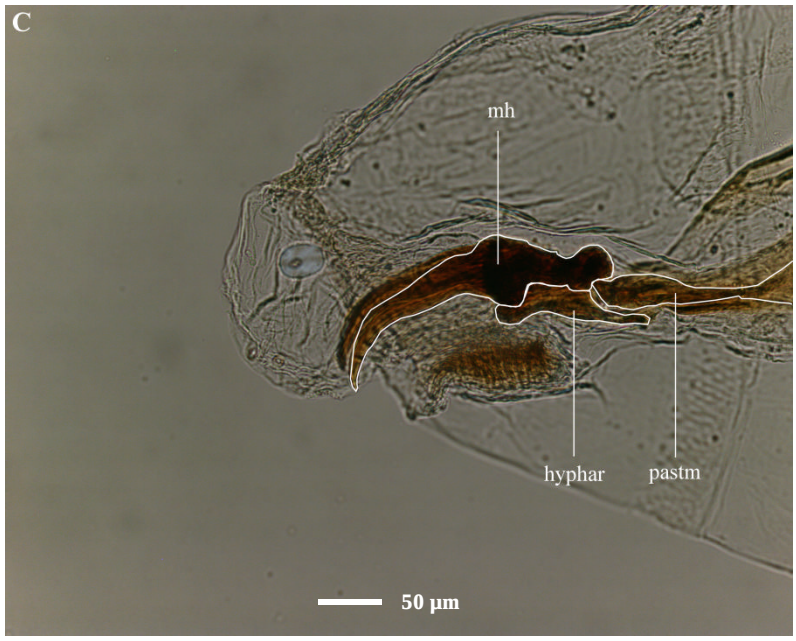


Fig. 29: Light micrographs of the lateral aspect of the cephalopharyngeal skeleton (CPS) of a first instar larva of *Sarcophaga cruentata*.
29A: Whole CPS.
29B: Posterior elements of the CPS.
29C: Anterior elements of the CPS.
Legend: d brg: dorsal bridge; d corn: dorsal cornu; hyphar: hypopharyngeal sclerite; mh: mouth hook; pastm: parastomal sclerite; tnt phgm: tentorial phragma; v corn: ventral cornu.

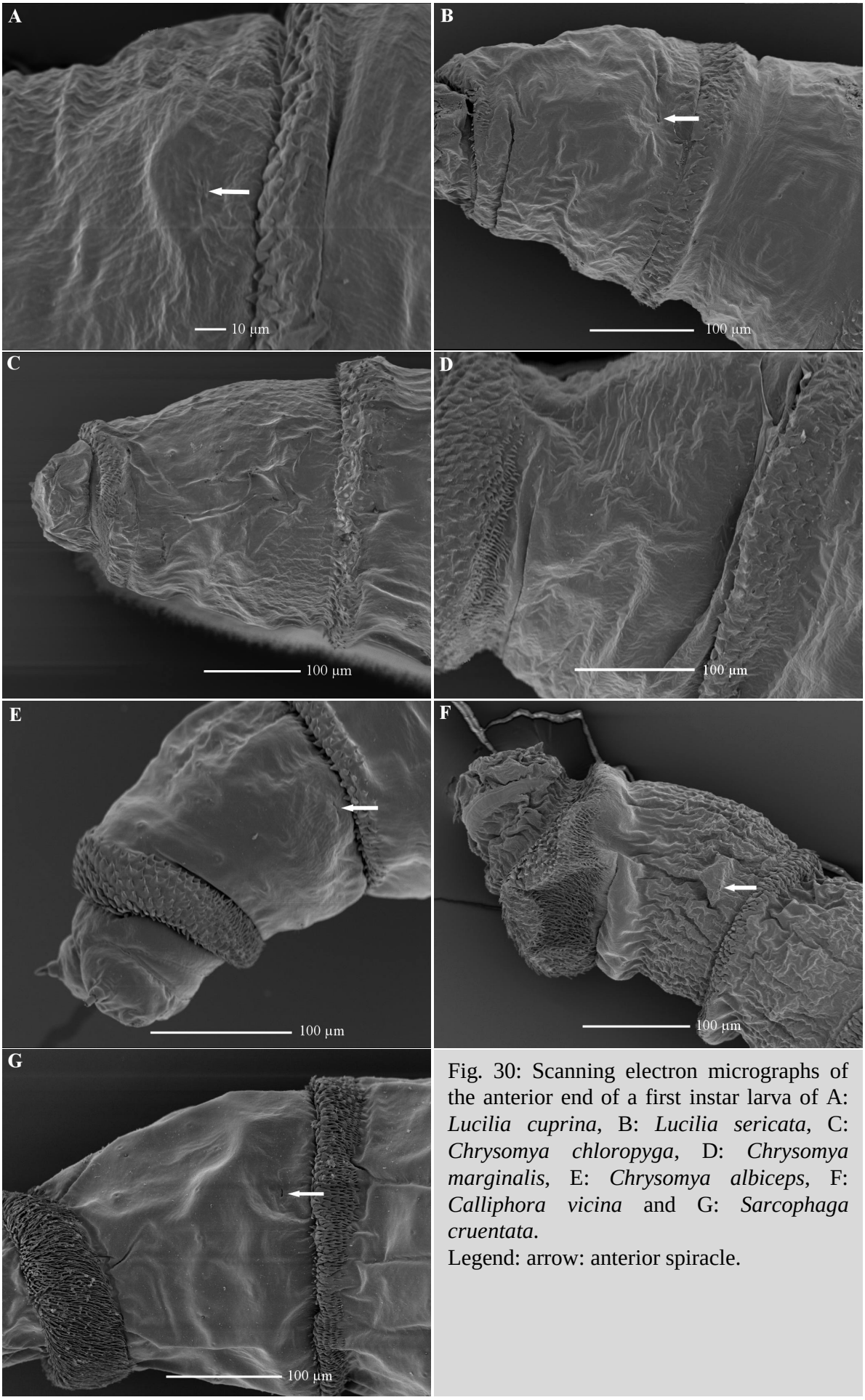


Fig. 30: Scanning electron micrographs of the anterior end of a first instar larva of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina* and G: *Sarcophaga cruentata*.
Legend: arrow: anterior spiracle.

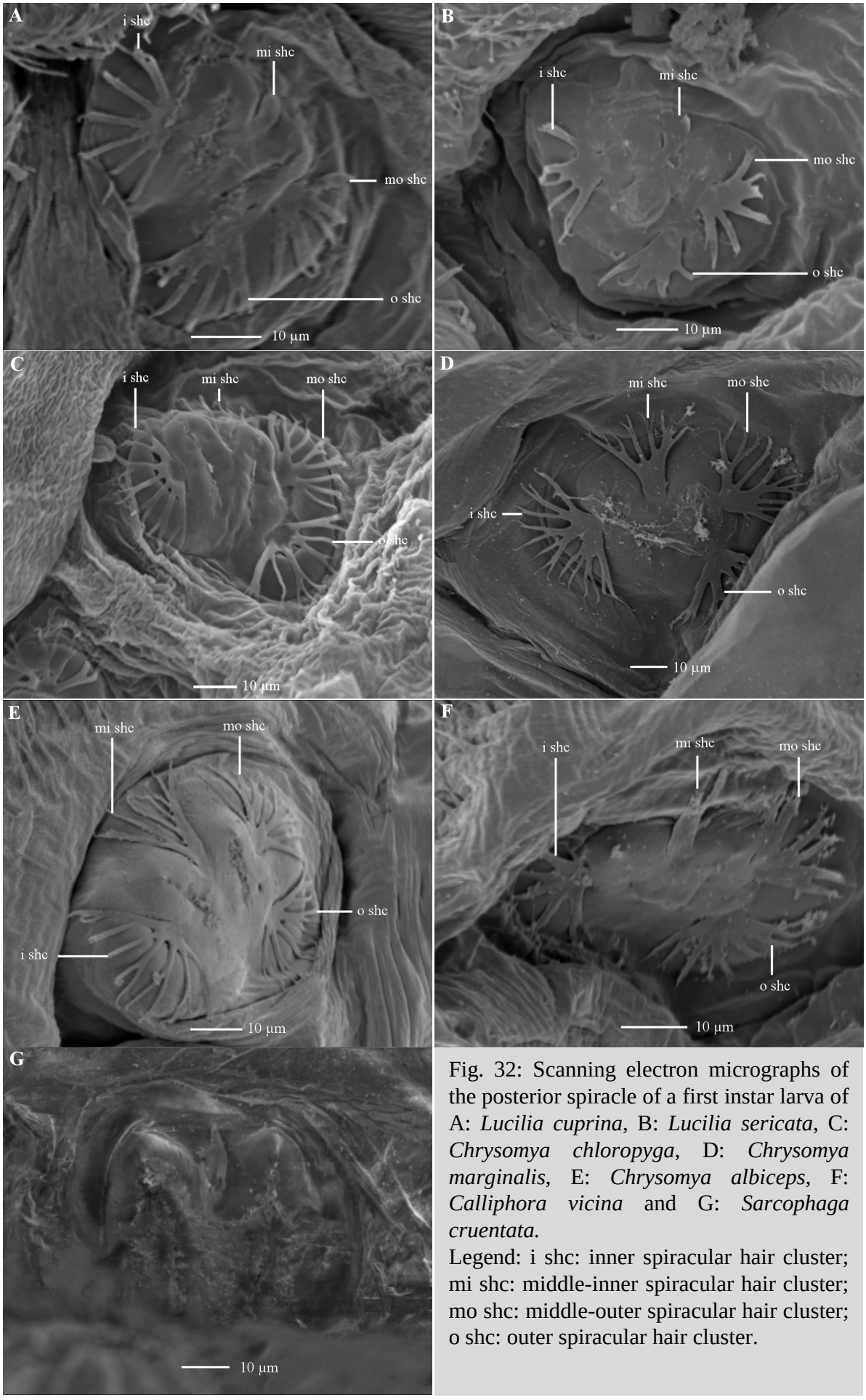


Fig. 32: Scanning electron micrographs of the posterior spiracle of a first instar larva of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina* and G: *Sarcophaga cruentata.*

Legend: i shc: inner spiracular hair cluster; mi shc: middle-inner spiracular hair cluster; mo shc: middle-outer spiracular hair cluster; o shc: outer spiracular hair cluster.

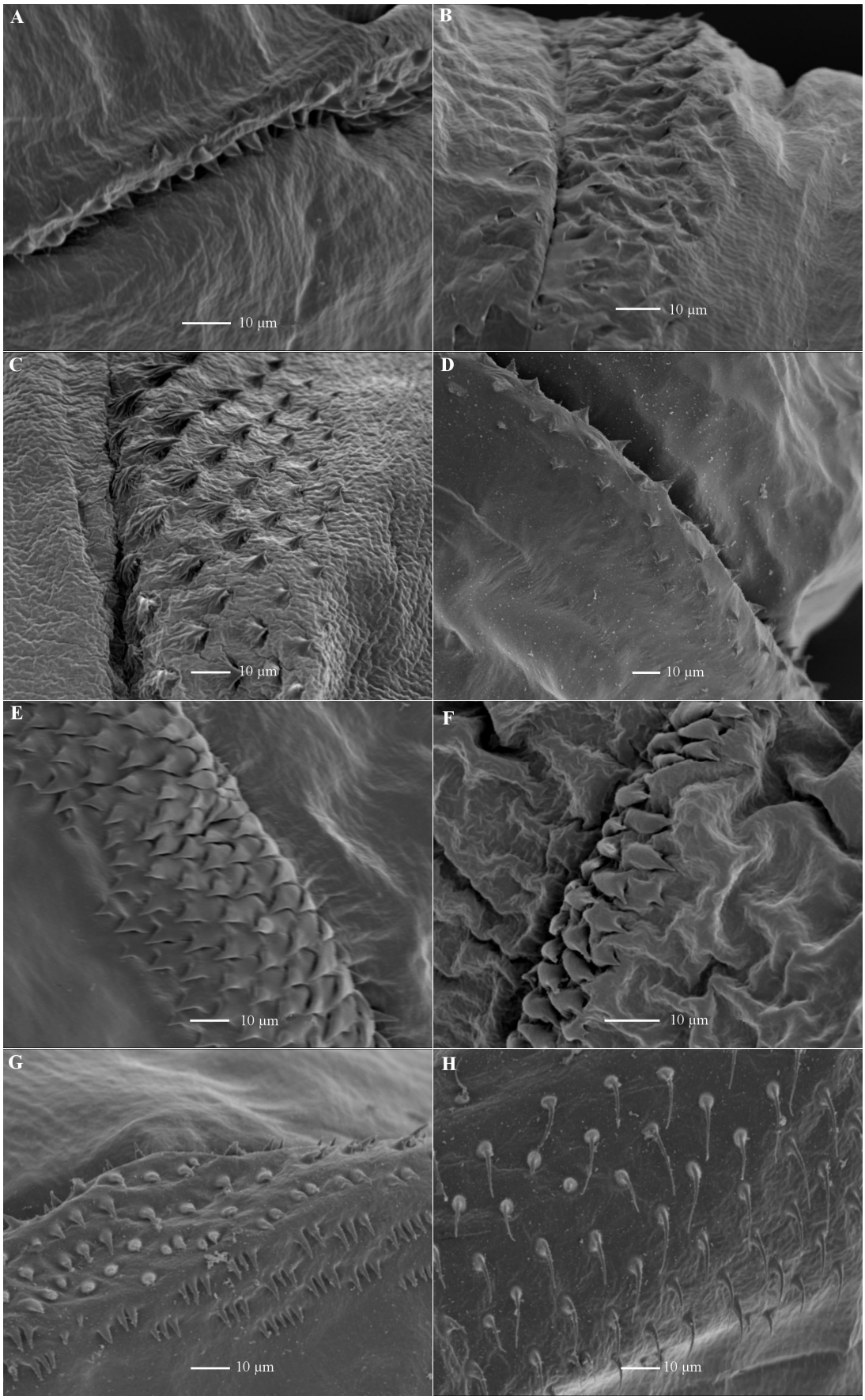


Fig. 33: Scanning electron micrographs of the spines of a first instar larva of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina*, G and H: *Sarcophaga cruentata.*

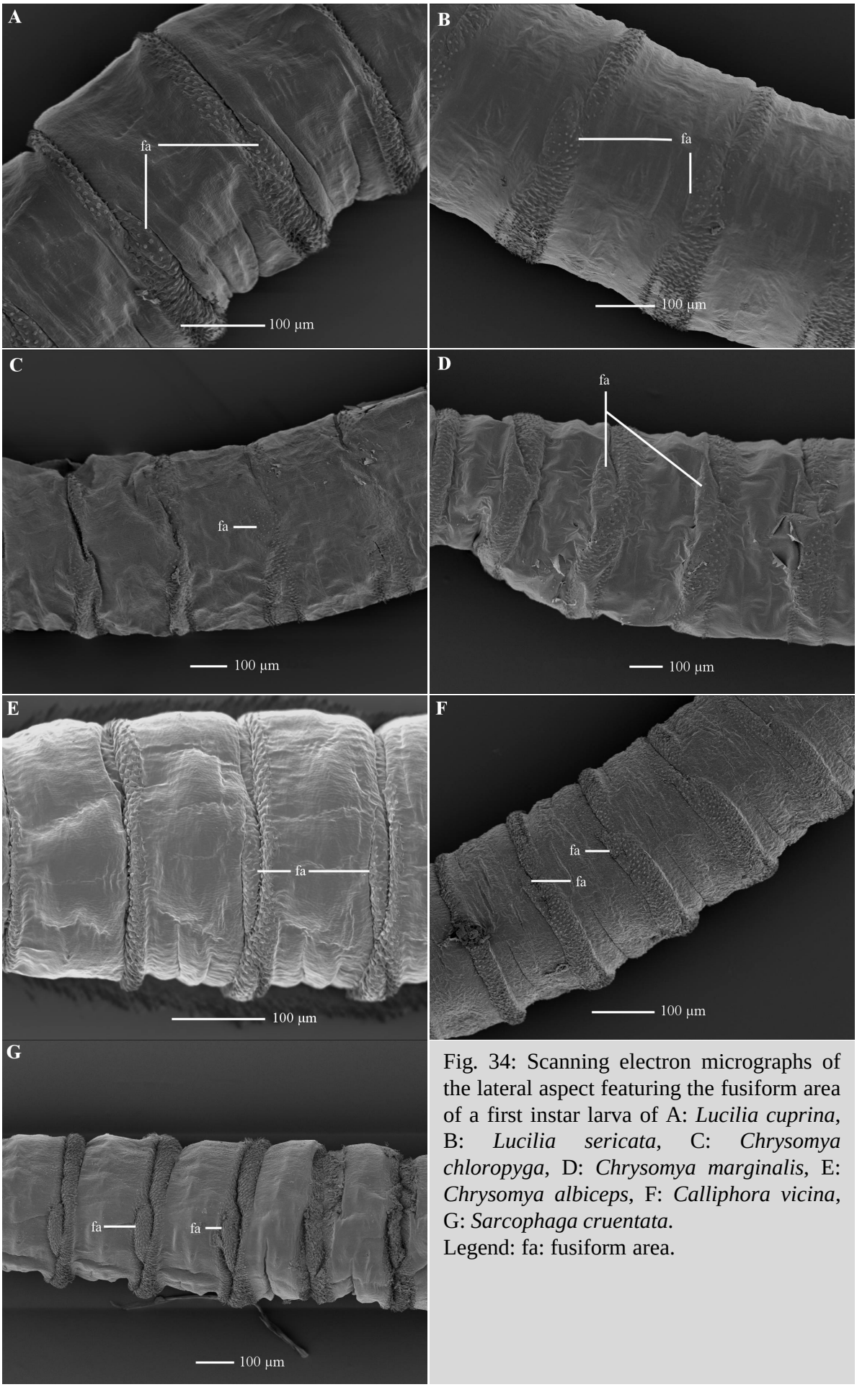


Fig. 34: Scanning electron micrographs of the lateral aspect featuring the fusiform area of a first instar larva of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina*, G: *Sarcophaga cruentata*.
Legend: fa: fusiform area.

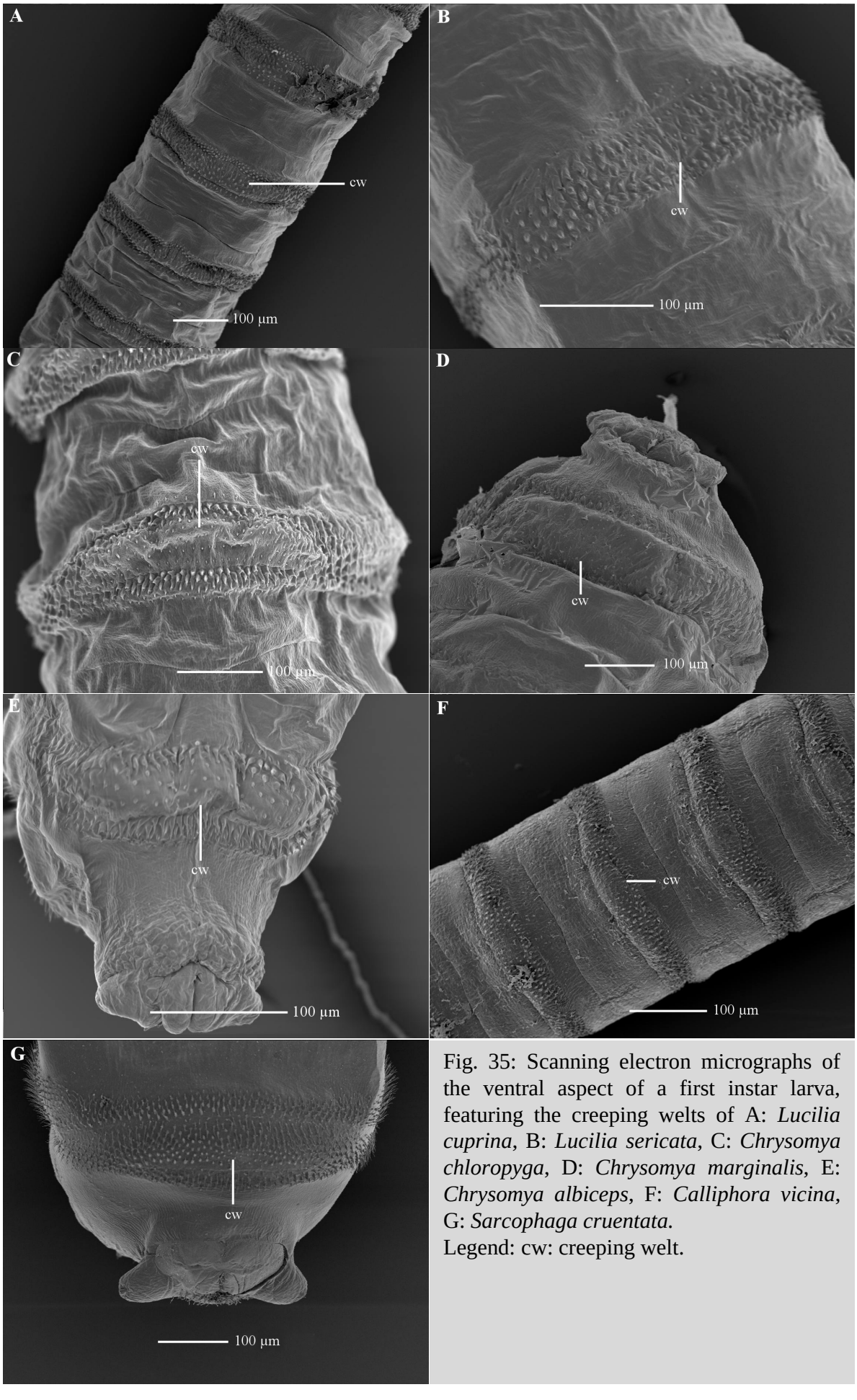


Fig. 35: Scanning electron micrographs of the ventral aspect of a first instar larva, featuring the creeping welts of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina*, G: *Sarcophaga cruentata.*
Legend: cw: creeping welt.

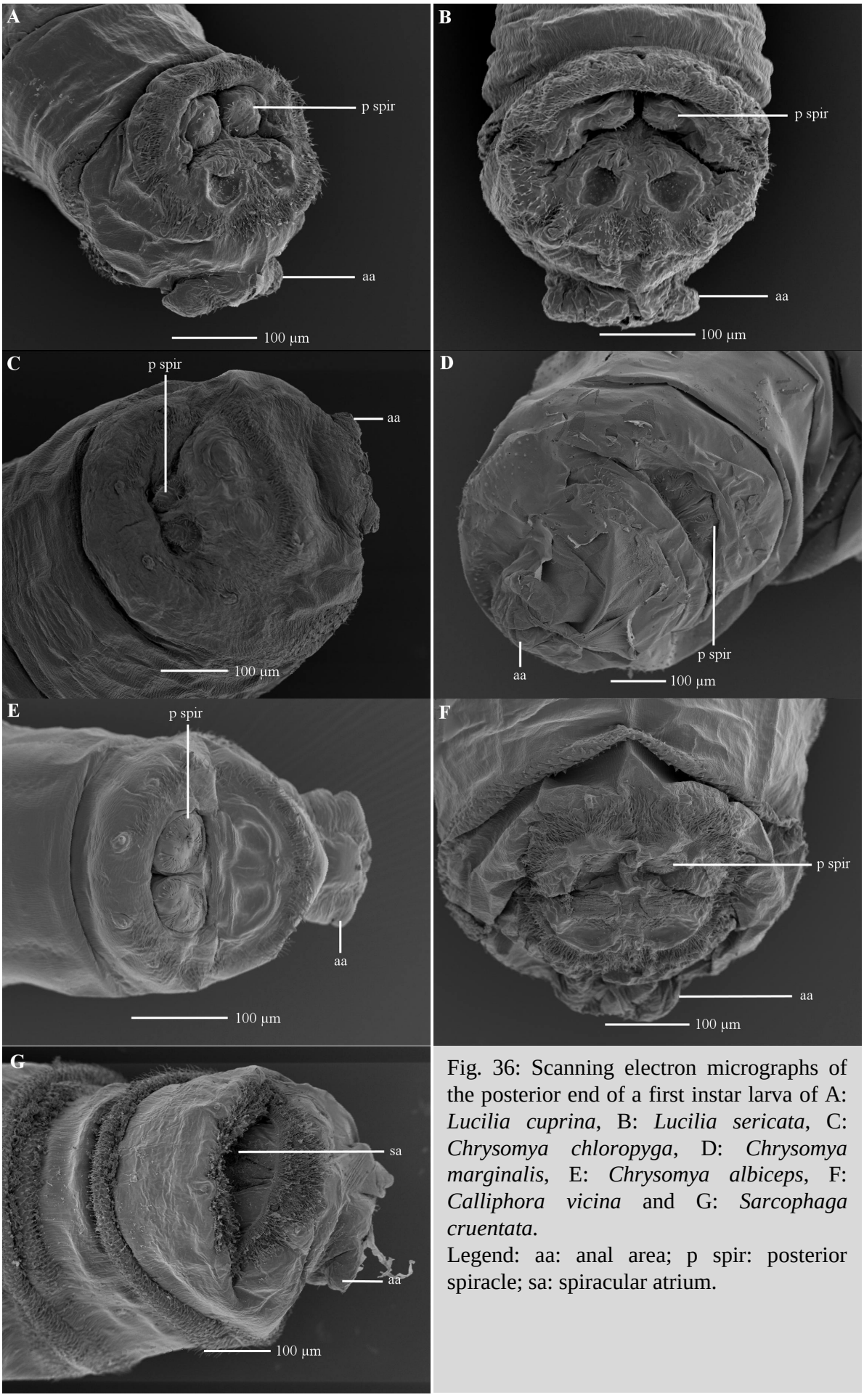


Fig. 36: Scanning electron micrographs of the posterior end of a first instar larva of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina* and G: *Sarcophaga cruentata*.
Legend: aa: anal area; p spir: posterior spiracle; sa: spiracular atrium.

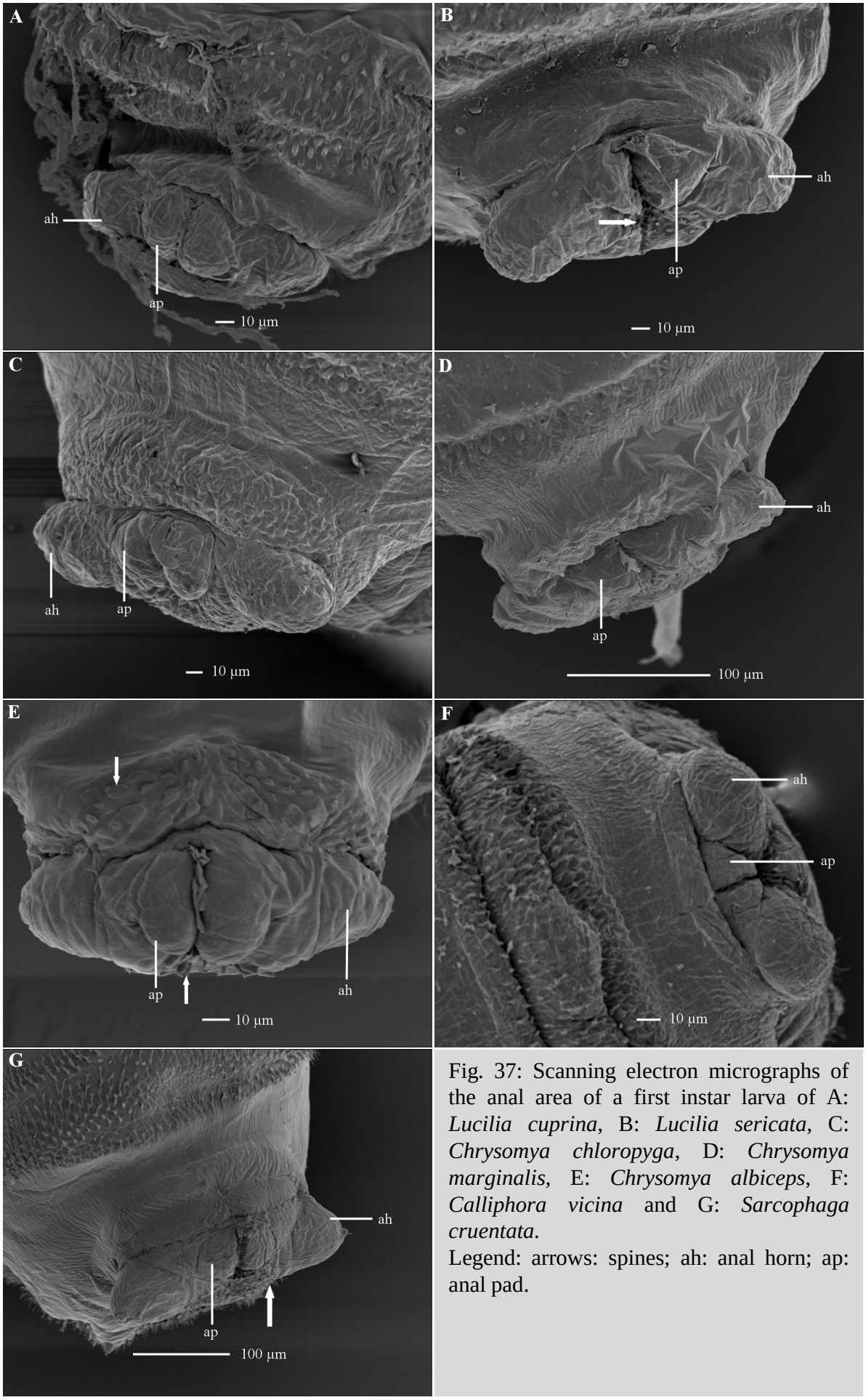


Fig. 37: Scanning electron micrographs of the anal area of a first instar larva of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina* and G: *Sarcophaga cruentata*.

Legend: arrows: spines; ah: anal horn; ap: anal pad.

3.4. MORPHOLOGY OF SECOND INSTAR LARVAE

3.4.1. INTEGUMENT (Figs. 38A to 38H and 39A to 39G)

Lucilia cuprina (Fig. 38A) **|** ***Lucilia sericata*** (Fig. 38B) **|** ***Calliphora vicina*** (Fig. 38G) **|** ***Sarcophaga cruentata*** (Fig. 38H)

The integuments of these second instar larvae were smooth (Figs. 38A, 38B, 38G and 38H). First instar larvae of these species also had smooth integuments (Figs. 17A, 17B, 17F and 17G).

Chrysomya chloropyga (Figs. 38C and 38D)

The integument of second instar *C. chloropyga* larvae were generally smooth (Fig. 38C), except for the hair-like {*hr*} growth seen on the dorsum of some segments. In most of the specimens examined, hair was noticed on the dorsum of only the most posterior segments, i.e. segments 8 to 12 (Fig. 38D). This feature was not constant and in a few specimens examined the dorsum was free of any hair, while in others hair was noticed on the dorsum of segments 5 to 12. No hair was noticed on the dorsum of first instar *C. chloropyga* larvae (Fig. 17C).

Chrysomya marginalis (Figs. 38E, 38F and 55D)

Second instar *C. marginalis* larvae were recognisable due to the rounded protrusions of the integument (Figs. 38E, 38F and 55D). These bulges {*blg*} were more defined on the lateral aspect of segments than on its dorsal or ventral aspects. Pit sensillae were conspicuous features on these bulges (Fig. 38F). This differed from first instar *C. marginalis* larvae, where no bulges of the integument were noticed (Fig. 17 D).

Chrysomya albiceps (Figs. 39A to 39G)

The integument of second instar *C. albiceps* larvae was strikingly different from the smooth integument of first instar *C. albiceps* larvae (Fig. 17E). It was also distinctly different from the general smooth integuments noticed in second instar larvae of the other species examined (Figs. 38A to 38H). Second instar *C. albiceps* larvae (Figs. 39A and 39B) were recognisable on a macroscopic level due to its “spiny” / “hairy” appearance. These “spines” / “hairs” were processes of the ventral, lateral and dorsal aspects of the segments. No processes were noted on

the ventral aspects of segments 1 to 4. The six ventral processes were first noticed on segment 5 (Fig. 39C) and were present up to segment 11. The four inner ventral processes of segment 5 contained no spines on their tips, whereas the two outer ventral processes were furnished with minute spines with rounded tips (Fig. 39D). On segment 6 the ventrally located processes were more developed in terms of their spines; spines adorned the tips of all ventrally located processes; the spines on the two inner processes being minute and broad tipped, whereas the spines on the four outer ventral processes being sharp-tipped (Fig. 39E). From segments 7 to 11 all spines on the tips of the ventral processes were sharp-tipped. No mention was made about the ventral processes by Zumpt (1965) for second instar *C. albiceps* larvae or by Liu & Greenberg (1989) for the congeneric *C. rufifacies* larvae. The lateral processes were first noticed on segment 4. Only the tip of the middle lateral process contained minute, blunt-tipped spines, whereas the other two processes were without spines on segment 4. From segments 5 to 11 all three lateral processes were adorned with sharp-tipped spines (Fig. 39F). Also present was a small process associated with the most ventrally located of the lateral processes (Fig. 39F). The dorsal processes were weakly developed on segment 4, i.e. just sprouts with no spines on its tips. Two fully developed dorsal processes were noted from segments 5 to 11 (Fig. 39G). The spinules crowning the tips of fully developed processes were short and robust (Fig. 39G). Sukontason *et al*. (2003) noticed that the tips of the processes contained approximately six robust spines in *C. rufifacies* larvae. This was different to the situation found in second instar *C. albiceps* larvae where the tips of fully developed processes contained a greater number of spines (Fig. 39G). Additional to the processes of the integument, the integument of second instar *C. albiceps* was covered in dome-shaped papillae (Figs. 39C to 39G); similar to the situation found in second instar *C. rufifacies* larvae as presented by Sukontason *et al.* (2003).

The integument as diagnostic feature

Not all species showed development regarding their integuments from their first instar stage. Only second instar larvae of *Chrysomya* species were distinctly different from their first instar larvae.

The integument was useful as a diagnostic feature distinguishing *Chrysomya* species from the rest of the species with their smooth integuments (Figs. 38A, 38B, 38G and 38H). Each one of

the *Chrysomya* species was unique regarding the distinguishing features of their integuments: (i) hair was noted on the dorsum on some segments (Fig. 38D) of most *C. chloropyga* specimens examined; (ii) the integument of *C. marginalis* larvae was prolapsed into rounded bulges (Figs. 38E and 38F) and (iii) dome-shaped papillae and distinct processes were noted on the integument of *C. albiceps* larvae (Figs. 39A to 39G).

3.4.2. ANTENNOMAXILLARY COMPLEX (Figs. 40A to 40G and 41A to 41G)

The antennae {*asc*} were located in an anterior position on the pseudocephalon (Figs. 40A to 40G). The morphology of the antennae of second instar larvae was similar to that of first instar larvae. The antennae were morphologically similar in the different species examined (Figs. 41A to 41G). The antennae were in essence a large encapsulated button sensillium, i.e. a dome-shaped base portion with a pointed distal portion.

The maxillary complex {*mxsc*} of second instar larvae (Figs. 41A to 41G) was different from that of the first instar larvae, but similar among the different calliphorid second instar larvae examined. Similar to the situation found in first instar larvae it was set on a raised area and consisted of a few encapsulated and a few un-encapsulated button sensillae located centrally. It was also similar to first instar larvae with regard to the two encapsulated button sensillae situated in an anterior position from the centralised grouping of sensillae {*cent sens*}. The first aspect where it differed from the first instar stage was with respect to the un-interrupted ring of the cuticle that encircled the central grouping of button sensillae. The maxillary complex of calliphorid larvae was distinctly different from the situation found in first instar larvae in that two rows of cuticular folds were present. These cuticular folds had a pleated appearance and it surrounded the centralised grouping of button sensillae. The two outlying button sensillae {*out sens*} were also enfolded by these cuticular folds. Whereas the outlying folds complete one to two rings in the calliphorid species, three to four rings were noted in second instar *S. cruentata* larvae. Similar to the complexity development seen from first – to second instar larvae regarding the maxillary complex in calliphorid species; second instar *S. cruentata* larvae showed progression in that the cuticular folds were pleated opposed to the uninterrupted nature of the folds in first instar *S. cruentata* larvae.

3.4.3. ORAL RIDGES (Figs. 40A to 40G and 42A to 42G)

The number of oral ridges {*or*} increased from the two observed in first instar larvae. The oral ridges (Figs. 40A to 40G and 42A to 42G) revealed no further variations to allow for it to be utilised as a diagnostic characteristic in second instar larvae.

3.4.4. LABIUM (Figs. 40A to 40G and 42A, 42B, 42D, 42F and 42G)

The morphology of the labium {*lbm*} of the first (Figs. 18A to 18G) and second instar larvae (Figs. 40A to 40G and 42A, 42B, 42D, 42F and 42G) was similar. The morphology of the labium was also similar in the various species examined. The labium was made up of two lateral lobes and a central lobe.

3.4.5. LABRUM {*lbr*} (Figs. 40A to 40G and 42A to 42G)

Lucilia cuprina (Figs. 40A and 42A) | ***Lucilia sericata*** (Figs. 40B and 42B) | ***Chrysomya chloropyga*** (Figs. 40C and 42C) | ***Chrysomya marginalis*** (Figs. 40D and 42D) | ***Calliphora vicina*** (Figs. 40F and 42F)

The anterior margin of the pre-oral cavity of these species (Figs. 40A to 40C, 40D and 40F and 42A to 42D and 42F) was simplified, lacking the tube-like extensions with cirri as noted in first instar larvae (Figs. 20A to 20D and 20F).

Chrysomya albiceps (Figs. 40E and 42E)

The labrum (Figs. 40E and 42E) was split along its central margin almost to its base to form two flaps. The two flaps were further split, but to a lesser extent than the centralised split. The distal ends of the flaps were broad. This differed from the situation found in first instar *C. albiceps* larvae where the labrum appeared as two tube-like extensions with cirri on its distal ends (Fig. 20E).

Sarcophaga cruentata (Figs. 40G and 42G)

The anterior margin of the pre-oral cavity had a distinct curvature to it (Figs. 40G and 42G). The curvature was more pronounced to what was seen in first instar *S. cruentata* larvae (Fig. 20G).

The labrum as diagnostic characteristic

The labrum was a distinct differentiating feature to distinguish first instar larvae from second instar larvae. This difference was more pronounced for the calliphorid species.

The labrum was of limited diagnostic use, with only two of the species examined (*C. albiceps* and *S. cruentata*) distinctly different from the other second instar species examined.

3.4.6. CEPHALOPHARYNGEAL SKELETON (Figs. 43 to 50)

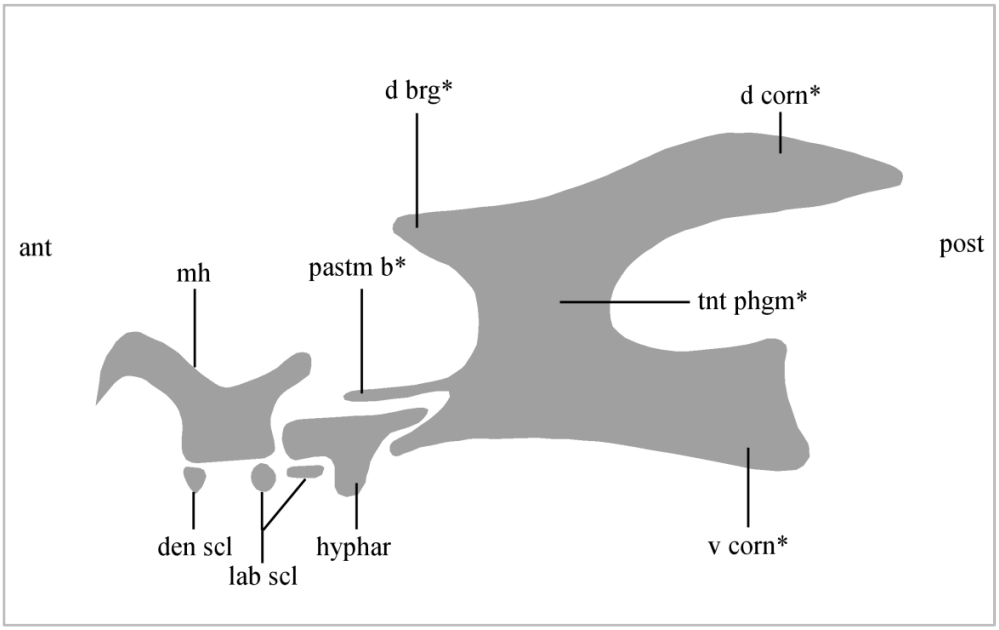


Fig. 43: Representation of the lateral aspect of a second instar cephalopharyngeal skeleton. Legend: ant: anterior end; d. brg*: dorsal bridge; d corn*: dorsal cornu; den scl: dental sclerite; hyphar: hypopharyngeal sclerite; lab scl: labial sclerite; mh: mouth hook; pastm b*: parastomal bar; post: posterior end; tnt phgm*: tentorial phragma; v corn*: ventral cornu. * = Elements of the tentoropharyngeal sclerite.

The terminology and diagrammatic representation used to describe the cephalopharyngeal skeleton was based on that presented by Teskey (1981). The description was of the lateral view of a cephalopharyngeal skeleton. The cephalopharyngeal skeleton (Fig. 43) is located within the anterior two segments of the larva. Three major sclerites constitute the cephalopharyngeal skeleton, namely the posterior located tentoropharyngeal sclerite, the anterior located mouth hooks {*mh*} and the hypopharyngeal sclerite {*hyphar*} located between these two elements. The tentoropharyngeal sclerite is the largest of these sclerites. A deep emargination of the posterior portion of the tentoropharyngeal sclerite forms the dorsal {*d corn*} and the ventral cornu {*v corn*}. The dorsal cornu stretches further posteriorly than the ventral cornu. The anterior emargination of the tentoropharyngeal sclerite is not as severe as the posterior emargination of the sclerite (Fig. 43). The anterio-dorsal portion of the tentoropharyngeal sclerite forms the dorsal bridge {*d brg*}. Between the anterior and posterior portions of the tentoropharyngeal sclerite is the tentorial phragma {*tnt phgm*}. The centrally located major sclerite, the hypopharyngeal sclerite, is a T-shaped structure. According to Erzinçlioglu (1985) the ventral protrusion of the hypopharyngeal sclerite is due to the medial section of this structure being at a lower plane than the rest of the sclerite. The parastomal bar {*pastm b*} was a thin rod-like structure. The parastomal bar originates from the tentoropharyngeal sclerite and projects above the hypopharyngeal sclerite. The most anterior located of the major sclerites are the mouth hooks. It is made up of a base section and curved hooks. Additional to the three major sclerites, a few smaller sclerites can be incorporated as part of the cephalopharyngeal skeleton: (i) a labial sclerite {*lab scl*} located obliquely below the hypopharyngeal sclerite, (ii) a second labial sclerite located below the posterior end of the basal section of the mouth hooks and (iii) a dental sclerite {*den scl*} located at the anterior end of the basal section of the mouth hook.

The cephalopharyngeal sclerite of second instar calliphorid larvae was distinctly different from that of the first instar calliphorid larvae. In first instar calliphorid larvae, the parastomal element manifested as a well defined, extended structure (Fig. 21) while it was much reduced and narrower in second instar larvae (Fig. 43). The hypopharyngeal sclerite did not have the distinct T- shape of second instar larvae in first instar larvae. No median tooth was noted in second instar larvae. The hook-like nature of the mouth hooks was a distinct feature of second instar larvae. Due to these distinct differences the first instar stage will not be compared as part of the

description and discussion of the second instar calliphorid larvae. The second instar sarcophagid larvae were not as distinctly different from its first instar stage; therefore, the differences between the two instars will be discussed as part of that species description.

Lucilia cuprina (Figs. 44A to 44B)

The dorsal cornu was solid (Fig. 44A). A horizontally orientated parastomal bar was extended above the hypopharyngeal sclerite (Fig. 44A). The top of the "T" of the hypopharyngeal sclerite was an extended narrow structure (Fig. 44A). The most anterior margin of this structure was relatively narrow in relation to the posterior margin of the base of the mouth hook (Fig. 44B). A robust, clearly defined labial sclerite extended from the ventral margin of the hypopharyngeal sclerite to the ventral margin of the base of the mouth hook (Figs. 44A and 44B). A faintly visible second labial sclerite was noted ventrally from the base of the mouth hook (Fig. 44B). An ill-defined dental sclerite was located below the anterio-ventral margin of the mouth-hooks (Fig. 44B). The base of the mouth hook had a posterior-dorsal projection to it (Fig. 44B). The base and hook portions of the mouth-hook were similar in size (Fig. 44B). The hook curved gradually away from the base portion for about two-thirds of its length, before curving into a sharp-tipped distal end (Fig. 44B). Erzincliogl (1989b) described the curvature of the tooth as narrower than the basal area; similar to the findings of the current study. The sketch presented by Greenberg & Szyska (1984) was very similar to that of the specimens examined during the current study. However, the distinctness of the posterior labial sclerite was not indicated as such.

Lucilia sericata (Figs. 45A to 45B)

The tentoropharyngeal - and the hypopharyngeal sclerite of *L. sericata* larvae (Figs. 45A and 45B) were similar to that seen in second instar *L. cuprina* larvae. A narrow labial sclerite was noted here, extending from the ventral margin of the hypopharyngeal sclerite to the base portion of the mouth hooks. This sclerite was less robust than in *L. cuprina*. A weakly defined dental sclerite was present (Fig. 45B). The posterio-dorsal projection of the base portion of the mouth hook was obvious (Fig. 45B). The base and hook portions of the mouth-hook were similar in size to each other. The hook portion curved gradually to the distal end of the structure (Figs. 45A and 45B) and similar to that reported by Erzincliogl (1989b); the area of curvature of the tooth was thickened.

Chrysomya chloropyga (Figs. 46A to 46B)

The dorsal cornu was solid (Fig. 46A). A horizontally orientated parastomal bar was extended above the hypopharyngeal sclerite (Fig. 46A); similar to that seen for the congeneric *C. putoria* by Greenberg & Szyska (1984). The top of the "T" of the hypopharyngeal sclerite was robust in relation to its other part (Figs. 46A and 46B). The anterior margin of this structure was only slightly narrower than the posterior margin of the base of the mouth hook (Fig. 46B). All three of the smaller sclerites were noted (Fig. 46B), i.e. the two labial sclerites and the dental sclerite. The most posterior of the labial sclerites was more distinct than the other two sclerites. This sclerite appeared to be thinner in the drawing presented of *C. putoria* by Greenberg & Szyska (1984). The posterio-dorsal projection of the base of the mouth-hooks was well defined (Figs. 46A and 46B). The mouth hook curved into its distal tip at approximately its half-way mark (Fig. 46B). This aspect was similar in *C. putoria*, except that the most distal portion seemed to be more curved in the drawing presented of *C. putoria* by Greenberg & Szyska (1984).

Chrysomya marginalis (Figs. 47A to 47B)

The dorsal cornu was solid (Fig. 47A). A horizontally orientated parastomal bar was extended above the hypopharyngeal sclerite (Fig. 47A). A thin, barely visible, elongated labial sclerite was noted stretching from the anterio-ventral edge of the hypopharyngeal sclerite to the posterio-ventral margin of the base portion of the mouth hook (Fig. 47B). The presence of the other smaller sclerites could not be confirmed in the specimens examined. The posterio-dorsal projection of the base portion of the mouth-hooks was relatively small, but distinct (Fig. 47B). The hook portion took off at an acute angle from the basal component of this sclerite, before curving at a point about a third into its extent into the distal tip; i.e. the mouth hook was distinctly sickle-shaped (Figs. 47A and 47B). Due to this configuration the base portion of the mouth-hook appeared to be an elongated structure. Generally, the mouth hooks were similarly indicated in the specimens of *C. marginalis* examined by Prins (1982).

Chrysomya albiceps (Figs. 48A to 48C)

A solid dorsal cornu was noted for this species (Fig. 48A). The parastomal bar was turned upwards (Fig. 48A); probably due to the robust hypopharyngeal sclerite. These aspects were not

similarly indicated by Liu & Greenberg (1989) for the congeneric *C. rufifacies*. All three of the smaller sclerites were noted (Fig. 48A), i.e. the two labial sclerites and a dental sclerite. The most posterior of the two labial sclerites was a thin, elongated structure. The curvature of the hook portion was gradual (Fig. 48A). At the base of the hook portion three protuberances were noted (Fig. 48A). These protuberances were also noted in scanning electron micrographs where the mouth hooks were pushed from the pre-oral cavity (Fig. 48C).

Calliphora vicina (Figs. 49A to 49B)

The dorsal cornu was solid (Fig. 49A). A horizontally orientated parastomal bar was extended above the hypopharyngeal sclerite (Fig. 49A). The top of the "T" of the hypopharyngeal sclerite was an extended narrow structure (Fig. 49A). A thin, elongated labial sclerite stretched from the anterio-ventral edge of the hypopharyngeal sclerite to the posterio-ventral margin of the base of the mouth hooks (Fig. 49B). A second labial sclerite was not noted in the specimens examined, but a lightly sclerotised dental sclerite was noted (Fig. 49B). Erzinçlioglu (1985) showed all three of the smaller sclerites in his drawing of *C. vicina*. The tooth portion of the mouth hook was acutely angled in relation to the base section (Fig. 49B). The tooth was a stout structure with a sharp tip. Aspects of the mouth hooks were comparable to that presented for *C. vicina* by Zumpt (1965), Erzinçlioglu (1985) and Liu & Greenberg (1989). The mouth hooks of the congeneric *C. croceipalpis* were different from that of *C. vicina* in that the projection to the posterio-dorsal margin of the base was pronounced in *C. croceipalpis* (Prins 1982). Erzinçlioglu (1985) also indicated this aspect of the base of the mouth hook in his drawing of *C. vicina*. Generally the mouth hooks of *C. croceipalpis* as indicated by Prins (1982) were similar to that of *C. vicina*.

Sarcophaga cruentata (Figs. 50A to 50B)

The dorsal cornu was split in this instar of *S. cruentata* (Fig. 50A). This was the most significant area of difference between first – and second instar *S. cruentata* larvae; the dorsal cornu was not split in first instar *S. cruentata* larvae. A horizontally orientated parastomal bar projected over the hypopharyngeal sclerite (Fig. 50A). All three of the smaller sclerites were noted (Fig. 50B), i.e. the two labial sclerites and the dental sclerite. The mouth-hook was an extended, slender structure (Figs. 50A and 50B). The curvature of the hook portion was gradual to the extent of it

being almost straight. The drawing presented by Zumpt (1965) for the second instar *S. haemorrhoidalis* cephalopharyngeal skeleton was similar to that presented for *S. cruentata* in the current study. Zumpt (1965) did not indicate the hypopharyngeal sclerite or the parastomal bar as separate, distinct structures. However, this is not a deviation from the findings of the current study since these sclerites were only discernable with careful focussing and use of contrast as separate from the tentoropharyngeal sclerite.

The cephalopharyngeal skeleton as diagnostic characteristic

A full separation was possible using the features of the cephalopharyngeal skeleton. *Sarcophaga cruentata* was unique due to its split dorsal cornu (Fig. 50A). Three features were unique to *C. albiceps:* (i) the orientation of the parastomal bar (Fig. 48A), (ii) the robust hypopharyngeal sclerite (Fig. 48A) and (iii) the bumps at the base of the hook portion of the mouth hooks (Figs. 48B and 48C). The curvature of the mouth hooks were the most important feature used to identify *C. marginalis* and *C. vicina*. The sickle-shape of the mouth hooks was typical *of C. marginalis* (Fig. 47B). *Calliphora vicina* (Fig. 49B) was unique due to the acutely-angled, thick-set hook portion of the mouth hooks. The mouth hooks were not definitive enough to be used to distinguish among *C. chloropyga* and the two *Lucilia species*. In these species the height of the anterior margin of the hypopharyngeal sclerite in relation to the height of the posterior margin of the mouth hooks were used to distinguish these species from each other. The height of the anterior margin of the hypopharyngeal sclerite was at least half of the height of the posterior margin of the mouth hook in *C. chloropyga* (Figs. 46A and 46B). The posterior margin of the hypopharyngeal sclerite was narrow in relation to the height of the posterior margin of the mouth hooks in the *Lucilia* species (Figs. 44 and 45). The cephalopharyngeal skeletons of the two *Lucilia* species were very similar to each other (Figs. 44 and 45), especially regarding the gradually curving mouth hooks (Figs. 44B and 45B). The two species were distinguished from each other by the most anterior of the labial sclerites, which were robust structures in *L. cuprina* larvae (Fig. 44A).

3.4.7. ANTERIOR SPIRACLES OF THE RESPIRATORY SYSTEM (Figs. 51A to 51G)

The anterior spiracles of the second instar larvae (Figs. 51A to 51G) were semi-circular disc-shaped structures, with button-like structures arranged on the distal ends. This differed from the situation seen in first instar larvae (Figs. 30A to 30G) where the anterior spiracles were in the form of a simple slit in the body wall of the larvae. The number of branches on the anterior spiracles for the different species investigated is given in Table 8.

Table 8: Comparison of the number of branches on the anterior spiracles for the species investigated(*) with that reported in other publications.

Species	*Number of branches on the anterior spiracles*
Lucilia cuprina	7 to 9* (Fig. 51A) 5 (Zumpt 1965); 7 to 9 (O'Flynn & Moorhouse 1980); 4 to 5 (Erzincliogl u 1989b)
Lucilia sericata	7 to 10* (Fig. 51B) 7 to 8 (Zumpt 1965); 7 to 9 (Erzincliogl u 1989b)
Chrysomya chloropyga	10 to 12* (Fig. 51C) 10 to 12 (Zumpt 1965)
Chrysomya marginalis	10 to 15* (Fig. 51D)
Chrysomya albiceps	8 – 11* (Fig. 51E) 9 to 10 (Zumpt 1965); 10 to 12 (de Carvalho Queiroz *et al.* 1997)
C. rufifacies	9 to 10 (O'Flynn & Moorhouse 1980) ; 9 to 12 (Sukontason *et al.* 2003)
Calliphora vicina	8 to 9* (Fig. 51F) 7 to 10 (Zumpt 1965 and Erzinçlioglu 1985)
C. croceipalpis	8 to 9 (Prins 1982)
Sarcophaga cruentata	12 to 15* (Fig. 51G)

The anterior spiracles as diagnostic feature

Using the number of buttons on the anterior spiracles as a diagnostic feature was of limited diagnostic value due to the large overlap in ranges (Table 8). This feature was only of use where specimens with a small number of buttons were found (*Lucilia* species) and for those specimens with a large number of buttons (*C. marginalis* and *S. cruentata*). Intraspecific variation was also

an issue since many of the ranges established during the current study were not an exact match to that found for other populations of the same species (Table 8).

3.4.8. POSTERIOR SPIRACLES OF THE RESPIRATORY SYSTEM (Figs. 52 and 53A to 53F)

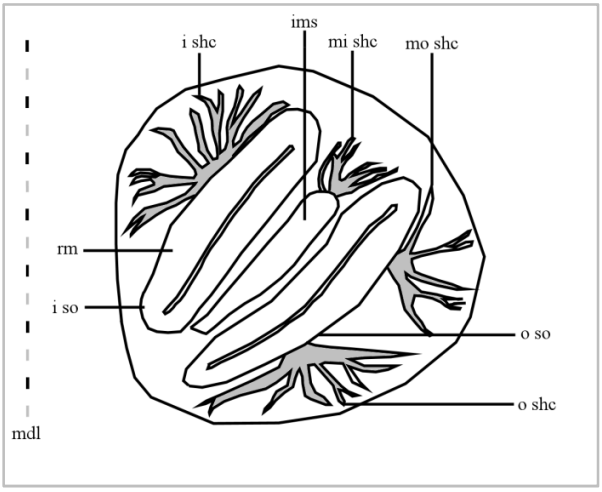


Fig. 52: Representation of a posterior spiracle of a second instar larva.
Legend: ims: intermediate structure; i shc: inner spiracular hair cluster; i so: inner spiracular opening; mdl: midline of spiracular field; mi shc: middle-inner spiracular hair cluster; mo shc: middle-outer spiracular hair cluster; o shc: outer spiracular hair cluster; o so: outer spiracular opening, rm: rima.

The posterior spiracles were not assessed by light microscopy for possible sclerotisation patterns since it was difficult to optimally mount the posterior spiracles of second instar larvae. All observations regarding the posterior spiracle were by scanning electron microscopy. The posterior spiracles of second instar larvae (Fig. 52) were similar to that of first instar larvae (Fig. 31), in that two spiracular openings were noticed in these instar larvae. However, whereas the rimae $\{rm\}$, i.e. the margins of the spiracular openings, of first instar larvae were ill-defined with the inner-ventral margins fused (Fig. 31); those of second instar larvae were separate, well defined structures. Determining the attachment positions of the spiracular hair clusters were possible due to the well defined nature of the rimae. The spiracular hair clusters were similar to

that of first instar larvae, namely: (i) the inner spiracular hair cluster {*i shc*} was attached to the outer margin of the inner spiracular opening {*i so*}, (ii) the middle-inner spiracular hair cluster {*mi shc*} was attached to a position between the two spiracular openings to the intermediate structure {*ims*}, (iii) the middle-outer spiracular hair cluster {*mo shc*} was attached to the upper margin of the outer spiracular opening {*o so*} and (iv) the outer spiracular hair cluster {*o shc*} was attached to the lower margin of the outer spiracular opening. It was found that the attachment areas of the middle-outer and the outer spiracular hair clusters to the outer spiracular opening were constant in the species examined. However, the attachment position on the inner spiracular hair cluster was somewhat varied in the species examined. Also of diagnostic value was the branching nature of the middle-inner spiracular hair cluster.

Lucilia cuprina (Fig. 53A)

The middle-inner spiracular hair cluster was generally not branched (Fig. 53A), whereas the other three spiracular hair clusters were branched. The inner spiracular hair cluster was attached slightly off-centre to its spiracular opening, i.e. to the upper-third portion of its spiracular opening (Fig. 53A).

Lucilia sericata (Fig. 53B) | ***Calliphora vicina*** (Fig. 53F)

Four branched spiracular hair clusters were noted in these two species (Figs. 53B and 53F). The inner spiracular hair cluster was attached to the middle of its spiracular opening (Figs. 53B and 53F).

Chrysomya chloropyga (Fig. 53C) | ***Chrysomya marginalis*** (Fig. 53D) | ***Chrysomya albiceps*** (Fig. 53E)

Four branched spiracular hair clusters were noted in these three species (Figs. 53C, 53D and 53E). The inner spiracular hair cluster was not attached to the middle of its spiracular opening, but more to the upper-third margin of the spiracular opening (Figs. 53C, 53D and 53E).

Sarcophaga cruentata (Fig. 57G)

The posterior spiracles of second instar *S. cruentata* larvae could not be evaluated due to the position these structures occupied within the deep-set cavity of the caudal segment (Fig. 57G).

The posterior spiracles as diagnostic feature

The posterior spiracles were only useful to achieve a partial distinction among the species examined. The posterior spiracles of *S. cruentata* were omitted from this diagnosis due to it being hidden from view in the deep-set spiracular atrium (Fig. 57G). *Lucilia cuprina* was recognisable due to the compacted nature of the middle-inner spiracular hair cluster (Fig. 53A). The remainder of the species could be broadly grouped as those where the inner spiracular hair cluster was attached to the middle of its spiracular opening (*L. sericata* and *C. vicina*) and those where this spiracular hair cluster was attached in a more off-centred position, i.e. to the upper-third margin of its spiracular opening (*Chrysomya* species). No other differentiated features were found to further distinguish the species from each other.

3.4.9. SPINES (Figs. 54A to 54H, 55A to 55G and 56A to 56G)

Whereas the spines of all first instar larvae were single-pointed (Figs. 33A to 33H), a few of the second instar larvae of the species examined contained multi-pointed spines. As with first instar larvae, further aspects considered regarding spines were (i) whether these were located on the anterior or posterior margins of segments and (ii) the completeness of the spine band around the circumference of segments. Features of the ventrally located creeping welts {*cw*} and the lateral fusiform areas {*fa*} (located between segments 5 and 6 up to between segments 11 and 12) were also noted. A typical creeping welt consisted of spines surrounding an area of no spines.

Lucilia cuprina (Figs. 54A, 55A and 56A)

Spines were single-pointed and sharp-tipped (Fig. 54A); a finding confirmed by O'Flynn & Moorhouse (1980). This aspect was similar in first instar *L. cuprina* larvae. Spine bands were located predominantly on the anterior margins of segments. The anterior spine bands were complete from segments 2 to 8; similar to that reported by Erzincliogl (1989b). Some specimens also had a complete anterior spine band for segment 9. However, generally no spines

were found on the anterio-dorsal aspects of segments 9 and 10; i.e. incomplete spine bands on segments 9 and 10. The posterior spine band was complete on segment 11. Various other accounts regarding the completeness of the anterior spine bands or regarding the presence of a posterior spine band of second instar *L. cuprina* larvae differed from each other and also from the account given for the current study. Zumpt (1965) indicated complete anterior spine bands on segments 2 to 9, comparable to the account of the current study. However, he reported differently from the account given for the current study regarding the complete posterior spine bands being present on segments 10 and 11. O'Flynn & Moorhouse (1980) presented comparable finding to Zumpt (1965); i.e. complete anterior spine band from segments 2 to 9, with very sparsely distributed spines on segment 10 and a posterior spine band for segment 11. Greenberg & Szyska (1984) reported complete spine bands on segments 2 to 7 and a posterior spine band on segment 11. The difference between first – and second instar larvae examined were that the anterior spine band ended at segment 7 and the lack of a confirmed complete posterior spine band for segment 11 in first instar larvae. The fusiform areas were weakly defined (Fig. 55A) and an indistinct longitudinal split was noted in creeping welts of some segments (Fig. 56A).

Lucilia sericata (Figs. 54B, 55B and 56B)

Spines were single-pointed and sharp-tipped (Fig. 54B); a finding confirmed by Liu & Greenberg (1989). Complete spine bands were located on the anterior margins of segments from segments 2 to 8, although very few spines were noted on the anterio-dorsal margin of segment 8. A complete posterior located spine band was noted for segment 11, although few spines were noted on the lateral aspect of this segment. Few to no spines were noted on the lateral aspects of segments 9 to 11. These findings were comparable to that reported by Zumpt (1965) and Liu & Greenberg (1989). Erzinclioglu (1989b) reported complete anterior spine bands for segments 2 to 6 and no complete posterior spine bands. The difference in the spine band pattern between first - and second instar larvae was that the anterior spine band ended at segment 7 and no complete posterior spine band was noted on segment 11 in first instar larvae. The fusiform areas of spines were not distinct (Fig. 55B). A small, indistinct longitudinal split was noted in the creeping welts of some segments (Fig. 56B).

Chrysomya chloropyga (Figs. 54C, 55C and 56C)

A few single-pointed spines were noted, but multi-pointed spines predominated (Fig. 54C). This was different from the first instar stage where only single-pointed spines were found. The multi-pointed nature of the spines was confirmed by Zumpt (1965). The anterior located spines bands were complete from segment 2 to 10. Although the spine bands were complete on segments 9 and 10, these bands were less well developed than the anterior ones. Zumpt (1965) reported complete spine bands from segments 2 to 11 of which the posterior bands were narrower. Greenberg & Szyska (1984) reported complete bands of spines for segments 2 to 8 and in a few instances a few spines on the dorsum of segment 9 to complete this band for the congeneric *C. putoria*. Additionally, they reported a complete posterior spine band for segment 11. The difference in the spine band pattern of first – and second instar larvae was that the complete anterior spine bands ended at segment 9 and the lack of spines from the lateral aspects of the posterior segments in first instar larvae. The fusiform areas were only distinctly visible on some segments (Fig. 55C) and the creeping welts were typical (Fig. 56C).

Chrysomya marginalis (Figs. 54D, 54E, 55D and 56D)

The spines were single-pointed and the tips of these spines were bluntly rounded and broad (Figs. 54D and 54E). Spines were sharp-tipped in first instar larvae of the species. All spine bands were located on the anterior margins of segments and were strongly developed. The spine bands were complete for all segments. This differed from the situation seen in the first instar larvae where the anterior spine bands were generally complete for segments 2 to 10, but sometimes incomplete on segment 10. The fusiform areas were well-defined (Fig. 55D) and the creeping welts were typical (Fig. 56D).

Chrysomya albiceps (Figs. 54F, 55E and 56E)

Predominantly multi-pointed spines (Fig. 54F) were present, but a few single-pointed spines were also noted; a finding confirmed by de Carvalho *et al.* (1997). This differed from the first instar stage where only single-pointed spines were noted. O'Flynn & Moorhouse (1980), Liu & Greenberg (1989) and Sukontason *et al.* (2003) presented comparable findings for the congeneric *C. rufifacies*. The split in the tip of the multi-pointed spine was pronounced. The completeness of the spine bands could only be confirmed with certainty for the first few anterior segments and

could not be ruled out from being present on other segments. The spine band's possible presence was obscured from view due to deep intersegmental folds. Zumpt (1965) referred to the three complete spine bands on anterior part of the larval body. O'Flynn & Moorhouse (1980) and Liu & Greenberg (1989) reported complete spine bands on segments 2 to 5 for *C. rufifacies*. Since the completeness of the spines band as reported here cannot be confirmed, a comparison with first instar larvae regarding this aspect cannot be made. The fusiform areas were well defined on some of the segments (Fig. 55E). The creeping welts were typical (Fig. 56E).

Calliphora vicina (Figs. 54G, 55F and 56F)

The spines were single-pointed (Fig. 54G) as in first instar larvae. Liu & Greenberg (1989) noted predominantly single-pointed spines, but also a few double-pointed spines. Anterior as well as posterior located spines were present. Generally, the spine bands were complete on the anterior margins of segments 2 to 8, although it was also complete on segment 9 in some specimens examined. Zumpt (1965), Erzinçlioglu (1985) and Liu & Greenberg (1989) reported complete anterior spine bands for segments 2 to 9 for *C. vicina*. Prins (1982) reported complete anterior spine bands for segments 2 to 8 (sometimes 9) for the congeneric *C. croceipalpis*. The posterior spine bands were first noticed on segment 8 and continued up to segment 11. In the odd specimen a posterior spine band was also noted on segment 7. Zumpt (1965) and Liu & Greenberg (1989) reported similar findings regarding complete posterior spine bands for segments 8 to 11. Erzinçlioglu (1985) differed from the account given of the posterior spine band from the previous two authors only in that he also observed a posterior spine bands on segment 7. Prins (1982) only reported a complete posteriorly located spine band for segment 11 for *C. croceipalpis*. The completeness of the spine band differed from those seen in first instar larvae with complete anterior bands from segments 2 to 10 and complete posterior bands from segments 6 to 11. The fusiform areas were not well defined (Fig. 55F) and the creeping welts were typical (Fig. 56F).

Sarcophaga cruentata (Figs. 54H, 55G and 56G)

Only single-pointed spines were noticed (Fig. 54H). The hair-like nature of the spines as noted on some segments in first instar *S. cruentata* larvae (Figs. 33G and 33H) were not observed in this instar. Segments 2 to 6 contained only anteriorly located complete spines bands. Both

anteriorly and posteriorly located complete spine bands were noticed from segments 7 to 11. The presence of an anterior spine band for segment 12 could not be confirmed. This differed from the first instar stage where the posterior spines bands were complete from segments 6 to 11. The fusiform areas were distinct (Fig. 55G) and the creeping welts were typical (Fig. 56G).

The spines as diagnostic feature

Not all species showed development from only single-pointed spines being present in first instar larvae to multiple-tipped spines in second instar larvae. Therefore, spines can only be used to distinguish specific first instar species from it second instar stage.

A complete distinction among the species examined was possible utilising the nature of spines and the spine band patterns. Two groups can be defined based on the nature of spines; (i) those with single-pointed spines (*L. cuprina, L. sericata, C. marginalis, C. vicina* and *S. cruentata*) and (ii) those with multi-pointed spines (*C. chloropyga* and *C. albiceps*). Of those with single-pointed spines, *C. marginalis* was the only one where the tips of the spines were rounded (Figs. 54D and 54E), whereas the tips of spines in the other species were sharp. *Lucilia* species presented with predominantly anteriorly located spine bands whereas the spines were located on the anterior and posterior margins of segments for *C. vicina* and *S. cruentata.* The two *Lucilia* species can be distinguished from each other based on lack of spines on the lateral aspect of the posterior segments (segments 9 to 11) in *L. sericata*. The anterior spine band extended further in *C. vicina* (from segments 2 to 8 and sometimes 9) than in *S. cruentata* (segments 2 to 6). Utilising the position of the posterior spine band as supportive diagnostic evidence is of no use for these two species since the ranges were very similar. For those species with multi-pointed spines the extent of the anterior spine bands were more extensive for *C. chloropyga* (segments 2 to 10) than the confirmed occurrence of it being present only on the most anterior segments on the larval body in *C. albiceps*. Due to the intraspecific variation noted regarding the presence of complete spine bands for specific segments, this feature should be utilised with the necessary caution when used as part of a diagnostic process.

3.4.10. CAUDAL SEGMENT (Figs. 57A to 57G and 58A to 58G)

As with first instar larvae the two aspects of the caudal segment assessed for their diagnostic value were (i) the spiracular field and (ii) the anal area {*aa*}. The same aspects considered for first instar larvae regarding the spiracular field were considered for this instar; (i) whether the spiracular field was relatively open or in the form of an atrium, (ii) the covering of hair on the rim of the spiracular field and (iii) the size of the perispiracular tubercles. The anal area was assessed with respect to the spines associated with this area and the shape and size of the anal area components (i.e. the anal pads {*ap*} and the anal horns {*ah*}).

Lucilia cuprina (Figs. 57A and 58A) | ***Lucilia sericata*** (Figs. 57B and 58B)

The perispiracular tubercles were small (Figs. 57A and 57B). A sparse covering of hair was present on the rim of the spiracular field (Figs. 57A and 57B). All aspects regarding the spiracular field were similar to that of first instar larvae. Single-pointed, straight spines surrounded the anal area (Figs. 58A and 58B). The anal pads were smaller than the anal horns and these elements of the anal area were rounded (Figs. 58A and 58B). First instar *L. cuprina* larvae differed from the second instar stage in that the anal pads and horns were similar in size and that spines were only noted on the inner margins of the anal pads. First instar *L. sericata* larvae only differed from second instar larvae regarding the spines being present only posterior from the anal pads.

Chrysomya chloropyga (Figs. 57C and 58C)

The perispiracular tubercles were small (Fig. 57C). Hair covered the entire rim of the spiracular field (Fig. 57C). First instar larvae differed from the second instar stage regarding less hair being noted on the rim of the spiracular field and that this hair did not cover the dorsal aspect of the spiracular field. Single – and multi-pointed, curved spines surrounded the anal area (Fig. 58C). No spines were noted associated with the anal area in first instar larvae. The anal pads and horns were similar in size and were rounded (Fig. 58C).

Chrysomya marginalis (Figs. 57D and 58D)

The perispiracular tubercles were clearly visible and of medium size (Fig. 57D). A moderate covering of hair was seen on the rim of the atrium (Fig. 57D). These hairs were sparse or absent

from the dorsal aspect of the rim in first instar larvae. A well defined ring of spines were noted around the anal area (Fig. 58D). These spines were curved and appeared hook-like. The anal pads were rounded and the anal horns triangular in shape (Fig. 58D). No spines were seen in the vicinity of the anal area and all elements of the anal area were rounded in first instar larvae.

Chrysomya albiceps (Figs. 57E and 58E)

Large perispiracular tubercles were noted on the rim of the spiracular disc (Fig. 57E), similar to those noted by Sukontason *et al.* (2003) in *C. rufifacies* second instar larvae. The perispiracular tubercles were similar to the processes of the body wall, i.e. triangular in shape with a crown of short, robust spines at its tips. The perispiracular tubercles on the rim of first instar larvae were small to almost not being visible. Fine hairs were noted on the entire rim of the atrium (Fig. 57E). In first instar *C. albiceps* larvae no hair was noted on the dorsal aspect of the spiracular atrium. Large robust, multi-pointed spines surrounded the anal area. The spines were curved and had a hook-like appearance (Fig. 58E). The anal pads were pointed structures and the anal horns were distinctly triangular in shape (Fig. 58E). The anal area in the first instar stage was simpler, i.e. the spines surrounding this area were single-pointed and the anal pads and horns were rounded.

Calliphora vicina (Figs. 57F and 58F)

The perispiracular tubercles were moderately sized and a moderate covering of hair was found on the entire rim of the spiracular field (Fig. 57F); similar to first instar larvae. Single-pointed, straight spines were noticed around the anal area (Fig. 58F). The anal horns were larger than the anal pads and both of these elements of the anal area were rounded (Fig. 58F). In first instar larvae spines were only found on the inside margin of the anal pads.

Sarcophaga cruentata (Figs. 57G and 58G)

A spiracular atrium was present in this species (Fig. 57G). The perispiracular tubercles were small, but distinctly visible on the rim of the atrium (Fig. 57G). A moderate covering of hair was present on the entire rim of the atrium (Fig. 57G). Straight, single-pointed spines were seen around the anal area (Fig. 58G). The anal pads were smaller than the anal horns. Both structures

were pointed with the horns prominently triangular in shape (Fig. 58G). In first instar larvae spines were only noted on the inside margin of the anal pads.

The caudal segment as a diagnostic characteristic

Spiracular field

A clear progression from the first – to the second instar stage was seen only for some species (*C. marginalis* and *C albiceps*) regarding the perispiracular tubercles. Although a few species showed progression with regard to the coverage of hair on the rim of the spiracular atrium, this feature was not as distinct as the change in the perispiracular tubercles.

The features of the spiracular field could only be utilised to achieve a partial separation among the species under investigation. *Sarcophaga cruentata* with its spiracular atrium was distinctly recognisable when compared to the rest of the species with their relatively open spiracular fields. Large perispiracular tubercles were unique to *C. albiceps*. The other species were grouped as those with a sparse covering of hair on the rim of the spiracular field (*L. cuprina, L. sericata* and *C. chloropyga*) and those with a moderate covering of hair on the rim of the spiracular field (*C. marginalis* and *C. vicina*). The spiracular field exhibited no further distinguishing features to differentiate amongst the species.

Anal area

The spines around the anal area were better developed in second instar larvae than in first instar larvae. The anal horns and pads were only distinctly different in second instar larvae of a few of the species examined.

The anal area also was not adequately differentiated to achieve a full separation. The species concerned were grouped as those where the anal horns were triangular in shape (*C. marginalis, C. albiceps* and *S. cruentata*) and those where the elements of the anal area were rounded (*L. cuprina, L. sericata, C. chloropyga* and *C. vicina*). For those species with rounded anal pads and anal horns, only a partial separation was possible; *C. chloropyga* was the only species here with multi-pointed spines, whereas *L. cuprina, L. sericata* and *C. vicina* all had single-pointed spines surrounding the anal area. The anal area was not differentiated furthermore to allow for a

distinction among these species. The nature of the spines around the anal area allowed for a full separation for those species where the anal horns were triangular in shape. Single-pointed spines surrounded the area in *S. cruentata* larvae; a mix of single-pointed spines with blunt tips and multi-pointed spines surrounded the anal area in *C. marginalis* larvae and large, robust, multi-pointed spines surrounded the anal area of *C. albiceps* larvae.

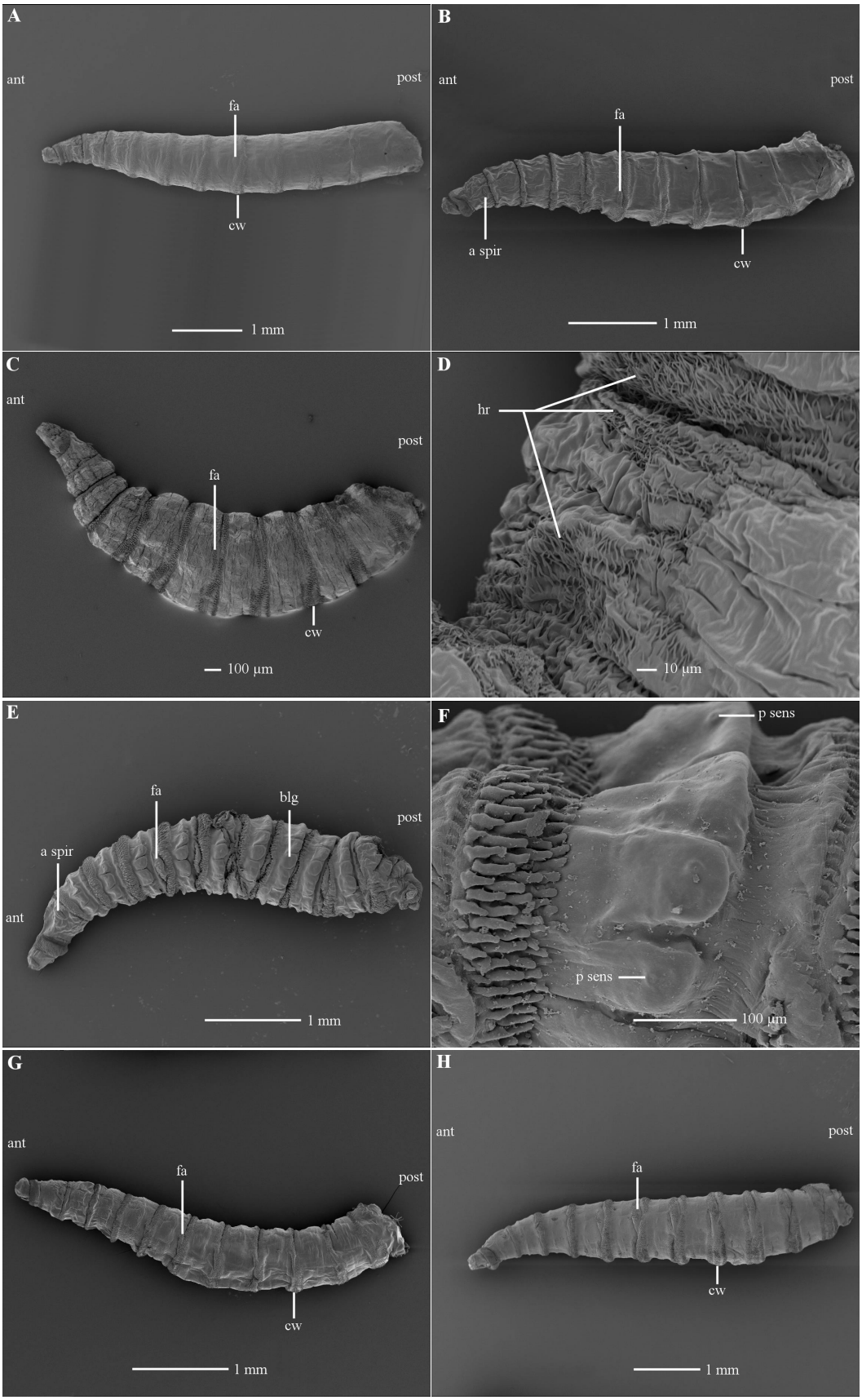


Fig. 38: Scanning electron micrographs of second instar larvae of A: *Lucilia cuprina*; B: *Lucilia sericata*; C and D: *Chrysomya chloropyga*; E and F: *Chrysomya marginalis*; G: *Calliphora vicina* and H: *Sarcophaga cruentata.*
Legend: ant: anterior; a spir: anterior spiracle; blg: bulge; cw: creeping welt; fa: fusiform area; hr: hair; p sens: pit sensillium; post: posterior.

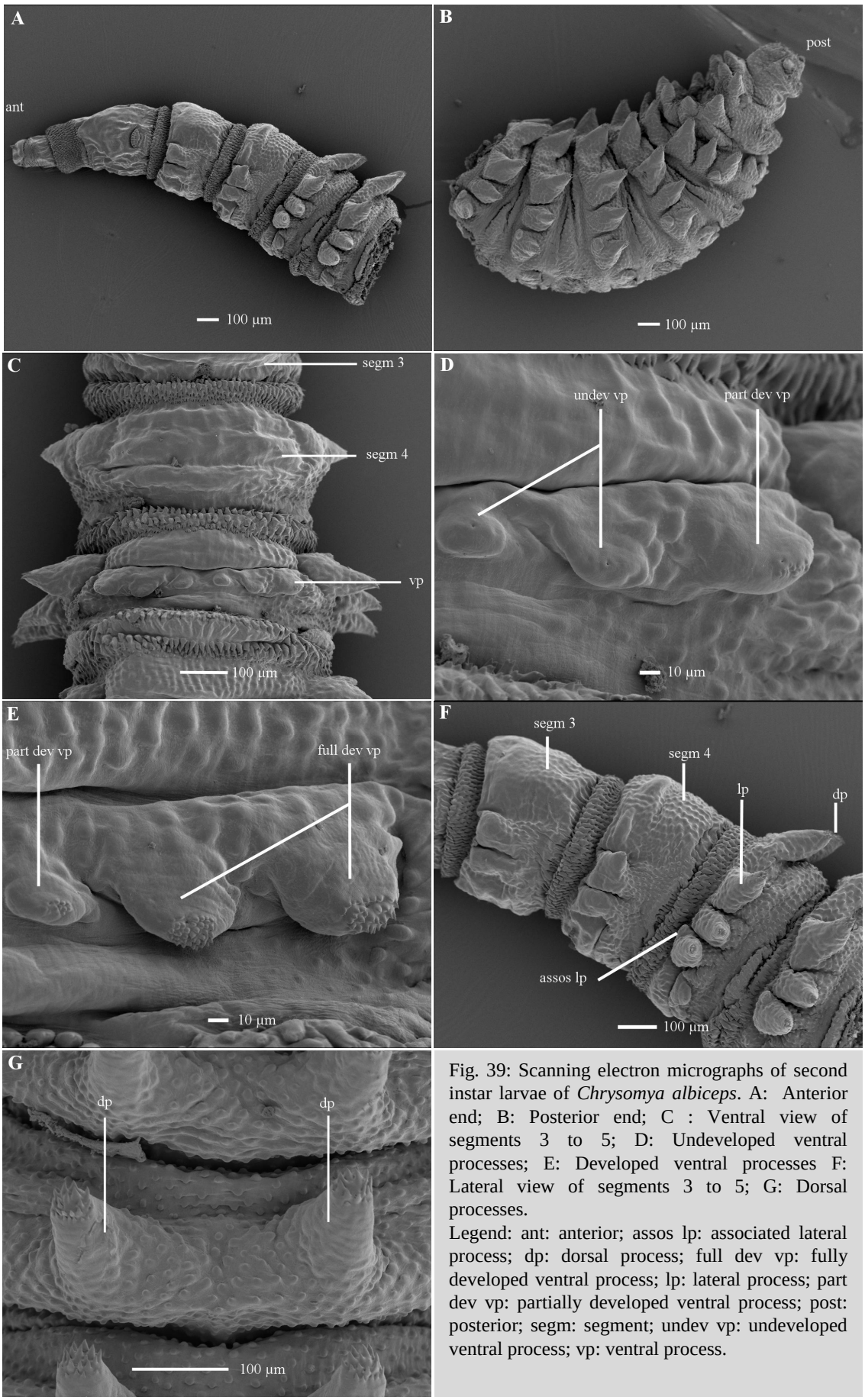


Fig. 39: Scanning electron micrographs of second instar larvae of *Chrysomya albiceps*. A: Anterior end; B: Posterior end; C : Ventral view of segments 3 to 5; D: Undeveloped ventral processes; E: Developed ventral processes F: Lateral view of segments 3 to 5; G: Dorsal processes.

Legend: ant: anterior; assos lp: associated lateral process; dp: dorsal process; full dev vp: fully developed ventral process; lp: lateral process; part dev vp: partially developed ventral process; post: posterior; segm: segment; undev vp: undeveloped ventral process; vp: ventral process.

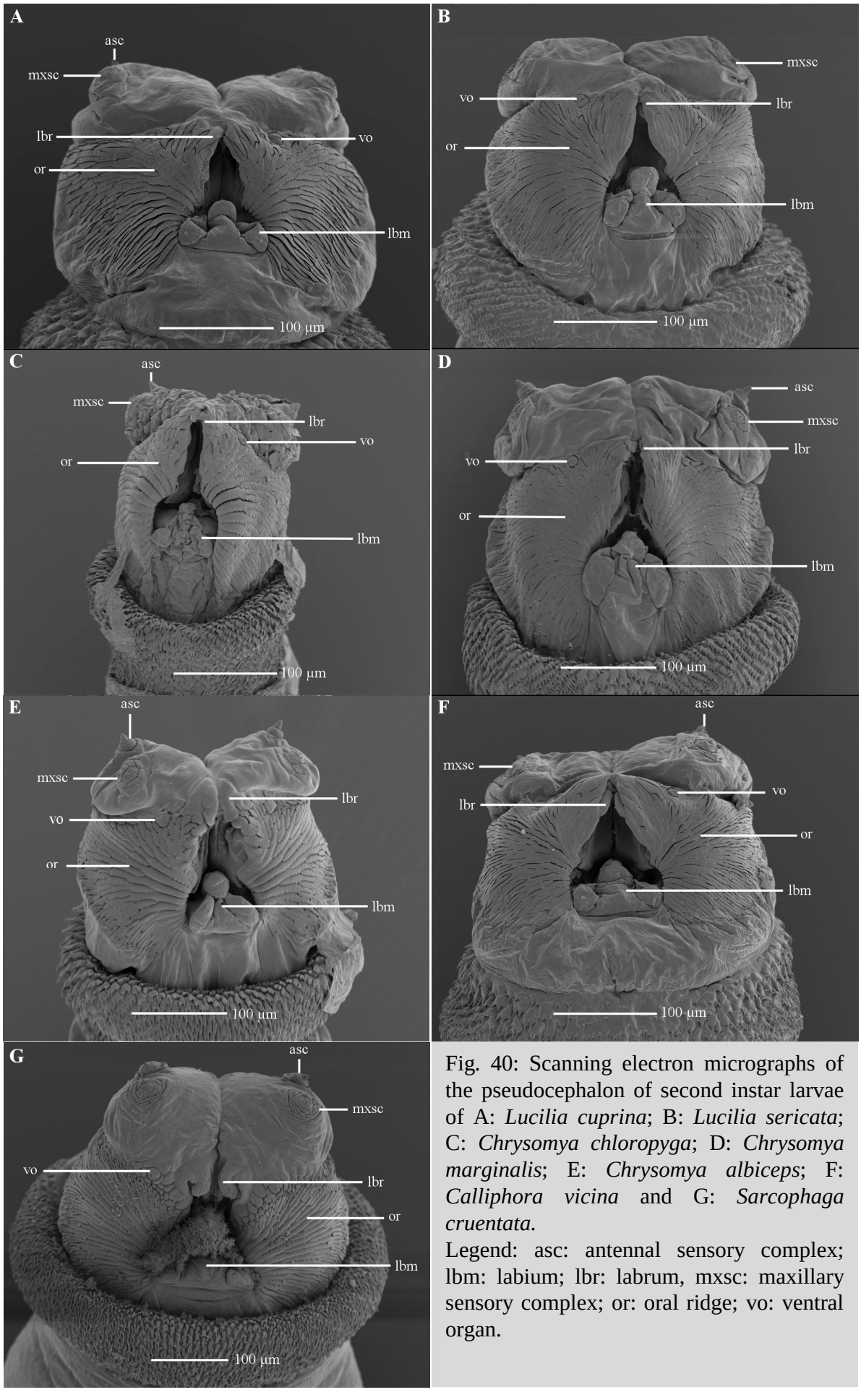


Fig. 40: Scanning electron micrographs of the pseudocephalon of second instar larvae of A: *Lucilia cuprina*; B: *Lucilia sericata*; C: *Chrysomya chloropyga*; D: *Chrysomya marginalis*; E: *Chrysomya albiceps*; F: *Calliphora vicina* and G: *Sarcophaga cruentata.*

Legend: asc: antennal sensory complex; lbm: labium; lbr: labrum, mxsc: maxillary sensory complex; or: oral ridge; vo: ventral organ.

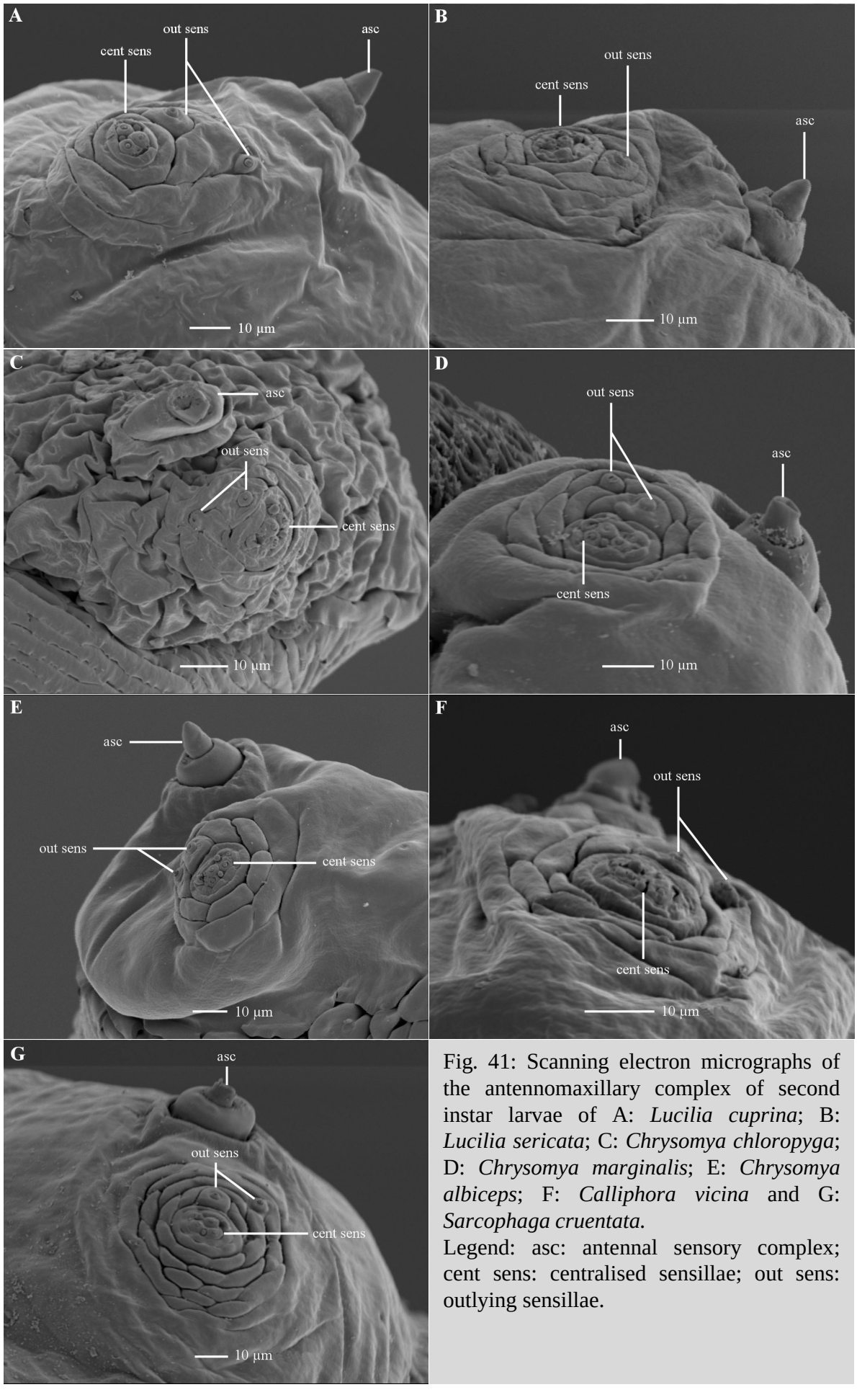


Fig. 41: Scanning electron micrographs of the antennomaxillary complex of second instar larvae of A: *Lucilia cuprina*; B: *Lucilia sericata*; C: *Chrysomya chloropyga*; D: *Chrysomya marginalis*; E: *Chrysomya albiceps*; F: *Calliphora vicina* and G: *Sarcophaga cruentata.*

Legend: asc: antennal sensory complex; cent sens: centralised sensillae; out sens: outlying sensillae.

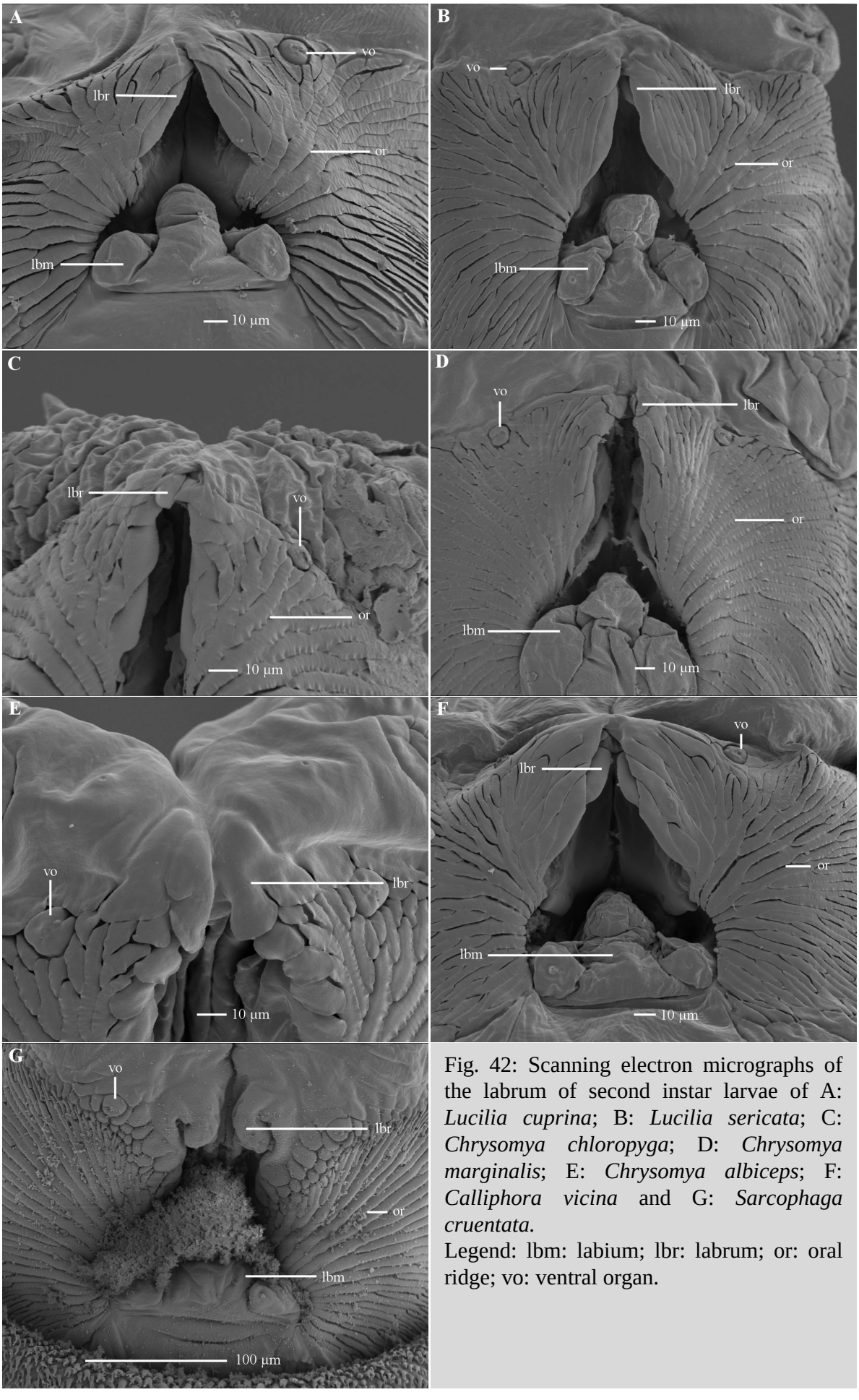


Fig. 42: Scanning electron micrographs of the labrum of second instar larvae of A: *Lucilia cuprina*; B: *Lucilia sericata*; C: *Chrysomya chloropyga*; D: *Chrysomya marginalis*; E: *Chrysomya albiceps*; F: *Calliphora vicina* and G: *Sarcophaga cruentata.*

Legend: lbm: labium; lbr: labrum; or: oral ridge; vo: ventral organ.

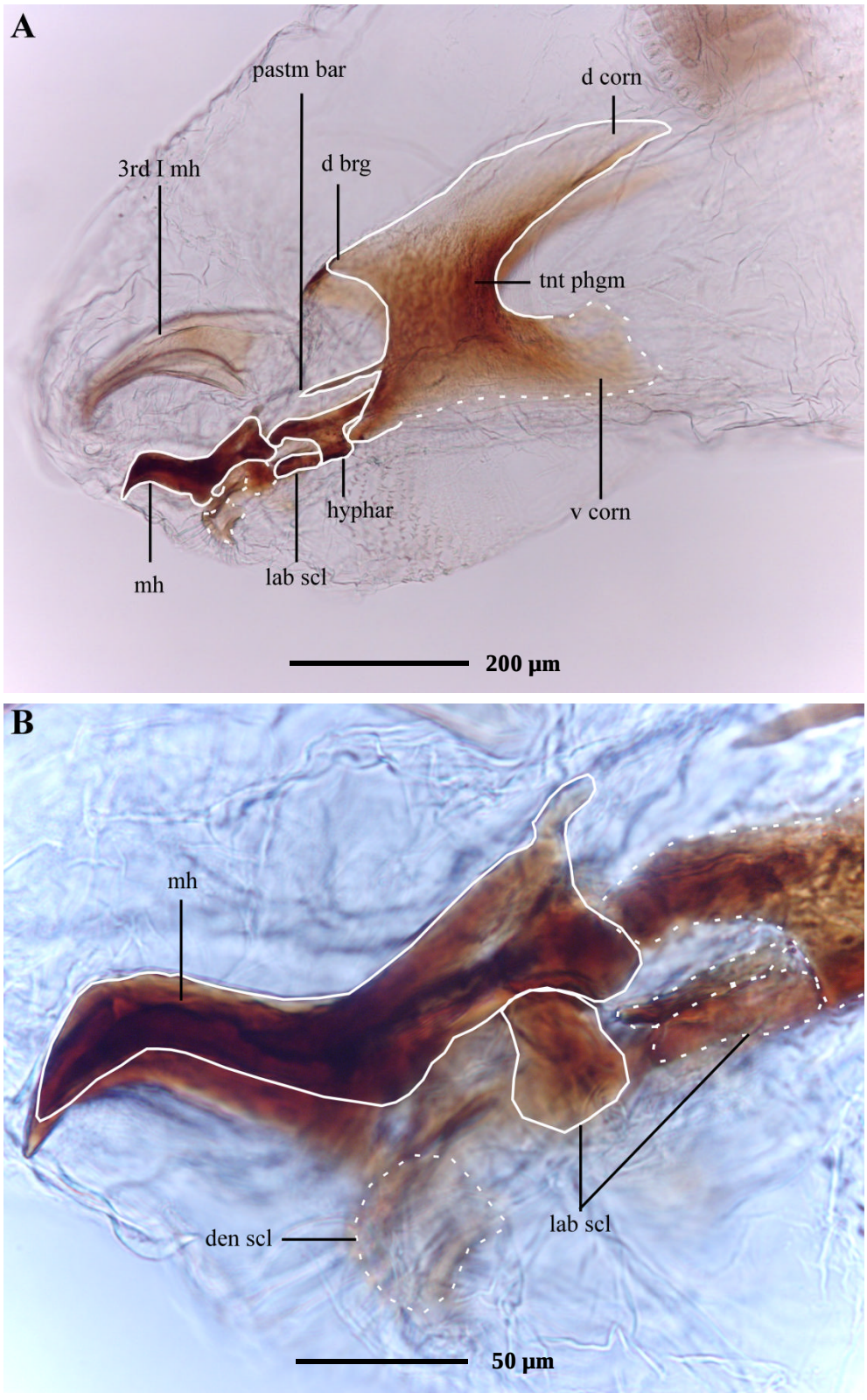


Fig. 44: Light micrographs of the lateral aspect of the cephalopharyngeal skeleton (CPS) of a second instar larva of *Lucilia cuprina*. 44A: Whole CPS; 44B: Anterior elements of the CPS.
Legend: 3rd I mh: emerging third instar mouth hook; d brg: dorsal bridge; d corn: dorsal cornu; den scl: dental sclerite; hyphar: hypopharyngeal sclerite; lab scl: labial sclerite; mh: mouth hook; pastm bar: parastomal bar; tnt phgm: tentorial phragma; v corn: ventral cornu.

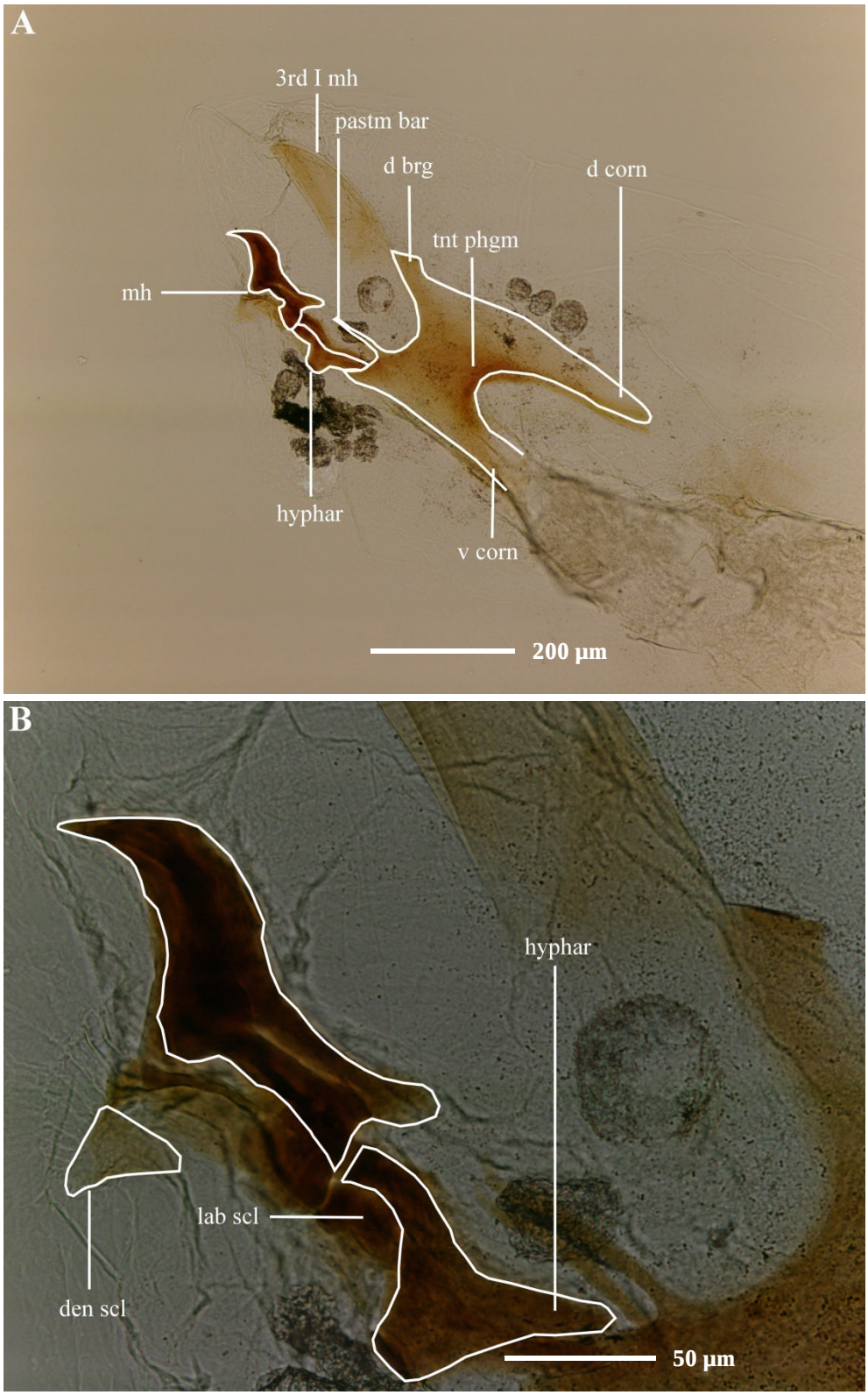


Fig. 45: Light micrographs of the lateral aspect of the cephalopharyngeal skeleton (CPS) of a second instar larva of *Lucilia sericata.* 45A: Whole CPS. 45B: Anterior elements of the CPS.
Legend: 3rd I mh: emerging third instar mouth hook; d brg: dorsal bridge; d corn: dorsal cornu; den scl: dental sclerite; hyphar: hypopharyngeal sclerite; lab scl: labial sclerite; mh: mouth hook; pastm bar: parastomal bar; tnt phgm: tentorial phragma; v corn: ventral cornu.

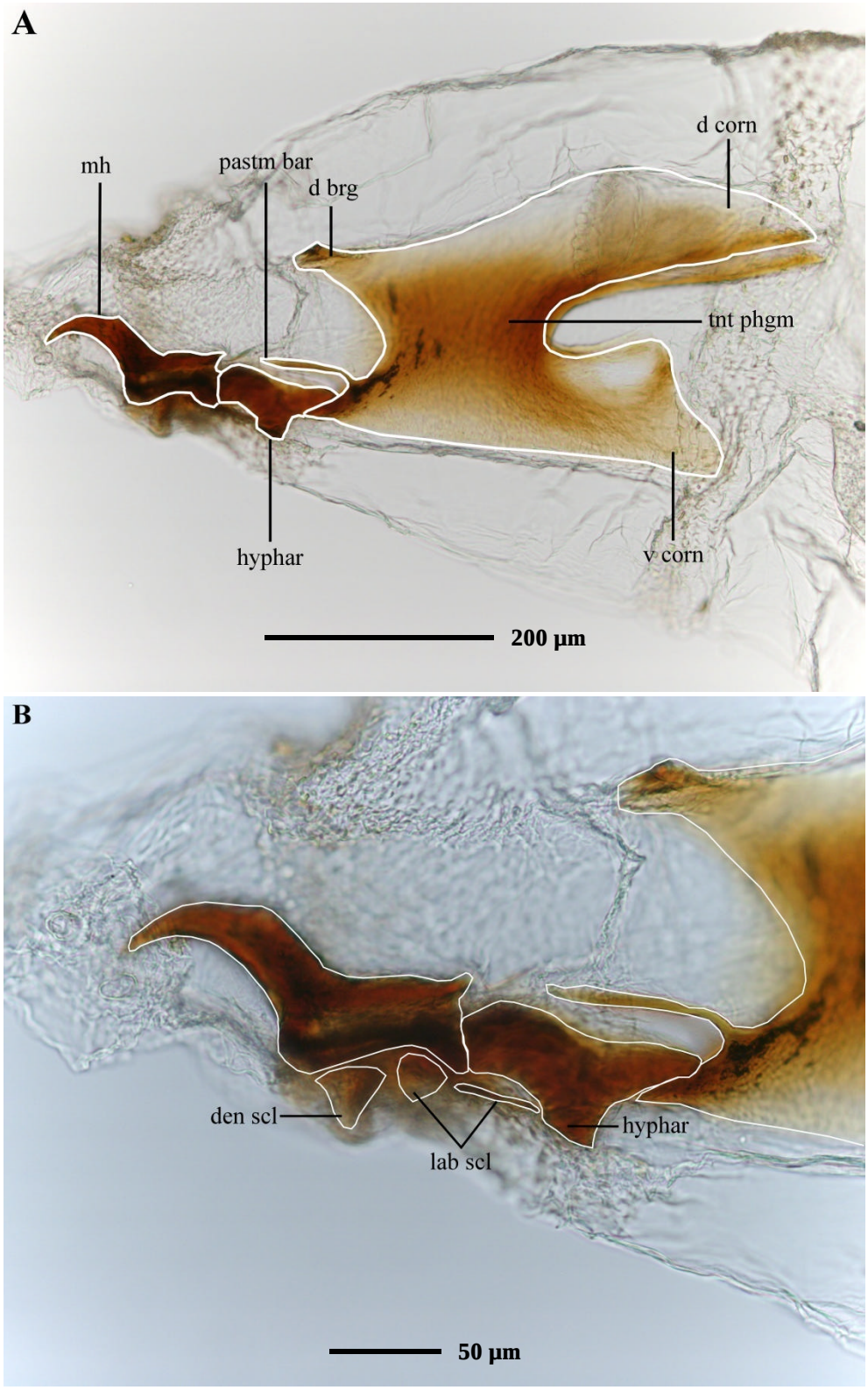


Fig. 46: Light micrographs of the lateral aspect of the cephalopharyngeal skeleton (CPS) of a second instar larva of *Chrysomya chloropyga*. 46A: Whole CPS. 46B: Anterior elements of the CPS.
Legend: d brg: dorsal bridge; d corn: dorsal cornu; den scl: dental sclerite; hyphar: hypopharyngeal sclerite; lab scl: labial sclerite; mh: mouth hook; pastm bar: parastomal bar; tnt phgm: tentorial phragma; v corn: ventral cornu.

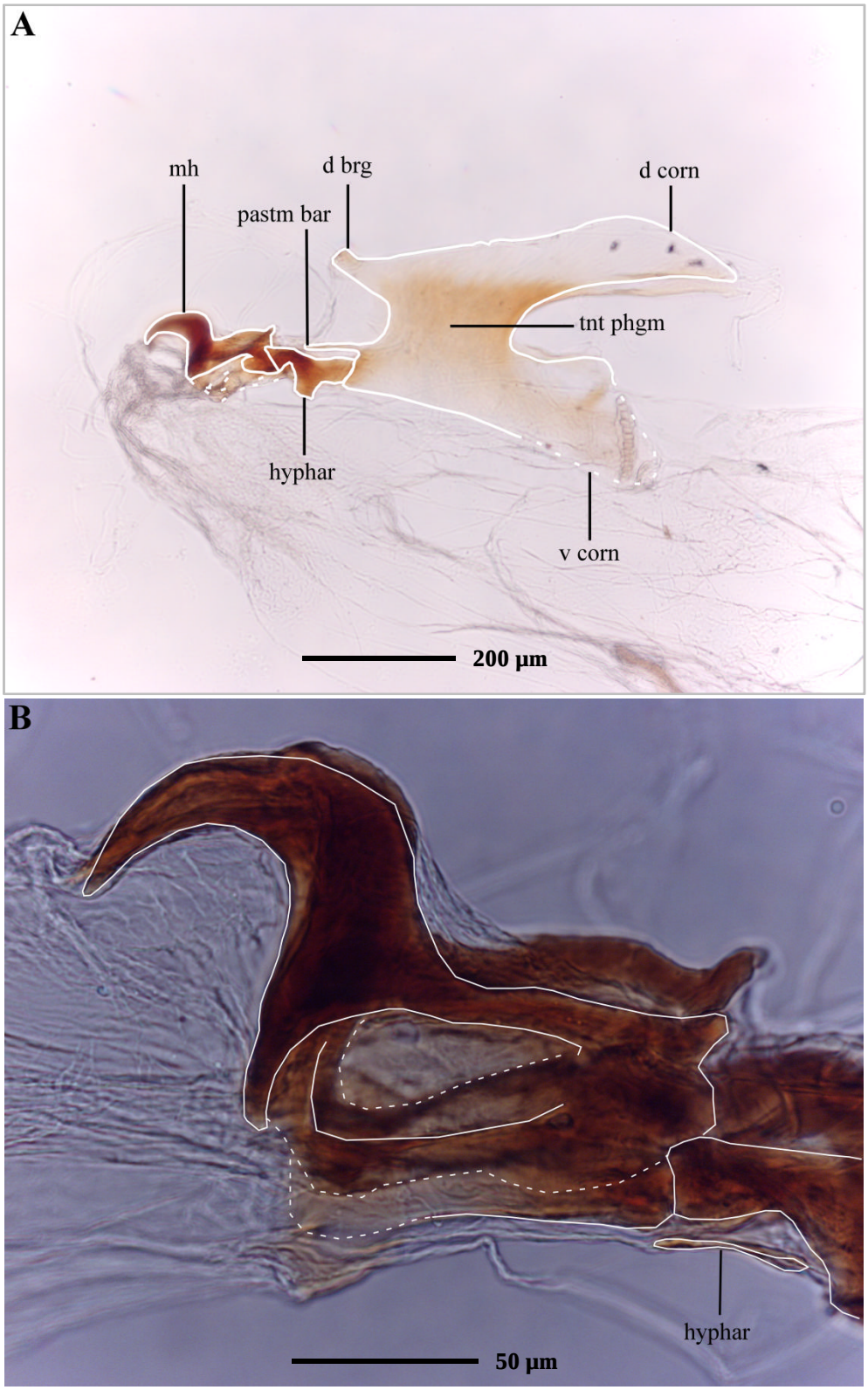
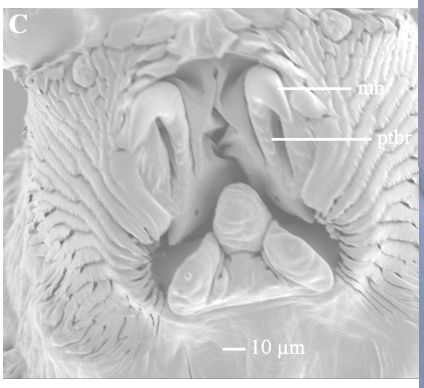


Fig. 47: Light micrographs of the lateral aspect of the cephalopharyngeal skeleton (CPS) of a second instar larva of *Chrysomya marginalis*. 47A: Whole CPS. 47B: Anterior elements of the CPS.
Legend: d brg: dorsal bridge; d corn: dorsal cornu; hyphar: hypopharyngeal sclerite; mh: mouth hook; pastm bar: parastomal bar; tnt phgm: tentorial phragma; v corn: ventral cornu.



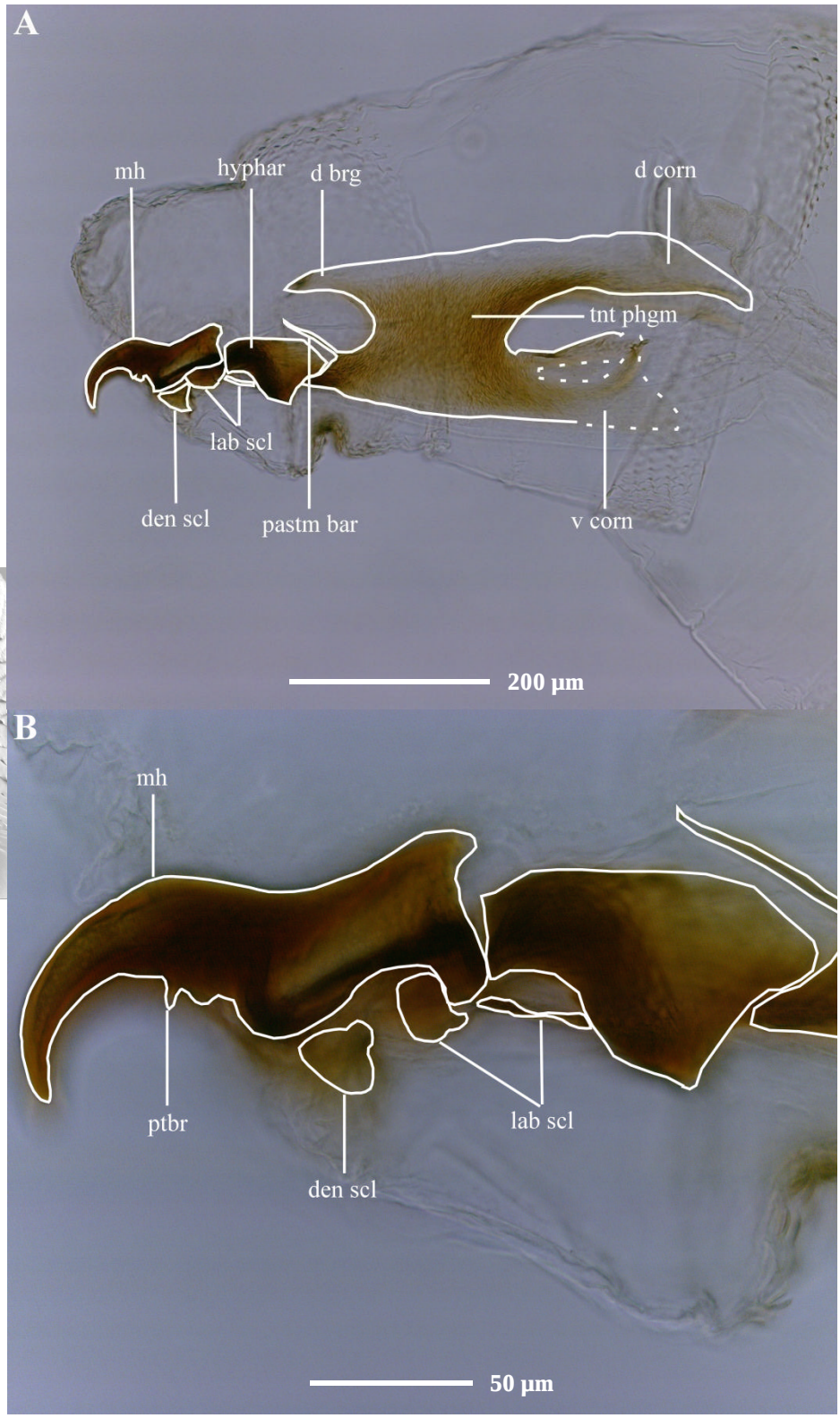
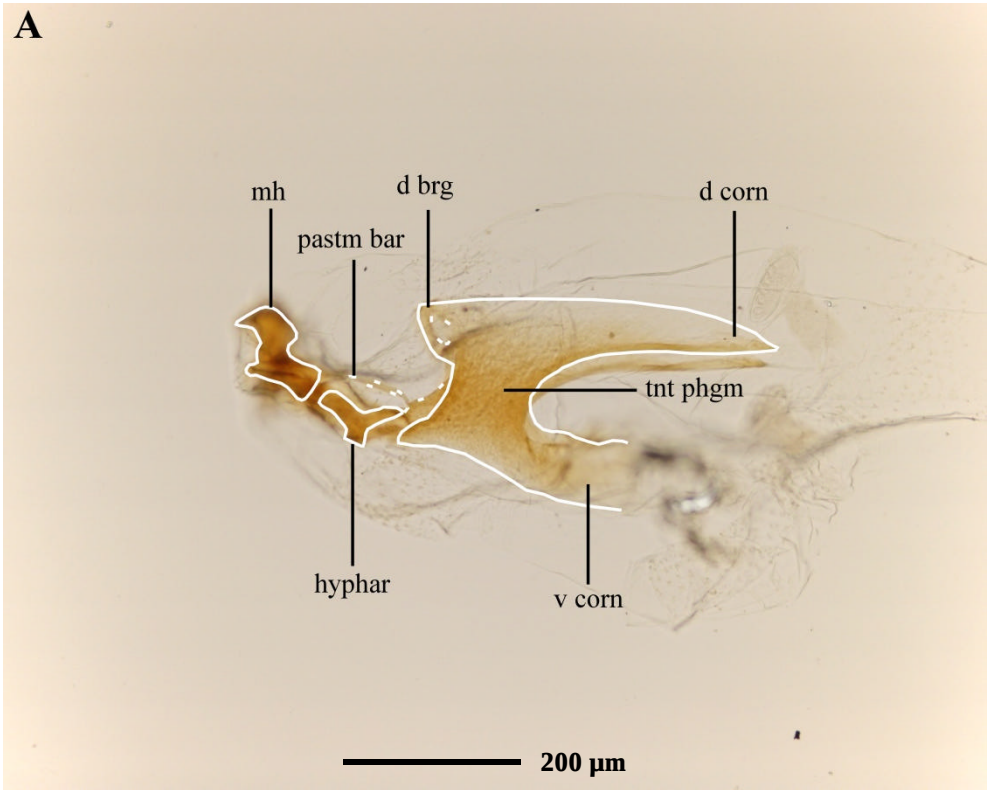


Fig. 48A and 48B: Light micrographs of the lateral aspect of the cephalopharyngeal skeleton (CPS) of a second instar larva of *Chrysomya albiceps*. 48A: Whole CPS. 48B: Anterior elements of the CPS.
Fig. 48C: Scanning electron micrograph of the pre-oral cavity with exposed mouth hooks of a second instar larva of *Chrysomya albiceps*.
Legend: d brg: dorsal bridge; d corn: dorsal cornu; den scl: dental sclerite; hyphar: hypopharyngeal sclerite; lab scl: labial sclerite; mh: mouth hook; pastm bar: parastomal bar; ptbr: protuberance; tnt phgm: tentorial phragma; v corn: ventral cornu.



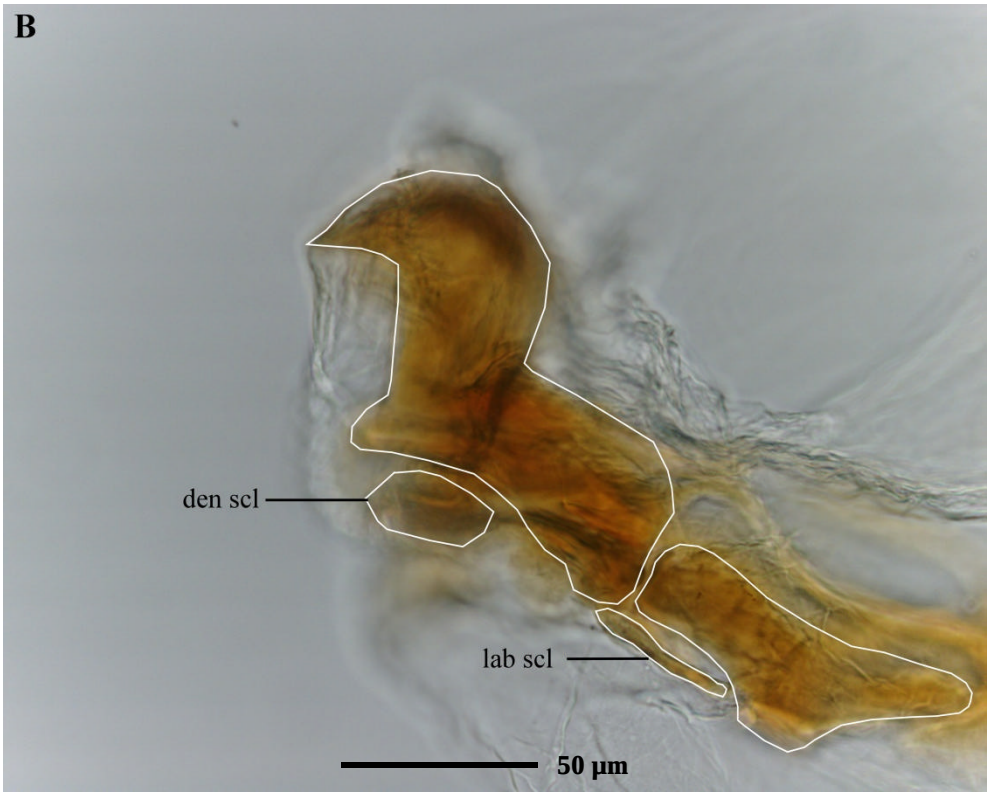


Fig. 49: Light micrographs of the lateral aspect of the cephalopharyngeal skeleton (CPS) of a second instar larva of *Calliphora vicina*. 49A: Whole CPS. 49B: Anterior elements of the CPS.
Legend: d brg: dorsal bridge; d corn: dorsal cornu; den scl: dental sclerite; hyphar: hypopharyngeal sclerite; lab scl: labial sclerite; mh: mouth hook; pastm bar: parastomal bar; tnt phgm: tentorial phragma; v corn: ventral cornu.

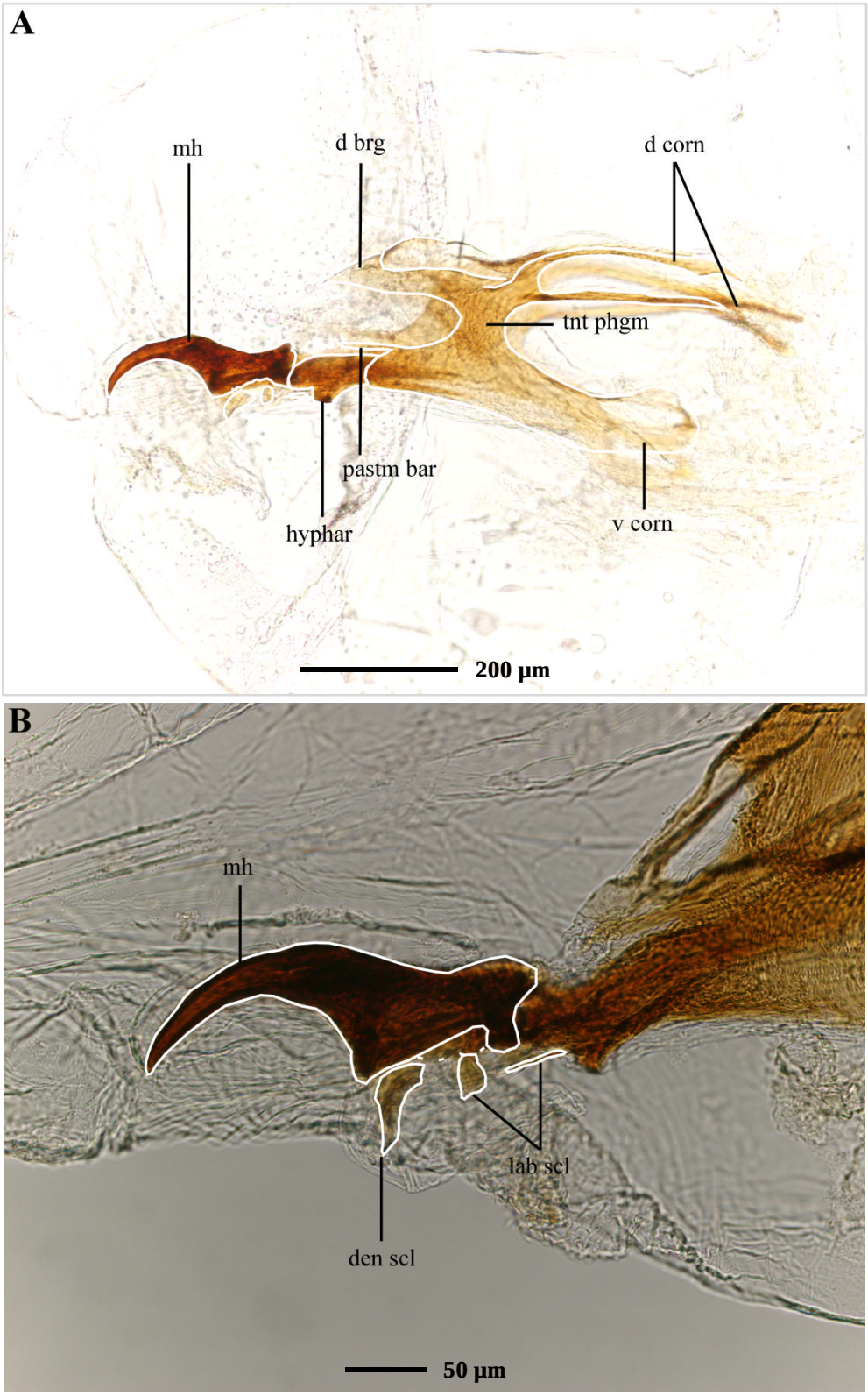


Fig. 50: Light micrographs of the lateral aspect of the cephalopharyngeal skeleton (CPS) of a second instar larva of *Sarcophaga cruentata*. 50A: Whole CPS. 50B: Anterior elements of the CPS.
Legend: d brg: dorsal bridge; d corn: dorsal cornu; den scl: dental sclerite; hyphar: hypopharyngeal sclerite; lab scl: labial sclerite; mh: mouth hook; pastm bar: parastomal bar; tnt phgm: tentorial phragma; v corn: ventral cornu.

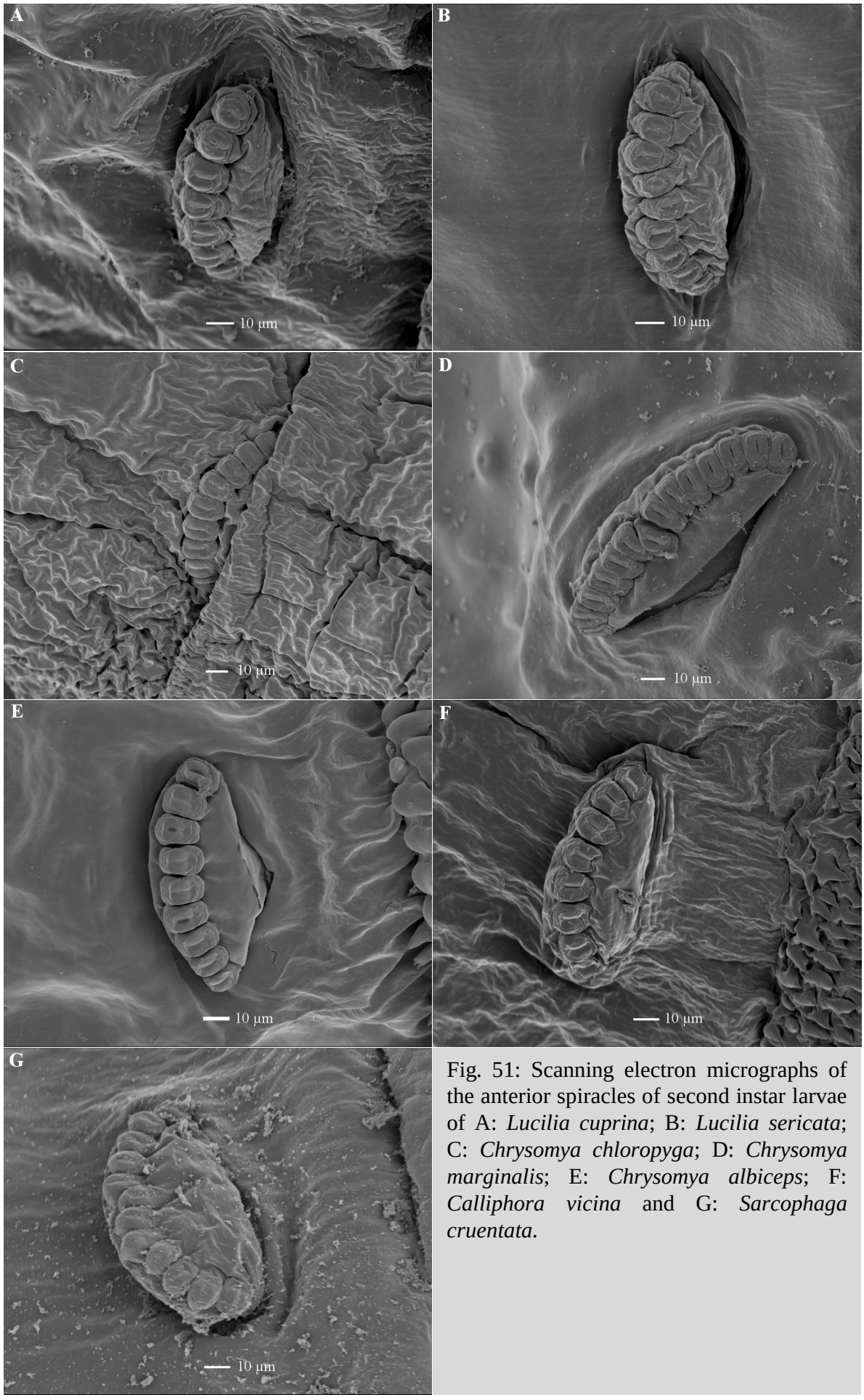


Fig. 51: Scanning electron micrographs of the anterior spiracles of second instar larvae of A: *Lucilia cuprina*; B: *Lucilia sericata*; C: *Chrysomya chloropyga*; D: *Chrysomya marginalis*; E: *Chrysomya albiceps*; F: *Calliphora vicina* and G: *Sarcophaga cruentata*.

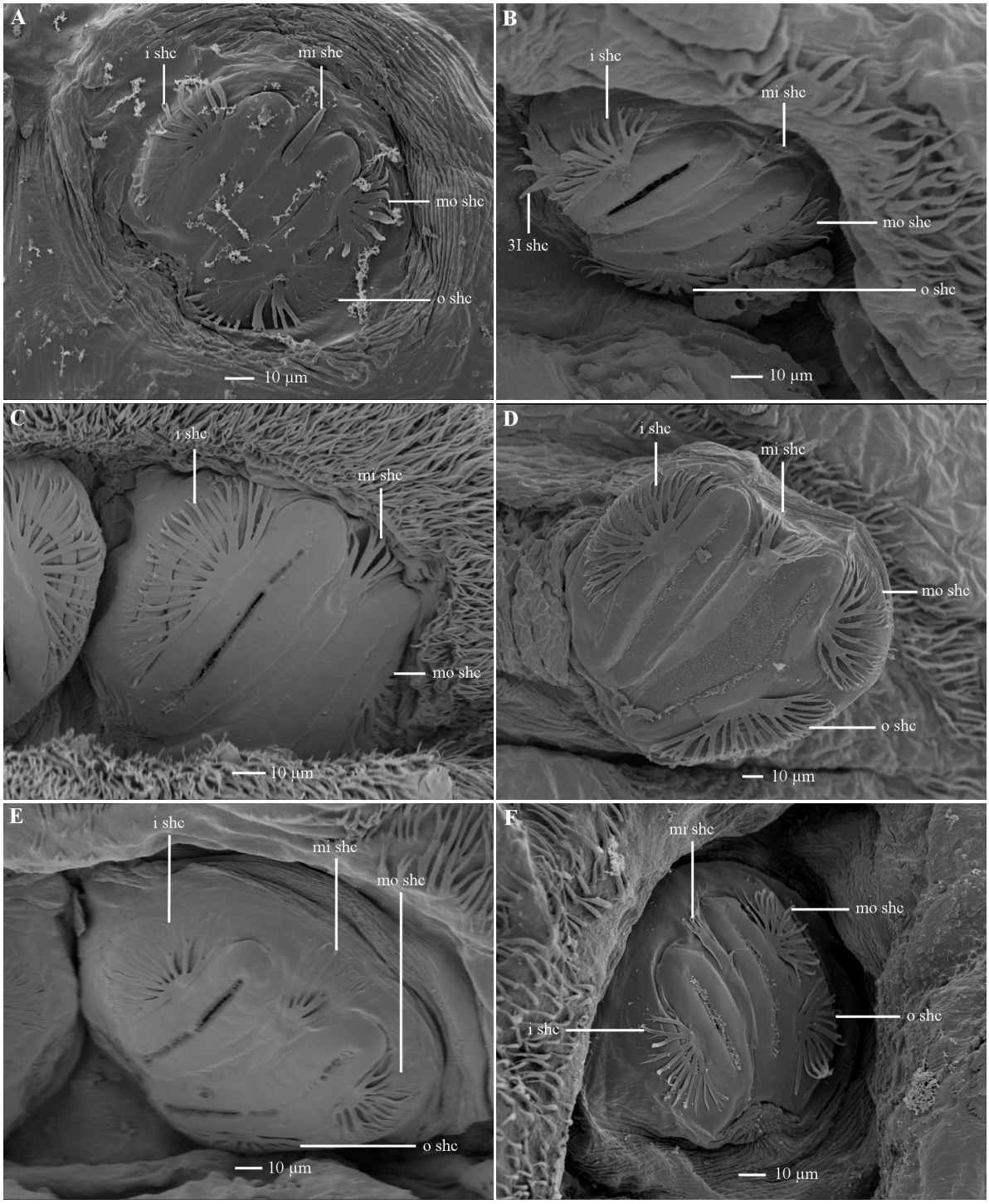


Fig. 53: Scanning electron micrographs of the posterior spiracle of second instar larvae of A: *Lucilia cuprina*; B: *Lucilia sericata*; C: *Chrysomya chloropyga*; D: *Chrysomya marginalis*; E: *Chrysomya albiceps* and F: *Calliphora vicina*.
Legend: 3I shc: emerging third instar spiracular hair cluster; i shc: inner spiracular hair cluster; mi shc: middle-inner spiracular hair cluster; mo shc: middle-outer spiracular hair cluster; o shc: outer spiracular hair cluster.

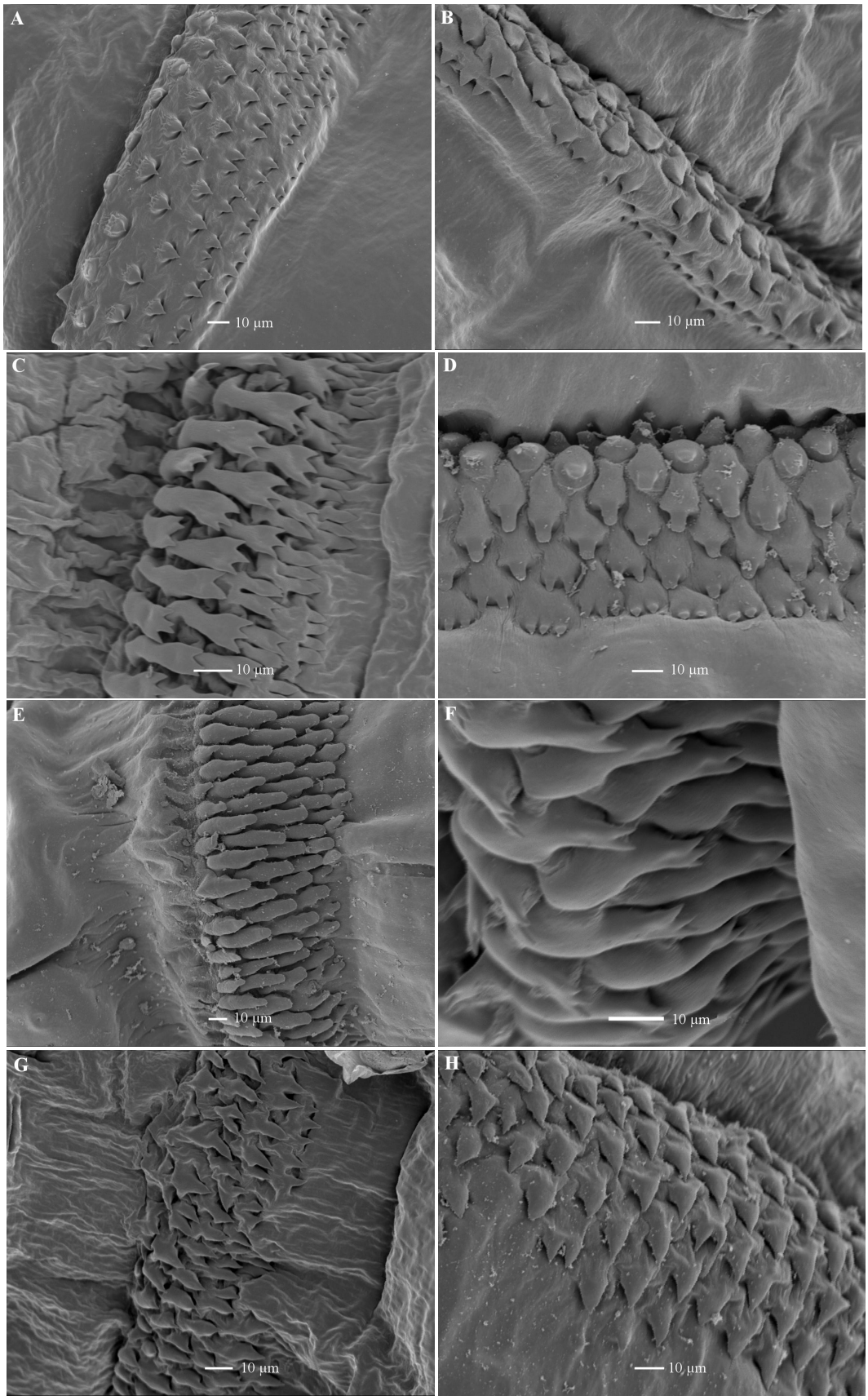


Fig. 54: Scanning electron micrographs of the spines of second instar larvae of A: *Lucilia cuprina*; B: *Lucilia sericata*; C: *Chrysomya chloropyga*; D and E: *Chrysomya marginalis*; F: *Chrysomya albiceps*; G: *Calliphora vicina*; H : *Sarcophaga cruentata.*

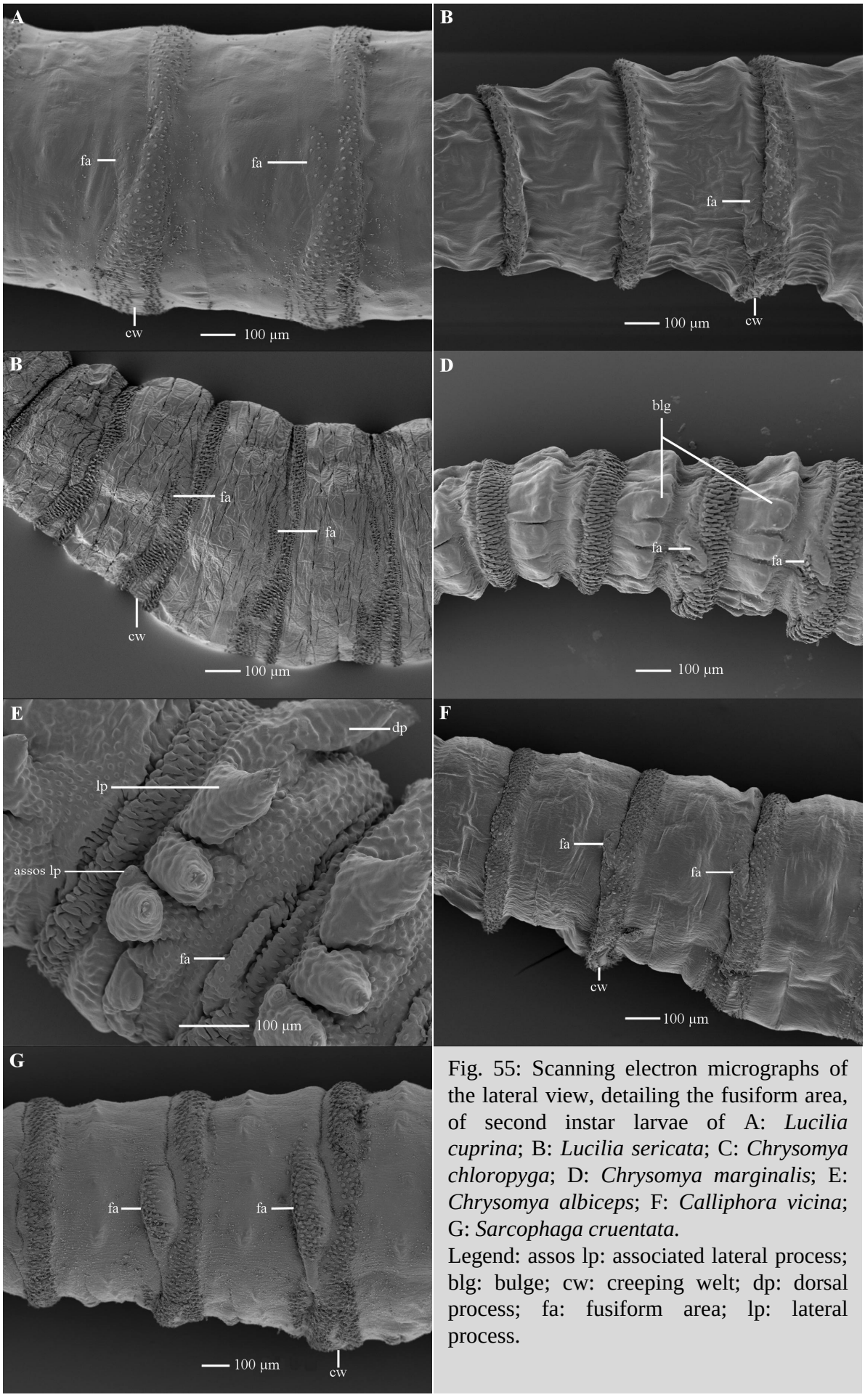


Fig. 55: Scanning electron micrographs of the lateral view, detailing the fusiform area, of second instar larvae of A: *Lucilia cuprina*; B: *Lucilia sericata*; C: *Chrysomya chloropyga*; D: *Chrysomya marginalis*; E: *Chrysomya albiceps*; F: *Calliphora vicina*; G: *Sarcophaga cruentata.*

Legend: assos lp: associated lateral process; blg: bulge; cw: creeping welt; dp: dorsal process; fa: fusiform area; lp: lateral process.

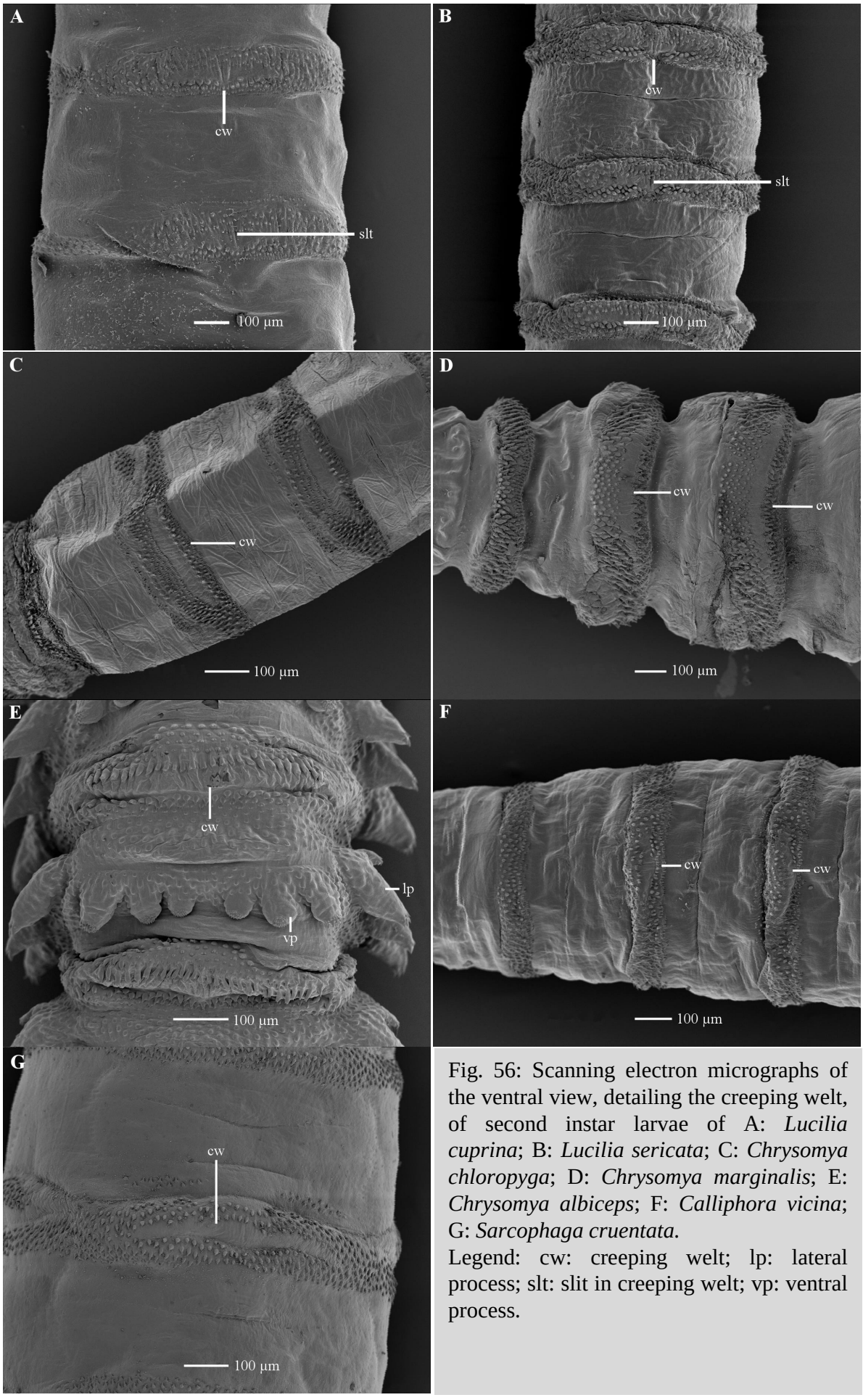


Fig. 56: Scanning electron micrographs of the ventral view, detailing the creeping welt, of second instar larvae of A: *Lucilia cuprina*; B: *Lucilia sericata*; C: *Chrysomya chloropyga*; D: *Chrysomya marginalis*; E: *Chrysomya albiceps*; F: *Calliphora vicina*; G: *Sarcophaga cruentata*.

Legend: cw: creeping welt; lp: lateral process; slt: slit in creeping welt; vp: ventral process.

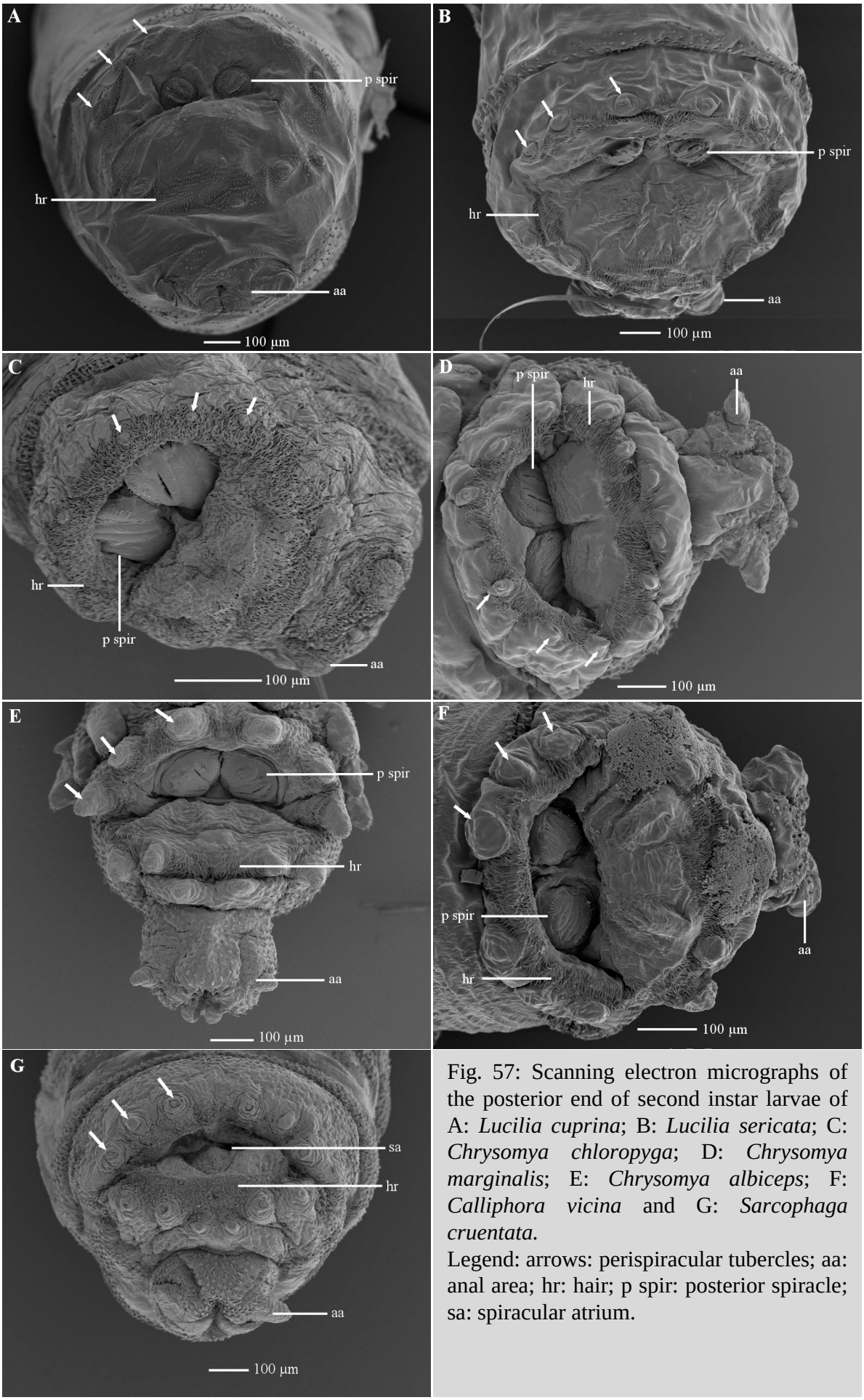


Fig. 57: Scanning electron micrographs of the posterior end of second instar larvae of A: *Lucilia cuprina*; B: *Lucilia sericata*; C: *Chrysomya chloropyga*; D: *Chrysomya marginalis*; E: *Chrysomya albiceps*; F: *Calliphora vicina* and G: *Sarcophaga cruentata.*

Legend: arrows: perispiracular tubercles; aa: anal area; hr: hair; p spir: posterior spiracle; sa: spiracular atrium.

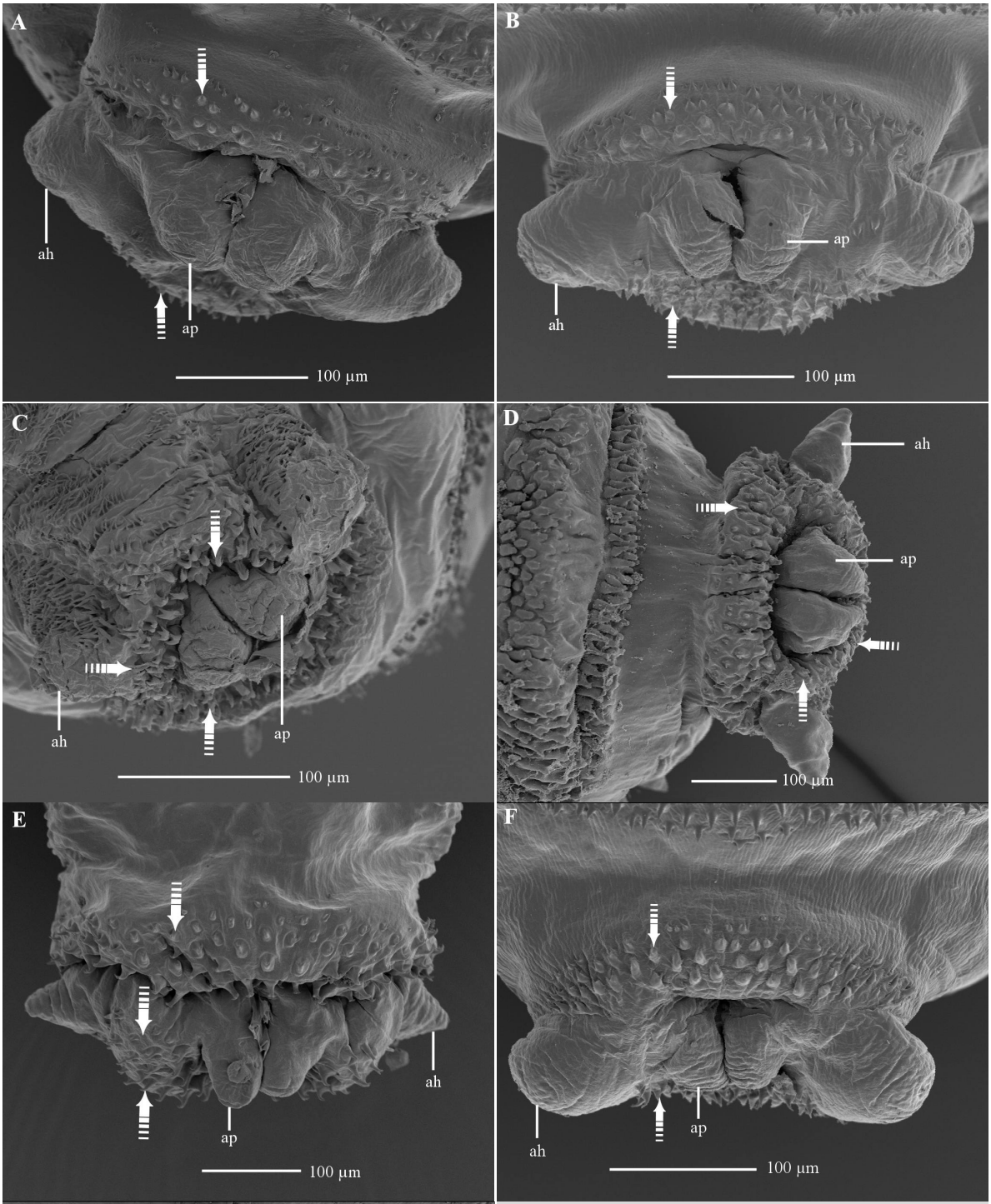


Fig. 58: Scanning electron micrographs of the anal area of second instar larvae of A: *Lucilia cuprina*; B: *Lucilia sericata*; C: *Chrysomya chloropyga*; D: *Chrysomya marginalis*; E: *Chrysomya albiceps*; F: *Calliphora vicina* and G: *Sarcophaga cruentata.*

Legend: arrows: spines; ah: anal horn; ap: anal pad.

3.5. MORPHOLOGY OF THIRD INSTAR LARVAE

3.5.1. INTEGUMENT (Figs. 59, 60, 78E, 79D and 79E)

Lucilia cuprina (Fig. 59A) **|** ***Lucilia sericata*** (Fig. 59B) **|** ***Calliphora vicina*** (Fig. 59F)

Third instar *L. cuprina* (Fig. 59A), *L. sericata* (Fig. 59B) and *C. vicina* (Fig. 59F) larvae had smooth integuments with no pronounced swellings of the integument. This was similar to the second instar larvae for these three species (Figs. 38A, 38B and 38G).

Chrysomya chloropyga (Fig. 59C)

The smoothness of the integument of third instar *C. chloropyga* larvae was in contrast to the fine hair noted by Wells *et al.* (1999) on the posterior dorsum of *C. putoria* larvae. Third instar *C. chloropyga* larvae was also different in this respect from second instar larvae (Fig. 38D) where hair was noted on the posterior dorsum of most specimens examined.

Chrysomya marginalis (Figs. 59D and 79D)

The lateral aspect of the integument on segments 3 to 11 was prolapsed into regular sack-like structures (Figs. 59D and 79D). A pit sensillium was located on these structures. The integument of third instar larvae was similar to that of second instar larvae (Figs. 38E and 38F).

Chrysomya albiceps (Figs. 59E, 60A to G, 78E, 79E)

With the exception of the relatively smooth integument of the pseudocephalon, the integument on the rest of the larval body was covered with dome-shaped papillae (Figs. 60A to 60E, 60G, 78E and 79E). Sukontason *et al.* (2003) found the papillae arranged in a pattern of smaller papillae surrounding a larger papilla on the integument of *C. rufifacies* larvae. This net-like arrangement of papillae was not observed on the integument of third instar *C. albiceps* larvae. The papillae were similar to that found in second instar *C. albiceps* larvae (Fig. 39).

As with second instar *C. albiceps* larvae, third instar larvae are recognisable on a macroscopic level due to their “spiny”/ “hairy” appearance (Fig. 59E). Microscopic evaluation of the larvae revealed that these “spines” / “hairs” were prominent processes of the integument. These processes were developed to various degrees. An undeveloped process {*undev proc*} was in the form of a raised bump with an aperture on its tip, but without spinules crowning the tip of the process (Fig. 60B). Short spinules crowned the tips of partially developed dorsal processes (Fig. 60G). The tips of fully developed dorsal and lateral processes bore long and slender spinules (Figs. 60D to 60F). Processes were absent or undeveloped on segments 2 to 4 with only the middle lateral process {*lp*} of segment 4 partially developed (Figs. 60B and 60C). In some specimens the dorsal process {*dp*} of segment 4 was partially developed, but this was rare (Fig. 60G). Segment 5 contained fully developed lateral and dorsal processes. The two inner ventral processes {*i vp*} of segment 5 were partially developed, furnished with a few blunt-tipped spinules and the middle and outer ventral processes were more developed, furnished with numerous spinules (Fig. 60A). In the second instar stage, only the outer ventral processes were partially developed, while the rest of the ventral processes on segment 5 were undeveloped. All processes were fully developed from segment 6 to 11 in third instar larvae. The processes were similarly arranged on third instar larvae as it was on second instar larvae. The arrangement of a full set of processes was as follows: six processes on the ventral surface (Fig. 78E); a group of three processes on the lateral surface (Fig. 79E) with a smaller process associated with the ventro-lateral process (Figs. 60C and 60D) and two dorsal processes (Figs. 60E, 79E). In some specimens a button sensillium {*b sens*} was noted in the vicinity of the crown of spinules of the dorsal process of segment 5 (Fig. 60F). The presence of this sensillium could not be established for other processes or for other segments.

Erzincliogl u (1987) distinguished between *C. albiceps* and *C. rufifacies* based on whether dorsal process 1 (this translates to the process on segment 4) was furnished with spinules or not. Tantawi & Greenberg (1993) warned against using this characteristic due to a high level of variability noted. The concern raised by Tantawi & Greenberg (1993) was confirmed by the results obtained during the current study where only a few specimens contained weakly developed (shorter) spinules on the dorsal process of segment 4 (Fig. 60G). Tantawi & Greenberg (1993) used the shape

of the outermost ventral process and the number of spinules crowning the tips of processes to distinguish between *C. albiceps* and *C. rufifacies*. The distinguishing characteristic of the triangular shape of the outer ventral process as described by Tantawi & Greenberg (1993) for *C. albiceps* was also observed in the larvae of *C. albiceps* examined during the course of the current study (Fig. 60A). The second distinguishing characteristic of *C. albiceps* described by Tantawi & Greenberg (1993) regarding the number of spinules crowning the tips of the processes was not evaluated specifically during the current study. Wells *et al.* (1999) identified a third characteristic to distinguish between *C. albiceps* and *C. rufifacies*. They noted that the spinules of the dorsal processes were small and pointed towards the centre in third instar larvae of *C. albiceps*, opposed to the large spinules pointing away from the centre of the process in third instar larvae of *C. rufifacies*. This was comparable to the findings of the present study, where the spinules pointed towards the centre of the dorsal processes (Figs. 60E to 60G).

Sarcophaga cruentata (Figs. 59G, 59H, 78G and 79G)

Small, pointed bulges were noted on the lateral and ventral aspects from segment 5 to 11 (Figs. 59G, 78G and 79G). A single pit sensillium was located on each of these bulges (Fig. 59H). These bulges were not clearly defined in second instar *S. cruentata* larvae (Fig. 55G).

The integument as diagnostic feature

The classic identification feature of smooth versus “hairy” / “spiny” larvae was used as the initial aspect to group the species accordingly. Those species with relatively smooth integuments were *L. cuprina*, *L. sericata*, *C. chloropyga*, *C. marginalis*, *C. vicina* and *S. cruentata*. *Chrysomya albiceps* was the only one of the species under investigation defined as a “hairy” / “spiny” larva (Fig. 59E). The integuments of *L. cuprina*, *L. sericata*, *C. chloropyga* and *C. vicina* offered no further distinguishing characteristics to allow for further separation of these species. The integuments of *C. marginalis* and *S. cruentata* were uniquely identified due to the sack-like structures of the integument of *C. marginalis* larvae (Figs. 59D and 79D) and the small, pointed bulges on the lateral and ventral aspects of the integument of *S. cruentata* larvae (Figs. 59G, 59H, 78G and 79G).

For most of the species investigated, it was not possible to assess intraspecific variation since the integument was rarely evaluated as such by other authors. The larval integument of third instar *C. albiceps* was evaluated by Tantawi & Greenberg (1993) to differentiate *C. albiceps* from *C. rufifacies*. The results of Tantawi & Greenberg (1993) formed the basis to illustrate that no intraspecific variation was found when evaluating the integument of *C. albiceps* specimens examined by these authors and specimens of *C. albiceps* examined during the course of the present study.

Based on the work of Tantawi & Greenberg (1993) on *C. rufifacies* and Wells *et al.* (1999) on *C. putoria*, the integument exhibited sufficient variations to respectively distinguish between the closely related *C. albiceps* and *C. rufifacies* and also between the closely related *C. chloropyga* and *C. putoria*. The variation noted in the processes and the papillae of the integuments were sufficiently different to distinguish *C. albiceps* third instar larvae from third instar *C. rufifacies* larvae. The absence of hair from the posterior dorsum of *C. chloropyga* larvae were the characteristic used to separate this species from the closely related *C. putoria* where hair was noted by Wells *et al.* (1999) on the posterior dorsum of larvae.

3.5.2. ANTENNOMAXILLARY COMPLEX (Figs. 61 and 62)

The antennal sensory complex {*asc*} was a pointed structure encircled within a socket-like outgrowth of the cuticle (Figs. 61 and 62). Its morphology was unchanged in all three larval instars and also in the different species under investigation.

The maxillary sensory complex {*mxsc*} was similar in third instar larvae (Fig. 61) to that of second instar larvae (Fig. 41). As in second instar larvae the maxillary sensory complex of sarcophagid larvae was different from that of the calliphorid larvae. The maxillary sensory complex (Fig. 61) was set on a raised area, similar to the situation found in first – and second instar larvae. The maxillary sensory complex consisted of a few encapsulated and a few un-encapsulated button sensillae located centrally {*cent sens*} in the maxillary sensory complex (Figs. 62A to 62G). Two un-interrupted rings of cuticular outgrowths encircled the central grouping of button sensillae (Figs. 62A to 62G). Dorso-laterally on the maxillary sensory complex, two encapsulated button sensillae (Figs. 62A to 62G) were noted. In calliphorids, the area around the ringed

central groupings of button sensillae was made up of a series of cuticular folds, having an interlaced appearance, forming two alternating rings on the periphery of the maxillary sensory complex (Figs. 62A to 62F). In the sarcophagid many rings were formed on the periphery of the maxillary sensory complex (Fig. 62G). This specific feature of the antennomaxillary complex can be used to distinguish the sarcophagid from the calliphorids. The dorso-lateral button sensillae were contained within the series of cuticular folds (Figs. 62A to 62G).

3.5.3. VENTRAL ORGAN (Figs. 61 and 63)

The ventral organ {*vo*} of third instar larvae (Figs. 61 and 63) was morphologically similar to that seen in first – and second (Fig. 42) instar larvae. The ventral organ was undifferentiated in all the species examined and was thus considered to be an unsuitable diagnostic feature.

3.5.4. ORAL RIDGES (Figs. 61 and 63)

As in second instar larvae (Fig. 42) numerous oral ridges {*or*} adorned the ventral surface of the pseudocephalon in third instar larvae for all the species under discussion (Figs. 61 and 63). The oral ridges revealed no further distinct variations to allow for it to be utilised as a diagnostic characteristic and was thus considered unsuitable as a diagnostic feature.

3.5.5. LABIUM (Fig. 61)

The morphology of the labium {*lbm*} of third instar larvae (Fig. 61) was similar to that seen in first – and second (Fig. 42) instar larvae. The labium was comprised of a centralised lobe and two lobes arranged bilaterally from the centralised lobe. Due to this structure being in various stages of contraction, this morphology was not distinctly expressed in all larvae examined. However, the overall triangular shape of this structure was evident in all specimens. The morphology of the labium was constant in the different species under discussion and was therefore not considered a suitable diagnostic characteristic.

3.5.6. LABRUM (Figs. 61 and 63)

The labrum {*lbr*} of third instar larvae (Figs. 61 and 63) was significantly different from the simplified form noted in second instar larvae (Fig. 42). Due to this, no discussion will be entered into regarding the specific difference between the second - and third instar stage in the discussion of the third instar stages that will follow.

Lucilia cuprina (Figs. 61A and 63A)

The overall shape of the labrum (Figs. 61A and 63A) was that of an arrow. The dominant proximal portion of the labrum was wedged between the antennomaxillary lobes. This portion was roughly triangular in shape, and terminated between the antennomaxillary lobes in a broad, blunt, but pointed tip. The lateral portions of this structure were attached relatively perpendicular to the centrally located aspect of this structure. The medially located distal end was a narrow pointed structure terminating with a slight curvature.

Lucilia sericata (Figs. 61B and 63B)

The overall shape of the labrum (Figs. 61B and 63B) was that of an arrow. The dominant proximal portion of the labrum was wedged between the antennomaxillary lobes. This portion was roughly triangular in shape. The lateral flanges of the structure had rounded distal edges and its attachment angle to the central aspect of the structure was acute. The medially located distal end did not narrow into a pointed end as in *L. cuprina* larvae, but were overall box-shaped. The distal portion of the labrum margin formed two lobes with rounded edges.

Chrysomya chloropyga (Figs. 61C and 63C)

The proximal portion of the labrum was in the form of a smooth convex shape and was wedged between the antennomaxillary lobes (Figs. 61C and 63C). The lateral edges of the distal end of the labrum were indented, giving it a scalloped appearance. The lateral portions of the labrum gradually merged into central portion of the labrum forming two lobes with rounded edges (Fig. 63C).

Chrysomya marginalis (Figs. 61D and 63D)

The proximal portion of the labrum (Figs. 61D and 63D) was roughly box-shaped and was wedged between the antennomaxillary lobes. The lateral margins of the distal portion of the labrum were slightly serrated (Fig. 63D). These lateral portions of the labrum merged with its central portion at a relatively perpendicular angle (Figs. 61D and 63D). From here it dropped into the medially located distal end of the labrum (Figs. 61D and 63D). The free distal end had a small notch to it (Figs. 61D and 63D). The two tips of this portion were blunt and the edges were slightly serrated (Fig. 63D).

Chrysomya albiceps (Figs. 61E and 63E)

The overall shape of the labrum (Figs. 61E and 63E) was that of a moustache on a lip. The proximal margin of the labrum was demarcated by an acute recession at its midline and was wedged between the antennomaxillary lobes (Figs. 61E and 63E). The lateral flanges of the "moustache" narrowed to its ends (Figs. 61E and 63E). The distal-medial end of the labrum had a slight curvature to its margin (Figs. 61E and 63E). All edges of the labrum were smooth (Fig. 63E).

Calliphora vicina (Figs. 61F and 63F)

The proximal portion of the labrum (Figs. 61F and 63F) was roughly half-moon-shaped and was wedged between the antennomaxillary lobes. The lateral aspects of this structure curved into the central portion of the labrum (Fig. 63F). The relatively narrow distal portion of the labrum was sharp-tipped with a slight notch at its tip (Fig. 63F). All edges of the labrum were smooth (Fig. 63F).

Sarcophaga cruentata (Figs. 61G and 63G)

The tips of the mouth hooks were visible in all specimens examined (Fig. 63G). The overall shape of the labrum (Figs. 61G and 63G) was M-shaped. The edges of the distally projecting portion were jagged (Fig. 63G). This distally projecting portion was partially split (Figs. 61G and 63G).

The labrum as diagnostic characteristic

The structure of the labrum was unique for each of the species under discussion. It is therefore considered a strong diagnostic feature, as identification can be made utilising this feature alone.

To observe this structure, the larvae had to be cleaned thoroughly. Blow - and flesh fly larvae generally burrow into the food medium and the liquefied food medium has a tendency to get stuck in and around the preoral cavity, masking the features in its vicinity from view.

3.5.7. CEPHALOPHARYNGEAL SKELETON (Figs. 64 to 71)

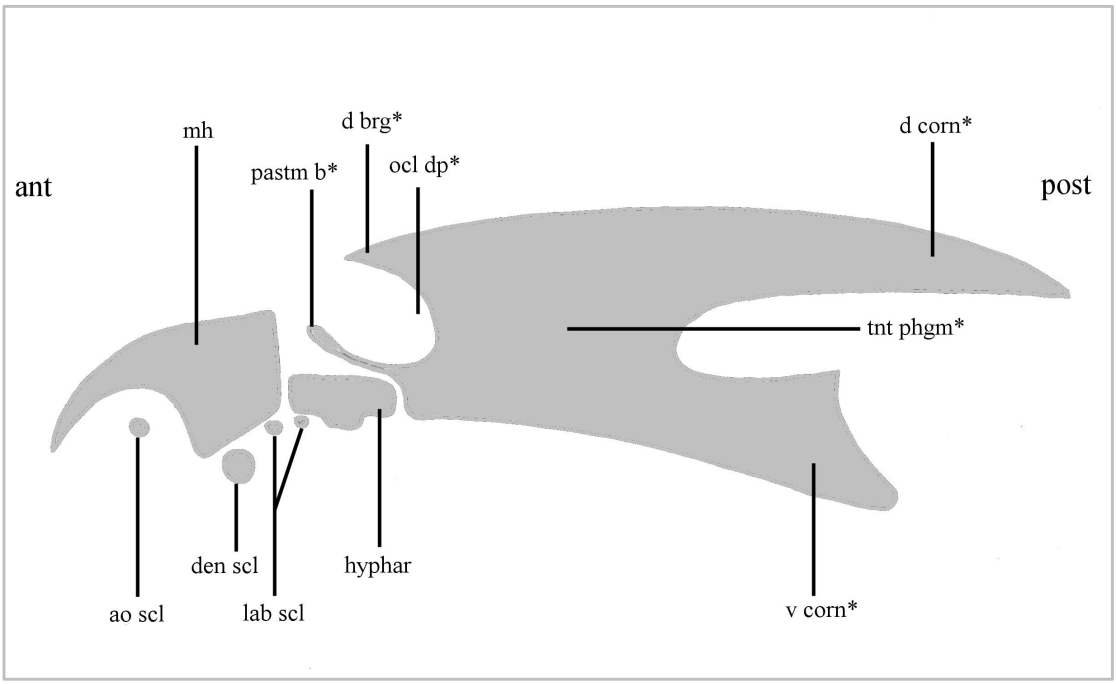


Fig. 64: Representation of the lateral aspect of a third instar cephalopharyngeal skeleton.
Legend: ant: anterior end; ao scl: accessory oral sclerite; den scl: dental sclerite; d brg*: dorsal bridge; d corn*: dorsal cornu; hyphar: hypopharyngeal sclerite; lab scl: labial sclerite; mh: mouth hook; ocl dp*: ocular depression; pastm b*: parastomal bar; post: posterior end; tnt phgm*: tentorial phragma; v corn*: ventral cornu. * = Elements of the tentoropharyngeal sclerite.

The cephalopharyngeal skeleton of third instar larvae (Fig. 64) was similar to that of second instar larvae (Fig. 43). As in second instar larvae, three major structures form the main body of the cephalopharyngeal skeleton. These structures were: the posterior

located tentoropharyngeal sclerite, the anterior located mouth hooks {*mh*} and the hypopharyngeal sclerite {*hyphar*} located between them. Similar to second instar larvae, the elements of the tentoropharyngeal sclerite are the dorsal - {*d corn*} and the ventral cornu {*v corn*}, ocular depression {*ocl dp*}, the dorsal bridge {*d brg*}, the tentorial phragma {*tnt phgm*} and the thin rod-like parastomal bar {*pastm b*} projecting above the hypopharyngeal sclerite. The juncture point between the anterio-ventral portion of the tentoropharyngeal sclerite and the hypopharyngeal sclerite is extremely narrow, to such an extent that these two sclerites appear to be fused when the entire cephalopharyngeal sclerite was viewed. Also similar to the second instar stage was the T-shape of the hypopharyngeal sclerite. The most obvious differences between second - and third instar larvae were found in the shape of the mouth hooks. Generally the hooks are more curved structures in second instar larvae, but this was not always the case in all species examined. The exact differences between second and third instar larvae regarding this structure will be discussed as part of the description of the different species. The smaller sclerites (the two labial {*lab scl*} and the dental sclerite {*den scl*}) were more clearly defined in third instar larvae than in second instar larvae. The accessory oral sclerite {*ao scl*}, was not present in all species examined. If present, it will be located below the hook section of the mouth hook. The accessory oral sclerite was not noted in any of the second instar larvae examined.

Lucilia cuprina (Fig. 65)

The dorsal cornu of the tentoropharyngeal sclerite was solid and its termination pattern was pointed (Fig. 65B). A well defined ocular depression was present. The parastomal bar was clearly visible (Figs. 65A and 65C). The hook portions of the mouth hooks were stout, robust structures (Figs. 65D and 65E). A small posterio-dorsal projection was observed at the base portion of the mouth hook (Fig. 65E). The level of sclerotisation of all elements of the mouth hooks was equal in most specimens examined, although a lighter sclerotised area was observed in the base section of the mouth hooks in some specimens (Figs. 65D and 65E). Easily discernable in all specimens was the strongly sclerotised dental sclerite (Figs. 65D and 65E). In contrast to this was the labial sclerites which were weakly sclerotised and not discernable in most specimens (Fig. 65D). The anterio-ventral margin of the base of the mouth hook curved slightly downwards around the dental sclerite (Fig. 65E). However, this

feature was not definitive in all specimens. Greenberg & Szyska (1984) presented an un-annotated drawing and offered no detailed description of the cephalopharyngeal skeleton of the specimens of *L. cuprina* examined by them. Therefore, although the major elements of the cephalopharyngeal skeleton of *L. cuprina* specimens examined during the present study could be confirmed to be similar to that of *L. cuprina* presented by Greenberg & Szyska (1984), similarities of the finer elements (minor sclerites) could not be confirmed. The most prominent difference between the second – and third instar cephalopharyngeal skeleton was noted regarding the mouth hooks which were more acutely curved in second instar larvae (Fig. 44).

Lucilia sericata (Fig. 66)

The dorsal cornu of the tentoropharyngeal sclerite was solid and its termination pattern was pointed (Fig. 66B). A well defined ocular depression was present (Figs. 66A and 66C). The parastomal bar was clearly visible (Figs. 66A and 66C). Long, slender hooks defined the mouth hooks (Figs. 66A and 66D). The curvature of the hooks was slight and the hooks appeared relatively straight along a large portion of their length (Figs. 66A and 66D). The dorsal, ventral and posterior margins of the base section of the mouth hooks were more sclerotised than the rest of the base and the hook (Fig. 66D). A small posterio-dorsal projection was noted at the base of the mouth hook (Fig. 66C). A well defined dental sclerite (Figs. 66A and 66D) was present and in the odd specimen a faintly sclerotised labial sclerite was observed obliquely below the hypopharyngeal sclerite. Only a dental sclerite and no labial sclerite were indicated in the drawings of the cephalopharyngeal skeleton of *L. sericata* presented by Prins (1982) and Trigo (2006). The mouth hooks were more robust and shorter in second instar *L. sericata* larvae (Fig. 45).

Chrysomya chloropyga (Fig. 67)

The dorsal cornu of the tentoropharyngeal sclerite was solid and its termination pattern was rounded (Figs. 67A and 67B). A well defined ocular depression was present (Figs. 67A and 67C). The parastomal bar was clearly visible (Figs. 67A and 67C). Sclerotisation of the mouth hook was not uniform, with the ventral, dorsal and posterior margins of the base section of the mouth hook slightly more sclerotised than the rest of the base and the hook section (Fig. 67D). The posterio-dorsal edge of the base of the mouth hook had a small projection to it (Fig. 67D). A clearly defined

dental sclerite was present (Figs. 67A and 67D). The presence of labial sclerites cannot be excluded since small lightly sclerotised structures were observed where these sclerites usually occur (Fig. 67D). In specimens of *C. chloropyga* presented by Zumpt (1965) and Prins (1982), only the dental sclerite was indicated. In the drawing of the cephalopharyngeal skeleton of *C. putoria* presented by Greenberg & Szyska (1984) two fully sclerotised labial sclerites were shown, in addition to the dental sclerite. Taking this into consideration, the presence of labial sclerites and / or the level of sclerotisation of these sclerites; these aspects are a possible distinguishing characteristic between *C. chloropyga* and *C. putoria*. The curvature of the mouth hooks was more distinguished in second instar larvae (Fig. 46) than in third instar larvae.

Chrysomya marginalis (Fig. 68)

The dorsal cornu of the tentoropharyngeal sclerite was solid and its termination pattern was rounded (Fig. 68B). A well defined ocular depression was present (Figs. 68A and 68C). The parastomal bar was clearly visible (Figs. 68A and 68C). The mouth hooks were robust structures (Fig. 68D). Sclerotisation of the mouth hook was not uniform with the dorsal, ventral and posterior margins of the mouth hook bases heavier sclerotised than the rest of the mouth hooks (Fig. 68D). The hook portion of the mouth hook was large in comparisons to its base portion (Figs. 68A and 68D). In the odd specimen, a small projection was noted on the posterio-dorsal edge of the base of the mouth hooks. A well-defined dental sclerite, an accessory oral sclerite and two lightly sclerotised labial sclerites were present (Fig. 68D). Prins (1982) also indicated all four of the smaller sclerites in the drawing of the cephalopharyngeal skeleton of *C. marginalis* presented by him. The most notable difference between second - and third instar larvae was that the mouth hooks were acutely angled and the absence of an accessory oral sclerite in second instar larvae (Fig. 47).

Chrysomya albiceps (Fig. 69)

The dorsal cornu of the tentoropharyngeal sclerite was solid and its termination pattern was rounded (Figs. 69A and 69B). Due to the large size of the hypopharyngeal sclerite, the space the ocular depression occupied was severely restricted (Figs. 69A, 69C and 69D). The parastomal bar was pushed dorsally to the point of almost touching the dorsal bridge (Fig. 69C). Furthermore, the parastomal bar was in such

close proximity to the hypopharyngeal sclerite that is was not visible as a separate structure when the whole cephalopharyngeal skeleton was viewed (Fig. 69A). The parastomal bar was only discernable as a separate structure under high magnification with careful focussing and use of contrast (Fig. 69C). De Carvalho Queiroz *et al*. (1997) did not indicate the parastomal bar in the drawing presented of *C. albiceps*. Similarly, Liu & Greenberg (1989) also did not indicate a parastomal bar in the drawing presented of *C. rufifacies*. The dorsal bridge was extended to the anterior, to the point of almost touching the posterio-dorsal aspect of the mouth hooks (Fig. 69A and 69C). Sclerotisation of the mouth hook was not uniform with the dorsal, ventral and posterior margins of the base of the mouth hook slightly darker than the rest of the mouth hook (Fig. 69D). A projection was noted at the posterio-dorsal edge of the base of the mouth hook and a smaller projection was also noted on its anterio-ventral margin in some specimens (Fig. 69D). A lightly sclerotised dental sclerite and two labial sclerites were present (Fig. 69D). De Carvalho Queiroz *et al*. (1997) did not indicate a second labial sclerite in their drawing of *C. albiceps*, possibly due to the lack of individualisation of the second sclerite. Liu & Greenberg (1989) only indicated one of the smaller sclerites (possibly a dental sclerite) in the drawing of *C. rufifacies*. This is a possible difference between *C. rufifacies* and *C. albiceps*. The most noticeable difference between second instar - and third instar larvae was that the parastomal bar and hypopharyngeal sclerites were still discernable as separate structures in second instar larvae (Fig. 48) and the hypopharyngeal sclerite was not occupying such a extensive space as in third instar larvae.

Calliphora vicina (Fig. 70)

The dorsal cornu of the tentoropharyngeal sclerite was solid and its termination pattern was ill defined in the specimens examined (Fig. 70B). A well defined ocular depression was present (Figs. 70A and 70C). The parastomal bar was visible (Figs. 70A and 70C). The level of sclerotisation of the base and hook sections of the mouth hooks was the same (Fig. 70D), but in some specimens a lightly sclerotised lumen was observed in the base section. A well-defined projection was noted on the posterio-dorsal edge of the base of the mouth hook (Fig. 70D). The hooks were small in relation to the base of the mouth hook (Fig. 70D). In the micrograph of the cephalopharyngeal skeleton of *C. vicina* presented by Trigo (2006) the hook of the mouth hooks appeared more robust than that of the specimen of *C. vicina* examined

during the course of this study. A major difference between second – and third instar larvae were found in the mouth hooks which were stout and acutely angled in second instar larvae (Fig. 49). Well-defined dental and accessory oral sclerites and in some specimens small, very faintly sclerotised labial sclerites were seen (Figs. 70A and 70D). Similar to the findings of the present study was that of Trigo (2006) on the cephalopharyngeal skeleton of *C. vicina* where only an accessory oral sclerite and a dental sclerite was indicated. No labial sclerites were indicated by Trigo (2006) for *C. vicina*, confirming the results of the present study where these sclerites were observed in only a few specimens by using very fine focussing and the use of contrast. The drawing of the cephalopharyngeal skeleton of the closely related *C. croceipalpis* larvae presented by Prins (1982) was similar to that of *C. vicina* examined during the course of the current study. Another major difference between second – and third instar larvae was that no accessory oral sclerite was present in second instar *C. vicina* larvae (Fig. 49).

Sarcophaga cruentata (Fig. 71)

The dorsal cornu of the tentoropharyngeal sclerite was split in two with the dorsal section of this split shorter than the ventral section (Figs. 71A and 71B). A split dorsal cornu appeared to be common in Sarcophagidae since most of the species studied by Cantrell (1981) had split dorsal cornu. The drawing of *Parasarcophaga crassipalpis* presented by Cantrell (1981) contained a very large lumen in the dorsal cornu, but no split of the dorsal cornu was indicated in that species. Zumpt (1965) also indicated a split dorsal cornu for *S. haemorrhoidalis*. Where the dorsal cornu was split, the dorsal section of the cornu was shorter than the ventral portion in all the species studied by Zumpt (1965) and Cantrell (1981). Observations made during the current study showed that the ventral section of the dorsal cornu emerged as a relatively thin structure from the tentorial phragma (Fig. 71B). It flared out to become broader halfway through its length to gradually narrow again into a pointed apex (Fig. 71B). Although the dorsal cornu was also split in second instar larvae (Fig. 50A), this aspect was more delicately structured in second instar larvae than in third instar larvae. An ocular depression was not noticed in the specimens examined (Figs. 71A and 71C). The parastomal bar was clearly visible (Figs. 71A and 71C), whereas it was not as clearly visible in second instar larvae (Fig. 50). The base of the mouth hook was more sclerotised than the hook section (Fig. 71D). A well-defined posterio-dorsal projection

of the posterior margin of the base was noted (Fig. 71D). The dorsal margin of the base of the mouth hook exhibited a slightly convex curvature (Fig. 71D). Long and slender hooks were typical of this species (Figs. 71A and 71D). The hooks gradually curved downwards (Fig. 71D). One of the major differences between second instar - and third instar larvae was that the base was more rectangular in second instar larvae (Fig. 50). The labial sclerite below the hypopharyngeal sclerite was more strongly sclerotised than the labial sclerite below the posterior margin of the base of the mouth hook and the dental sclerite (Figs. 71A and 71D).

The cephalopharyngeal skeleton as diagnostic characteristic

The different species forming part of the current study could be identified using only the characteristics of the cephalopharyngeal skeleton.

Sarcophaga cruentata was distinguished from the other species based on the split dorsal cornu (Figs. 71A and 71B) compared to the solid dorsal cornu seen in the other species under investigation.

The parastomal bar was clearly visible in all the species, except in *C. albiceps*. In this species the parastomal bar was weakly individualised from the large hypopharyngeal sclerite (Figs. 69A and 69C). Furthermore, this was the only species where the dorsal bridge was in close proximity to the posterio-dorsal margin of the mouth hooks (Figs. 69A and 69C).

Chrysomya marginalis (Figs. 68A and 68D) and *C. vicina* (Figs. 70A and 70D) were the only two species with an accessory oral sclerite. The two species were distinguished from each other based on the size of the hooks and the shape of the base of the mouth hook. In *C. marginalis* the hooks were robust in relation to its base portion (Fig. 68D), while these were small and slender in relation to its base portion in *C. vicina* (Fig. 70D). Furthermore, the prominent posterior-dorsal projection of the base of the mouth hook (Fig. 70D) distinguished *C. vicina* from all the other species under investigation.

A specimen without a split dorsal cornu, an indiscernible parastomal bar or an accessory oral sclerite was either, *L. sericata*, *L. cuprina* or *C. chloropyga*. *Lucilia*

cuprina and *L. sericata* were distinguished from *C. chloropyga* based on the termination pattern of the dorsal cornu. The termination pattern of the dorsal cornu was rounded in *C. chloropyga* (Fig. 67B) and pointed in the other two species (Figs. 65B and 66B). Long, slender, gradually curving hooks were typical for *L. sericata* (Fig. 66D) while more robust hooks were typical of *L. cuprina* (Fig. 65D). Furthermore, the anterio-ventral portion of the base of the mouth hook slightly curved around the dental sclerite in most specimens of *L. cuprina*, while this portion exhibited no curvature in *L. sericata*.

It was difficult to determine intraspecific variation for the species under discussion. It was equally difficult to identify differences between the closely related *C. chloropyga* and *C. putoria*, as well as the closely related *C. albiceps* and *C. rufifacies*. This could mainly be attributed to the fact that the bulk of previous studies contained un-annotated drawings of cephalopharyngeal skeletons and lacked detailed descriptions.

3.5.8. ANTERIOR SPIRACLES OF THE RESPIRATORY SYSTEM (Fig. 72)

The overall morphology of the anterior spiracles of third instar calliphorid larvae (Figs. 72A to 72F) was similar to that of second instar calliphorid larvae (Figs. 51A to 51F). *Sarcophaga cruentata* third instar larvae (Fig. 72G) differed from third instar calliphorids (Figs. 72A to 72F) and from its second instar stage (Fig. 51G) in that not all the buttons were aligned. Due to this, together with fact that most specimens were not orientated perfectly, the maximum count for this species could not be established with certainty. The number of branches on the anterior spiracles for the different species investigated is provided in Table 9.

The anterior spiracles as diagnostic feature

The overlapping in the ranges of the number of branches of the anterior spiracles in the different species, as well as the intraspecific variation found with other populations, rendered this feature unsuitable for identification purposes. Kitching (1976a) expressed the same sentiment; i.e. that the use of spiracles is inadequate if used in isolation. The ranges observed for second instar larvae differed somewhat from that of third instar larvae. This difference followed no general pattern.

Table 9: Comparison of the number of branches on the anterior spiracles of third instar calliphorid and sarcophagid larvae for the species investigated (*) with that reported in other publications.

Species	*Number of branches on the anterior spiracles*
Lucilia cuprina	7 – 10* (Fig.72A)
	5 – 6 (Greenberg & Szyska 1984)
Lucilia sericata	8 – 9* (Fig.72B)
	7 – 8 (Zumpt 1965)
Chrysomya chloropyga	9 – 12* (Fig.72C)
	10 – 12 (Zumpt 1965)
Chrysomya putoria	10 – 12 (Greenberg & Szyska 1984)
Chrysomya marginalis	12 – 14* (Fig.72D)
	11 – 15 (Prins 1982)
Chrysomya albiceps	10 – 12* (Fig.72E)
	11 – 12 (Zumpt 1965)
Calliphora vicina	8 – 10* (Fig.72F)
	7 – 10 (Zumpt 1965)
Calliphora croceipalpis	8 – 9 (Prins 1982)
Sarcophaga cruentata	13 - 16 (but could be more)* (Fig.72G)
	11 – 13 (Aspoas 1991)

3.5.9. POSTERIOR SPIRACLES OF THE RESPIRATORY SYSTEM (Figs. 73 to 76 and 80)

The major difference between second – and third instar larvae is that three spiracular openings are present in third instar larvae (Fig. 73) opposed to the two present in second instar larvae (Fig. 52). The margin of a posterior spiracle is stabilised by a sclerotised ring called the peritreme {*pt*}. The margins of the spiracular openings, the rimae {*rm*}, are also sclerotised. Located off-centre on the margin of the peritreme was the button {*bt*} (ecdysial scar). Associated to the rimae are the spiracular hair clusters.

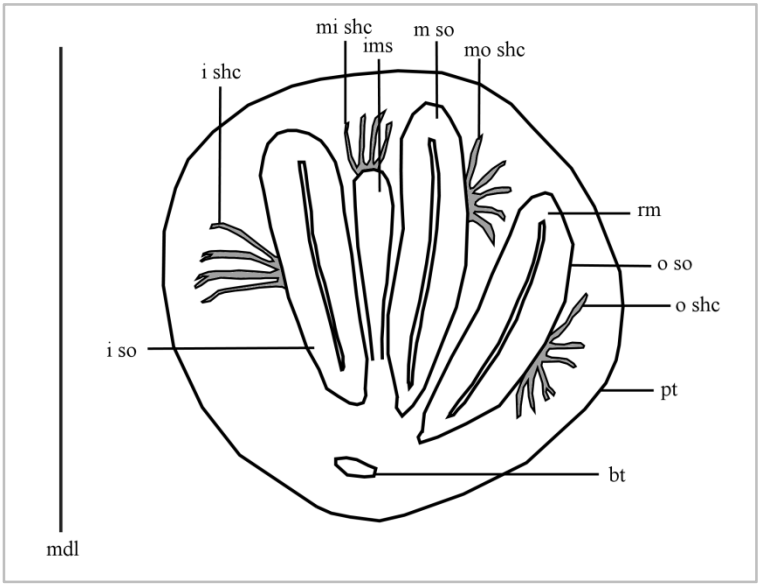


Fig. 73: Representation of a posterior spiracle of a third instar larva. Legend: bt: button; ims: intermediate structure; i shc: inner spiracular hair cluster; i so: inner spiracular opening; mdl: midline of the spiracular plate; mi shc: middle-inner spiracular hair cluster; mo shc: middle-outer spiracular hair cluster; m so: middle spiracular opening; o shc: outer spiracular hair cluster; o so: outer spiracular opening; pt: peritreme; rm: rima.

The posterior spiracles were assessed by light - and scanning electron microscopy. An overall assessment was made employing both of these media regarding (i) the orientation of the buttons of the two posterior spiracles in relation to each other on the spiracular disc, (ii) the shape of the posterior spiracles and (iii) the size of the spiracular openings. The spiracular opening size was defined as being large where a spiracular opening cannot be fitted between adjacent pairs of spiracular openings. It was defined as narrow when a spiracular opening could be fitted between adjacent pairs of spiracular openings. Examination by light microscopy primarily revealed those aspects concerning sclerotisation of the posterior spiracles that could not be evaluated by scanning electron microscopy. One of the first aspects considered was whether the peritreme completely enclosed the spiracles or not. Another aspect considered here was the thickness (a qualitative measure) and the pattern of sclerotisation of the peritreme. Examination by scanning electron microscopy, revealed the details of the posterior spiracles that could not be assessed by light microscopy. One of these aspects involved the area between the dorsal edge of the spiracular plate and the middle spiracular openings, here forth referred to as the dorsal spiracular plate area. This area was considered to be infringed upon if the rimae of the nearest spiracular opening could not be fitted into this area. Another feature not

visible by light microscopy was the four clusters of spiracular hairs (Fig. 73) associated with the spiracular openings. The inner spiracular hair cluster {*i shc*} was associated with the outer margin of the inner spiracular opening {*i so*}, the middle-inner spiracular hair cluster {*mi shc*} was located between the inner and middle spiracular openings, attached to the intermediate structure {*ims*}, the middle-outer spiracular hair cluster {*mo shc*} was associated to the outer margin of the middle spiracular opening {*m so*} and the outer spiracular hair cluster {*o shc*} was associated to the outer margin of the outer spiracular opening {*o so*}. The position of the spiracular hair in relation to the spiracular openings, the attachment area it occupied and the extent of the branching of the spiracular hairs were considered for diagnostic purposes.

Lucilia cuprina (Figs. 74A, 75A, 76A and 80A)

The buttons of the two posterior spiracles were orientated to a ventro-medial position (Figs. 76A and 80A). The posterior spiracles were a basic O-shape and contained large spiracular openings (Figs. 74A and 75A). The peritreme (Fig. 75A) formed a complete ring and was of substantial thickness. It slightly projected in between the spiracular openings (Fig. 75A). The peritreme of the specimens of *L. cuprina* presented by Greenberg & Szyska (1984) was similar to that of the specimens of *L. cuprina* examined during the present study, i.e. a closed structure with the peritreme arching in between the spiracular openings. However, the subjective nature of using criteria regarding the thickness of the peritreme was illustrated when considering the drawing of the peritreme of *L. cuprina* presented by Greenberg & Szyska (1984), where the peritreme appeared to be thin walled.

The dorsal spiracular plate area was slightly infringed upon by the spiracular openings (Fig. 74A). The spiracular hairs of the middle-inner spiracular hair cluster were compacted into a solid structure in most specimens examined (Fig. 74A). In the odd specimen examined, the middle-inner spiracular hair cluster was split into two at its apex. The inner spiracular hair cluster and the outer spiracular hair cluster were attached to a small area in a position at the midline of their respective spiracular openings (Fig. 74A). The middle-outer spiracular hair cluster was attached to a small area in a position at the upper third of its spiracular opening (Fig. 74A).

Lucilia sericata (Figs. 74B, 75B, 76B and 80B)

The buttons of the two posterior spiracles were orientated to a ventro-medial position (Figs. 76B and 80B). The posterior spiracles were a basic O-shape and contained large spiracular openings (Figs. 74B and 75B). The peritreme was thin-walled and formed a complete ring (Fig. 75B). The peritreme gently arched in between the spiracular openings, with a pronounced projection between the middle - and outer spiracular openings (Fig. 75B); an observation also noted as such by Zumpt (1965) for *L. sericata*. However, Erzinçlioglu (1985) noted that many *L. sericata* specimens examined by him lacked this projection.

The dorsal spiracular plate area was not infringed upon by the spiracular openings (Fig. 74B). Most aspects of the spiracular hair clusters were comparable to that of *L. cuprina*. The spiracular hairs of the middle-inner spiracular hair cluster were compacted into a solid structure (Fig. 74B). Spiracular hair of the inner - and outer spiracular hair clusters were attached to the middle portions of their respective spiracular openings (Fig. 74B). The attachment area of the spiracular hair cluster was slightly larger at the outer spiracular opening than at the inner spiracular opening (Fig. 74B). Spiracular hair of the middle-outer spiracular hair cluster was attached to a small area in a position on the upper third portion of the spiracular opening (Fig. 74B). The attachment area of the middle-outer spiracular hair cluster was similar to that of the inner spiracular hair cluster (Fig. 74B).

Chrysomya chloropyga (Figs. 74C, 75C, 76C and 80C)

The buttons of the two posterior spiracles were orientated to a ventro-medial position (Figs. 76C and 80C). The posterior spiracles were a basic O-shape and contained large spiracular openings (Figs. 74C and 75C). Large spiracular openings were also illustrated in the drawings presented by Prins (1982) for *C. chloropyga* and by Greenberg & Szyska (1984) for *C. putoria*. In the present study the peritreme was found to be substantial and formed an incomplete ring (Fig. 75C). The projection of the inner margin of the peritreme between the inner and middle spiracular openings was knob-like and the projection between the middle and outer spiracular openings was pointed (Fig. 75C). Prins (1982) also illustrated projections between the spiracular openings of the posterior spiracles for *C. chloropyga*. No inner projections

of the peritreme between the spiracular openings were illustrated for the specimen of *C. putoria* examined by Greenberg & Szyska (1984). Caution was exercised to identify this as a possible distinguishing characteristic for the two species, as Greenberg & Szyska (1984) might not have evaluated the projections of the peritreme specifically.

The dorsal spiracular plate area was infringed upon by the spiracular openings (Fig. 74C). Spiracular hair (Fig. 74C) of the inner spiracular hair cluster was associated with a small portion of the top third of its spiracular opening. The middle-inner spiracular hair cluster was associated with the intermediate structure (Fig. 74C). The middle-outer spiracular hair cluster was associated with a small portion on the upper third portion of its spiracular opening whereas the outer spiracular hair cluster was associated with a large portion of its spiracular opening spiracular (Fig. 74C). Spiracular hair of all clusters was typically arranged in a branching fan-like pattern.

Chrysomya marginalis (Figs. 74D, 74H, 75D, 76D and 80D)

The buttons of the two posterior spiracles were orientated in a ventro-medial position (Figs. 76D and 80D). The posterior spiracles were a basic O-shape and contained large spiracular openings (Figs. 74D and 75D). A thin walled peritreme forming an incomplete ring was present (Fig. 75D). The peritreme did not arch-in significantly between the spiracular openings (Fig. 75D). Prins (1982) reported similar findings for most aspects of the posterior spiracles, but illustrated slight arching-in of the peritreme between the spiracular openings.

Due to the somewhat sunken-in nature of the spiracular plate, the whole structure of posterior spiracle of third instar larvae could not be observed. During pupariation, the posterior spiracles of third instar larvae were kept in-tact in puparia. The benefit of viewing these structures in the puparium was that the spiracular plate was exposed in the puparium, which enabled a clear view of the posterior spiracles. The dorsal spiracular plate area was slightly infringed upon by the spiracular openings (Figs. 74D and 74H). The extent of the attachment areas and the positions of the spiracular hair clusters (Figs. 74D and 74H) were as follow: (i) the inner spiracular hair cluster was attached to a small area of the upper third of the spiracular opening, (ii) the middle-inner spiracular hair cluster was attached to the intermediate structure, (iii) the

middle-outer spiracular hair cluster was attached to a small area of the upper third of its spiracular opening and (iv) the outer spiracular hair cluster was attached to a large area, occupying about a half of the spiracular opening margin, to the middle of its spiracular opening. The spiracular hair branched extensively into tertiary branches (Figs. 74D and 74H).

Chrysomya albiceps (Figs. 74E, 75E, 76E and 80E)

The buttons of the two posterior spiracles were orientated in a ventro-medial position (Figs. 76E and 80E). The posterior spiracles were a basic O-shape and contained large spiracular openings (Figs. 74E and 75E). A well defined peritreme, with thick margins, was present (Fig. 75E). The inner margin of the peritreme formed small projections between the spiracular openings (Fig. 75E). Furthermore, the peritreme bifurcated around the button, cupping it, but not completely enclosing it (Fig. 75E). Zumpt (1965) and De Carvalho Queiroz *et al*. (1997) reported similar findings regarding the thickness of the peritreme and its projections for the specimens of *C. albiceps* examined by them. However, only Zumpt (1965) showed the bifurcation of the peritreme around the button.

The dorsal spiracular plate area was infringed upon by the spiracular openings (Fig. 74E). The extent of the attachment areas and the positions of the spiracular hair clusters (Fig. 74E) were as follow: (i) the outer – and inner spiracular hair clusters were attached to a small area to the midline of its respective spiracular openings, (ii) the middle-outer spiracular hair cluster was attached to a small area at a position about a third from the top of its spiracular opening and (iii) the middle-inner spiracular hair cluster was attached to the intermediate structure. The hair of the clusters branched extensively into tertiary branches (Fig. 74E).

Calliphora vicina (Figs. 74F, 75F, 76F and 80F)

The two buttons of the posterior spiracles faced each other, pointing to the midline of the spiracular plate (Figs. 76F and 80F). This characteristic distinguishes this species from the rest of the species under discussion where the buttons of the posterior spiracles pointed to a ventro-medial position. The posterior spiracles were a basic O-shape and contained large spiracular openings (Figs. 74F and 75F). The wall of the peritreme was substantial and formed a complete ring (Fig. 75F). The peritreme

slightly arched in between the spiracular openings (Fig. 75F). All of these aspects evaluated by light microscopy were similar to that presented by Prins (1982) for *C. croceipalpis*.

The dorsal spiracular plate area was not infringed upon by the spiracular openings (Fig. 74F). The extent of the attachment areas and the positions of the spiracular hair clusters (Fig. 74F) were as follow: (i) the inner - and outer spiracular hair clusters were attached to small areas to the middle of their respective spiracular openings, (ii) the middle-inner spiracular hair cluster was attached to the intermediate structure and (ii) the middle-outer spiracular hair cluster was attached to a small area of the top third of its spiracular opening. The branching of the spiracular hair was extensive, forming tertiary branches (Fig. 74F).

Sarcophaga cruentata (Figs. 74G, 75G, 76G and 80G)

The buttons of the two posterior spiracles were orientated in a ventro-medial position (Figs. 76G and 80G). The posterior spiracles were ear-shaped structures (Figs. 74G and 75G); a characteristic that distinguishes it from the other species forming part of the current study. The spiracular openings were thin, elongated structures (Figs. 74G and 75G). A thin walled peritreme, forming an open ring, was present (Fig. 75G). The peritreme was uniform in thickness, with no projections to its margins (Fig. 75G). A tear-shaped protrusion was formed at the ventro-medial margin of the posterior spiracle (Figs. 74G and 75G). The drawing of the posterior spiracles presented by Zumpt (1965) for *S. haemorrhoidalis* was similar to that observed during the current study with regard to the ear-shaped form of the posterior spiracles, the thin elongated spiracular openings and the open peritreme. Zumpt (1965) illustrated distinct projections of the inner margin of the peritreme, which could not be confirmed for the specimens examined during the current study. The distinct tear-shaped projection at the ventro-medial margin of the posterior spiracle as observed in the specimens examined during the course of the present study was not illustrated by Zumpt (1965). By examining a vast range of sarcophagids, Cantrell (1981) established those characteristics common in the subfamily Sarcophaginae. Common features were (i) the ear-shape of the posterior spiracles, (ii) the thin elongated spiracular openings and (iii) the open structure of the peritreme. Taking into consideration those species presented by Cantrell (1981), possible features that can be investigated as

distinguishing characteristics for Sarcophaginae were (i) projections to the inner margin of the peritreme and (ii) the distinct tear-shaped projection to the ventro-medial margin of the posterior spiracle.

The dorsal spiracular plate area was not infringed upon by the spiracular openings (Fig. 74G). The inner -, middle-outer - and outer spiracular hair clusters were attached to small areas to the middle of their respective spiracular openings (Fig. 74G). The middle-inner spiracular hair cluster was attached to the intermediate structure (Fig. 74G). The inner spiracular hair cluster was made up of four primary branches of which two of these branched into tertiary branches, one into secondary branches and one with only the primary trunk. The middle-inner spiracular hair cluster had one primary trunk that branched into a few secondary branches. The middle-outer spiracular hair cluster had three branches with the outer two branches branching into secondary trunks and the middle one just with a primary trunk. The outer spiracular hair cluster had five branches with the ventral three with just the primary trunks and the dorsal two with secondary branches. Aspoas (1991) showed the spiracular hairs to be distinct for the different species of Sarcophagidae examined by her. A comparison could not be made between the results obtained by Aspoas (1991) and that obtained during the course of the present study, since Aspoas (1991) did not indicate to what spiracular opening the spiracular hair clusters belonged to that she described. Only the inner spiracular hair cluster exhibited a similar branching pattern to that presented by Aspoas (1991) for *S. cruentata*. However, the branching patterns of the different spiracular hair clusters examined during the present study were not similar to each other. The other spiracular hair clusters of *S. cruentata* larvae examined during the current study, revealed similarities to the branching patterns established for the species other than *S. cruentata* examined by Aspoas (1991).

The posterior spiracles as diagnostic feature

Based on light microscopy evaluation, two groups were identified (i) those with a complete peritreme (*L. cuprina, L. sericata* and *C. vicina*) and (ii) those with an incomplete peritreme (*C. chloropyga, C. marginalis, C. albiceps* and *S. cruentata*). In the grouping of those species with a complete peritreme, *L. sericata* was uniquely identified due to its relatively thin peritreme but more so due to the significance of the projection of the peritreme plunging in between the middle and outer spiracular

openings (Fig. 75B). *Calliphora vicina* (Fig. 75F) and *L. cuprina* (Fig. 75A) had relatively thicker peritremes than that of *L. sericata* (Fig. 75B). Although it appeared as if the peritreme of *C. vicina* was more substantial than that of *L. cuprina*, caution was exercised to utilise qualitative measures to base the diagnostic on. The orientation of the posterior spiracles was considered a more appropriate feature to separate *L. cuprina* (Figs. 74A and 80A) from *C. vicina*. *Calliphora vicina* was unique in this regard from all of the other species examined, in that the buttons of its posterior spiracles were orientated more to the midline of the spiracular plate (Figs. 74F and 80F) than the ventro-medial position these exhibited in the other species investigated (Figs. 80A to 80E and 80G). Those with an incomplete peritreme were initially separated based on the size of the spiracular openings, with *S. cruentata* being uniquely identified by its thin, elongated spiracular openings (Fig. 75G). An additional feature unique to *S. cruentata* was the distinct ear-shaped posterior spiracles (Fig. 75G), opposed to the basically O-shaped spiracles noted in the other species forming part of the current study. The other species were separated from each other based on the thickness of the peritreme, with *C. marginalis* being isolated from the other two due to its thin peritreme with no distinct projections to the peritreme (Fig. 75D). The other two species with their substantial peritreme were separated from each other due to the projections of the peritreme. In *C. albiceps* the peritreme cupped around the button (Fig. 75E), and although the opening in the peritreme was slight in this area, the peritreme was not a closed ring. Furthermore, the peritreme was only slightly arching in between the spiracular opening in *C. albiceps*. In *C. chloropyga*, the projections between the spiracular openings were unique, with a knob-like projection between the inner - and middle spiracular openings and sharp-pointed projection between the middle - and outer spiracular openings (Fig. 75C).

Based on scanning electron microscopy, the orientation of the spiracles was used as an initial defining characteristic. The buttons of the posterior spiracles faced to a more medial position in *C. vicina* (Fig. 80F), separating this species from the others under discussion, where the buttons of the posterior spiracles faced in a ventro-medially position (Figs. 80A to 80E and 80G). The shape of the posterior spiracles allowed *S. cruentata* (Fig. 74G) with its ear-shaped spiracles to be separated from the others with their basically O-shaped posterior spiracles. The characteristics of the spiracular hair were subsequently used to separate the rest of the species from each other. *Lucilia*

cuprina and *L. sericata* was unique due to the middle-inner spiracular hair cluster generally being reduced to a single trunk (Figs. 74A and 74B respectively), opposed to the branched pattern observed in the other species under investigation. The two *Lucilia* species were distinguished from each other based on the infringement of the middle spiracular opening into the dorsal spiracular plate area. This area was infringed upon in *L. cuprina* (Fig. 74A) and not so in *L. sericata* (Fig. 74B). In the remainder of the species where the middle-inner spiracular hair cluster was branched, a separation was achieved on the basis of the attachment area extent of the outer spiracular hair clusters. The attachment area was small in *C. albiceps* (Fig. 74E), but relative large in comparison to the other spiracular hair clusters for *C. chloropyga* (Fig. 74C) and *C. marginalis* (Fig. 74D). These two species were distinguished from each other based on the infringement on the dorsal spiracular plate area. This area was totally infringed on in *C. chloropyga* (Fig. 74C) and not to such an extent in *C. marginalis* (Figs. 74D and 74H).

In conclusion, observations made from both light- and scanning electron microscopy allowed for a full separation among the species under discussion.

Intraspecific variation for the species under discussion, as well as differences between the closely related *C. chloropyga* and *C. putoria*, as well as the closely related *C. albiceps* and *C. rufifacies* were difficult to determine. This could mainly be attributed to the fact that the features used during the current study to distinguish between the different species were not evaluated as such in most other publications.

Qualitative measures like the volume of the spiracular openings and the thickness of the peritreme were utilised to separate the species concerned in groups. However, these characteristics were not used singularly as definitive characteristics to base identification on.

3.5.10. SPINES (Figs. 77 to 79)

Lucilia cuprina (Figs. 77A, 78A and 79A)

A combination of single- and multi-pointed spines was present in the spine band area (Fig. 77A). The multi-pointed spines had a comb-like appearance, with a broad base,

with two to three tips which were split to almost the base of the comb-like structure. This was in contrast to the situation found for second instar larvae where only single-pointed spines were found (Fig. 54A). The spine bands were located on the anterior margin of segments. Spine bands were complete from segments 2 to 11; a bit more extensive than in second instar larvae. A longitudinal groove was etched in the spine-free zone of the creeping welt (Fig. 78A). The fusiform area was not distinct and was furnished with only a few spines (Fig. 79A). The fusiform area was similarly weakly defined in second instar larvae (Fig. 55A), but the longitudinal groove was more developed in third instar larvae (Fig. 78A) than what it was in second instar larvae (Fig. 56A).

Lucilia sericata (Figs. 77B, 78B and 79B)

A combination of single- and multi-pointed spines was present in the spine band area (Fig. 77B). Similar to *L. cuprina*, the multi-pointed spines were arranged as comb-like structures. However, unlike in *L. cuprina*, three to more tips were noted on this comb-like structure. This aspect of the spines was more developed than in second instar larvae, where only single-pointed spines were found (Fig. 54B). The spine bands were located on the anterior margin of the segments. The spine band was complete from between segments 2 to 11. Only the creeping welt situated between segments 5 and 6 had a weakly defined longitudinal groove, while the creeping welt between the other segments contained no longitudinal groove (Fig. 78B). The fusiform area (Fig. 79B) was weakly defined with only a few spines noted on it. The fusiform area and the creeping welt were comparable to that found for second instar larvae (Figs. 55B and 56B).

Chrysomya chloropyga (Figs. 77C, 78C and 79C)

A variety of single- and multi-pointed (two-, three- and four-pointed) spines was present in the spine band area (Fig. 77C). Some of the multi-pointed spines were similar to that found for *L. cuprina* and *L. sericata*, i.e. spines were arranged as comb-like structures, with the tips split severely to almost its base. The other multi-pointed spines were more elongated structures, compared to the broad-based comb-like structures, with only slightly notched distal tips. The spine bands were located on the anterior, as well as the posterior margins of the segments with the anterior band being more prominent. This was in contrast to the situation found in second instar *C.*

chloropyga larvae where spines were only found on the anterior margins of segments. Small, single-pointed spines were located on the posterior margin of segments and the large, mainly multi-pointed spines were located on the anterior margin of segments. The spine band was complete on all the larval body segments, although fewer spines were found on the dorsal and lateral aspects from segment 8 onwards. This distribution of the spines was also reported as such by Prins (1982) for *C. chloropyga* larvae and by Greenberg & Szyska (1984) for *C. putoria* larvae. The creeping welt was typical, with no longitudinal groove present in the spine-free zone of the creeping welt (Fig. 78C). The fusiform area was prominent with a good coverage of spines (Fig. 79C). The creeping welt was similar to that of second instar larvae (Fig. 56C), but the fusiform area of third instar larvae (Fig. 79C) was better developed than that of second instar larvae (Fig. 55C).

Chrysomya marginalis (Figs. 77D, 78D and 79D)

Spines were single-pointed, with rounded, blunt tips (Fig. 77D). The spine band areas were located on the anterior margin of segments. The spine band was complete on possibly all body segments, although this could only be confirmed for segments 2 to 10. The creeping welt was typical, with no longitudinal groove present in the spine-free zone of the creeping welt (Fig. 78D). The fusiform area was prominent with a good coverage of spines (Fig. 79D). All aspects pertaining to spines were comparable to the second instar stage (Figs. 54D, 54E, 55D and 56D).

Chrysomya albiceps (Figs. 77E, 78E and 79E)

Single-pointed - as well as double-pointed spines were present in the spine bands (Fig. 77E). The notch in the double-pointed spines was slight (Fig. 77E), in contrast to the more pronounced split noticed in the tips of spines of second instar larvae (Fig. 54F). The single-pointed spines had rounded (blunt) tips. The spine band areas were located on the anterior margins of the segments. The completeness of the spine band could not be ascertained for most segments due to the intersegmental areas not being visible. The creeping welt (Fig. 78E) in *C. albiceps* larvae differed from the typical structure seen in the other species. This is due to the excessive folding of the intersegmental area. The creeping welt appeared to be in the form of a ridge and the anterior located spines of this area appeared to be perched on top on this raised area. The fusiform area (Fig. 79E) was not observed on all segments due to excessive folding of the

intersegmental areas. However, where it was visible, it was prominent (Fig. 79E). The creeping welt and fusiform area was comparable to that found for second instar larvae (Figs. 56E and 55E).

Calliphora vicina (Figs. 77F, 78F and 79F)

The sharp-tipped spines were mostly single pointed with a few being double pointed (Fig. 77F). Spine bands were located on the anterior margins as well as the posterior margins of some segments. The creeping welt was typical, with no longitudinal groove present in the spine-free zone of the creeping welt (Fig. 78F). The lateral fusiform area could not be clearly ascertained in the specimens examined (Fig. 79F). All aspects pertaining to spines were comparable to that of second instar larvae (Figs. 54G, 55F and 56F).

Sarcophaga cruentata (Figs. 77G, 78G and 79G)

The spines were sharp-tipped and single-pointed (Fig. 77G). Spines were located on the anterior margin as well as the posterior margins of segments. The creeping welt was typical, with no longitudinal groove present in the spine-free zone of the creeping welt (Fig. 78G). The fusiform area was well defined, with a good coverage of spines (Fig. 79G). All aspects pertaining to spines were comparable to that of second instar larvae (Figs. 54H, 55G and 56G).

Spines as a diagnostic characteristic

The species were grouped into two groupings, those with only single pointed spines (*C. marginalis*, *C. vicina* and *S. cruentata*) and those that also have multi-pointed spines (*L. cuprina*, *L. sericata*, *C. chloropyga* and *C. albiceps*). For those with single-pointed spines, *C. marginalis* was unique due to the blunt tips of its spines (Fig. 77D). The clearly defined fusiform area in *S. cruentata* distinguished this species from *C. vicina* where the fusiform area was not as clearly defined. For those species with multi-pointed spines, the initial basis of separation was regarding whether some of the spines had notched tips (*C. chloropyga* and *C, albiceps*) or not (*L. cuprina* and *L. sericata*). In those species with notched tips the extent of the notch separated the two species, with the spine tips only slightly notched in *C. albiceps* (Fig. 77E) and more severe in *C. chloropyga* (Fig. 77C). If the difference noted in this characteristic were deemed too slight, the distinct form of the creeping welt in *C. albiceps* (Fig. 78E)

could confirm the identification. The *Lucilia* species were distinguished from each other based on the distinct longitudinal groove noted in most creeping welts of *L. cuprina* (Fig. 78A) compared to the indistinct longitudinal groove noted in one or two creeping welts in *L. sericata* (Fig. 78B).

Utilising the various aspects of spines allowed for a full separation of the species examined.

3.5.11. CAUDAL SEGMENT (Figs. 80 and 81)

Two aspects of the perispiracular tubercles were evaluated namely (i) the prominence of the major tubercles and (ii) the relation between the various groupings of the dorsal perispiracular tubercles. The anal area was evaluated for: (i) the shape of the anal pads and the anal horns, (ii) the size relation between these two components of the anal area and (iii) the occurrence of spines in this area.

Lucilia cuprina (Figs. 80A and 81A)

The spiracular disc was an open, shallow structure (Fig. 80A) that was somewhat angled / hinged at its horizontal midline. Clearly visible perispiracular tubercles were located on the rim of the spiracular field (Fig. 80A); a feature also observed in the drawing presented by Greenberg & Szyska (1984) for *L. cuprina*. The perispiracular tubercles were more defined than that found in second instar larvae (Fig. 57A). In the specimens examined during the course of the present study it was found that the distance between the two inner dorsal tubercles was approximately equal to the distance between the inner - and the outer dorsal tubercle (Fig. 80A) – a ratio confirmed by Zumpt (1965) and Greenberg & Szyska (1984) for *L. cuprina*. The middle dorsal tubercle was equidistant from the inner – and the outer dorsal tubercles (Fig. 80A).

The anal pads were slightly smaller than the triangular (equilateral) anal horns. No spines were found on the anal pads or horns, but the area surrounding the anal area was furnished with spines (Fig. 81A). The anal area of second instar larvae (Fig. 58A) was comparable to that of third instar larvae. The most noticeable difference was that the anal horns were more rounded structures in second instar larvae (Fig. 58A).

Lucilia sericata (Figs. 80B and 81B)

The spiracular disc was an open, shallow structure with small, almost indistinct perispiracular tubercles (Fig. 80B). The perispiracular tubercles were slightly more developed than in second instar larvae (Fig. 57B). The distance between the inner - and outer dorsal tubercles was greater than the distance between the two inner dorsal tubercles (Fig. 80B). The middle dorsal tubercle was closer to the outer dorsal tubercle than to the inner dorsal tubercle (Fig. 80B). Zumpt (1965) noted that the distance between the inner dorsal tubercles were approximately equal to the distance between the inner and middle dorsal tubercle.

The anal pads were smaller than the anal horns (Fig. 81B). Both elements of the anal area were globular (Fig. 81B). No spines were present on the pointed anal pads and lateral horns (Fig. 81B). Spines were found in the area around the anal area (Fig. 81B). The anal area of second instar larvae (Fig. 58B) were similar to that of third instar larvae, but appeared to be relatively smaller structures on the ventral aspect of the terminal segment.

Chrysomya chloropyga (Figs. 80C and 81C)

The spiracular disc was an open, somewhat angled / hinged structure, with noticeable perispiracular tubercles on the rim of the spiracular plate (Fig. 80C). These perispiracular tubercles were larger than those seen in second instar larvae (Fig. 57C). The perispiracular tubercles appeared more pronounced in the specimens of *C. putoria* presented by Greenberg & Szyska (1984) than that of the specimens of *C. chloropyga* examined during the course of the current study. The distance between the inner dorsal tubercles was approximately equal to the distance from the inner dorsal tubercle to the outer dorsal tubercle (Fig. 80C). This was in contrast to that reported for *C. putoria* by Greenberg & Szyska (1984) where the distance between the inner dorsal tubercles was approximately equal to or slighter greater than the distance from the inner dorsal tubercle to the middle dorsal tubercle. In the specimens examined during the course of the present study it was found that the outer dorsal tubercle was closer to the middle dorsal tubercle than what the inner dorsal tubercle was to the middle dorsal tubercle (Fig. 80C). This was similar to that reported by Greenberg & Szyska (1984) for *C. putoria*. Thus the size of the tubercles and the distances between

some groupings of the dorsal tubercles would allow for separation between *C. putoria* and *C. chloropyga*.

The anal pads were rounded and the anal horns triangular (an isosceles triangle) in shape. The triangular shape of the anal horns was more pronounced than those seen in second instar larvae (Fig. 58C). The anal pads were slightly smaller than the anal horns (Fig. 81C). The area surrounding the anal pads was adorned with large, curved, multi-pointed spines (Fig. 81C). Small, single-pointed spines were arranged in a concentric pattern on the anal pads (Fig. 81C).

Chrysomya marginalis (Figs. 80D and 81D)

The spiracular disc was somewhat sunken in, with clearly noticeable perispiracular tubercles on its rim (Fig. 80D). The spiracular plate was somewhat angled at its horizontal plane (Fig. 80D). The size of the perispiracular tubercles was similar to those illustrated by Prins (1982) for *C. marginalis*. These aspects of the posterior end were similar to that of the second instar larvae (Fig. 57D). The distance between the two inner dorsal tubercles were similar to the distance between the inner and outer tubercles (Fig. 80D). The middle dorsal tubercle was equidistant from the inner - and outer dorsal tubercles (Fig. 80D).

The triangular (an isosceles triangle) anal horns were larger than the globular anal pads (Fig. 81D). It appeared to be larger in comparison to the anal pads what was found for second instar larvae (Fig. 58D). Stubby, single-pointed spines surrounded the anal area (Fig. 81D).

Chrysomya albiceps (Figs. 80E and 81E)

The spiracular disc was an angled / hinged structure and appeared to be slightly sunken in Fig. 80E. Large perispiracular tubercles, similar to the processes on the rest of the larval body, were noted on the rim of the spiracular disc (Fig. 80E). The spinules crowning the tips of the tubercles were short and did not extend past the tips of the perispiracular tubercles. These aspects of the posterior end were similar to that of second instar larvae (Fig. 57E). The distances between the different groupings of dorsal tubercles were equal (Fig. 80E); equal distances between the inner dorsal

tubercles, between the inner and middle dorsal tubercles and between the middle and the outer dorsal tubercles.

The anal pads were rounded and the anal horns were triangular (an isosceles triangle) in shape (Fig. 81E). The anal pads and horns were similar in size (Fig. 81E). Robust, curved spines surrounded the anal area (Fig. 81E). On the inside walls of the anal pads small, single-pointed spines were noted. The tips of the anal pads were adorned with small, stubby spines forming a more or less concentric pattern on the anal pads (Fig. 81E). The anal area of second instar larvae (Fig. 58E) was comparable to that of third instar larvae.

Calliphora vicina (Figs. 80F and 81F)

The spiracular disc was an open, shallow structure with distinct perispiracular tubercles on its rim (Fig. 80F). The perispiracular tubercles were more defined in third instar larvae than those found in second instar larvae (Fig. 57F). In the specimens examined during the course of the present study it was found that the distance between the inner dorsal tubercles was approximately the same as the distance between the inner dorsal tubercle to the outer dorsal tubercle (Fig. 80F). The middle dorsal tubercle was approximately equidistant to the outer dorsal tubercle and the inner dorsal tubercle. This was comparable to that reported for *C. vicina* by Liu & Greenberg (1989) where the distance between the inner dorsal tubercles was greater than the distance between the inner and middle dorsal tubercles.

The anal pads and triangular (an equilateral triangle) anal horns were similar in size (Fig. 81F). The anal elements were globular in second instar larvae (Fig. 58F). Single-pointed spines surrounded the anal area (Fig. 81F).

Sarcophaga cruentata (Figs. 80G and 81G)

The spiracular disc was deeply recessed, forming a spiracular atrium (Fig. 80G). The posterior spiracles were obscured from view in this cavity (Fig. 80G). This deep cavity might be due to the feeding behaviour of these larvae. The feeding medium showed no signs of larvae on or around it when inspected. Larvae were only noted when the medium was lifted from the substrate. Teskey (1981) postulated that the formation of an atrium is an adaptation in response to an aquatic environment.

Although not an aquatic environment, the submerged environment these larvae were found in, i.e. in the feeding medium, might have necessitated some sort of adaptation. A deep spiracular atrium might allow for the protection of the spiracles from the feeding medium clogging it. Alternatively, a deep-set spiracular atrium may act as an oxygen reservoir in such an environment. Overall the spiracular atrium was spindle-shaped, with the dorsal margin of the spiracular atrium rounded and the two sides of the ventral margin forming a slight angle to its ventral midpoint (Fig. 80G). The margins of the atrium were more defined than those found in second instar larvae (Fig. 57G). The perispiracular tubercles were indistinct (Fig. 80G); similar to the situation found in second instar larvae (Fig. 57G). The distance between the inner dorsal tubercles was approximately the same as the distance from the inner dorsal tubercle to the outer dorsal tubercle (Fig. 80G). The middle dorsal tubercle was equidistant from the inner - and the outer dorsal tubercle (Fig. 80G).

The anal horns were elongated, tube-like structures, without any spines. The anal horns were triangular in second instar larvae (Fig. 58G). The medial area around the anal slit, the anal pads as well as the area around the anal area was covered with small, single-pointed spines (Fig. 81G). The anal pads were not covered with spines in second instar larvae.

The caudal segment as a diagnostic characteristic

Spiracular field

Sarcophaga cruentata was uniquely identified due to its deeply recessed atrium (Fig. 80G). Aspects of the perispiracular tubercles were used to separate the species further. The perispiracular tubercles were indistinct in *L. sericata* (Fig. 80B), whereas it was clearly visible in the remainder of the species. In this grouping with clearly visible perispiracular tubercles, the unique morphology of the perispiracular tubercles of *C. albiceps* (Fig. 80E) distinguished it from the rest of the species examined. The rest of the species were distinguished from each other based on the distance among the various groupings of dorsal tubercles. The middle dorsal process was equidistant from the inner dorsal tubercle and the outer dorsal tubercle in all of the species except *C. chloropyga* (Fig. 80C). The exposure level of the spiracular plate clearly differentiated *Lucilia cuprina*, *C. marginalis* and *C. vicina*. The spiracular plate was

sunken-in in *C. marginalis* (Fig. 80D), it was relatively flat and opened-up in *C. vicina* (Fig. 80F), whereas it was slightly angled in *L. cuprina* (Fig. 80A).

Anal area

The first basis of distinction was whether the anal horns were triangular (*L. cuprina*, *C. chloropyga*, *C. marginalis*, *C. albiceps* and C. vicina) or not (*L. sericata* and *S. cruentata*). In the species where the anal horns were triangular it was noted that the triangle was an equilateral triangle in *L. cuprina* and *C. vicina* and an isosceles triangle in *C. chloropyga*, *C. marginalis* and *C. albiceps*. For those species where the anal horns were equilateral the size of the anal pads in comparison to the anal horns were used to distinguish between the two species. The anal pads were smaller than the horns in *L. cuprina* (Fig. 81A) and approximately equal in size for *C. vicina* (Fig. 81F). In these species where the triangle was an isosceles triangle the size of the two elements in relation to each other was also used to distinguish the species from each other. The anal pads were much smaller than the horns for *C. marginalis* (Fig. 81D), compared to it being similar in size for *C. chloropyga* and *C. albiceps*. These two species were distinguished from each other based on the nature of the spines in the anal pads. These spines were fine in *C. chloropyga* (Fig. 81C) and robust in *C. albiceps* (Fig. 81E). The spines surrounding the anal area were also more robust and a larger number were present in *C. albiceps* opposed to the situation found for *C. chloropyga*. In those species where the anal horns were not triangular, the anal horns were elongated structures in *S. cruentata* (Fig. 81G), whereas it was more compact in *L. sericata* (Fig. 81B). An additional area of distinction was the fine spines noted on the anal pads in *S. cruentata* (Fig. 81G), whereas no spines were noted on the anal pads of *L. sericata* (Fig. 81B).

From this analysis it can be seen that a full separation can be achieved by utilising any of the elements of the terminal segment.

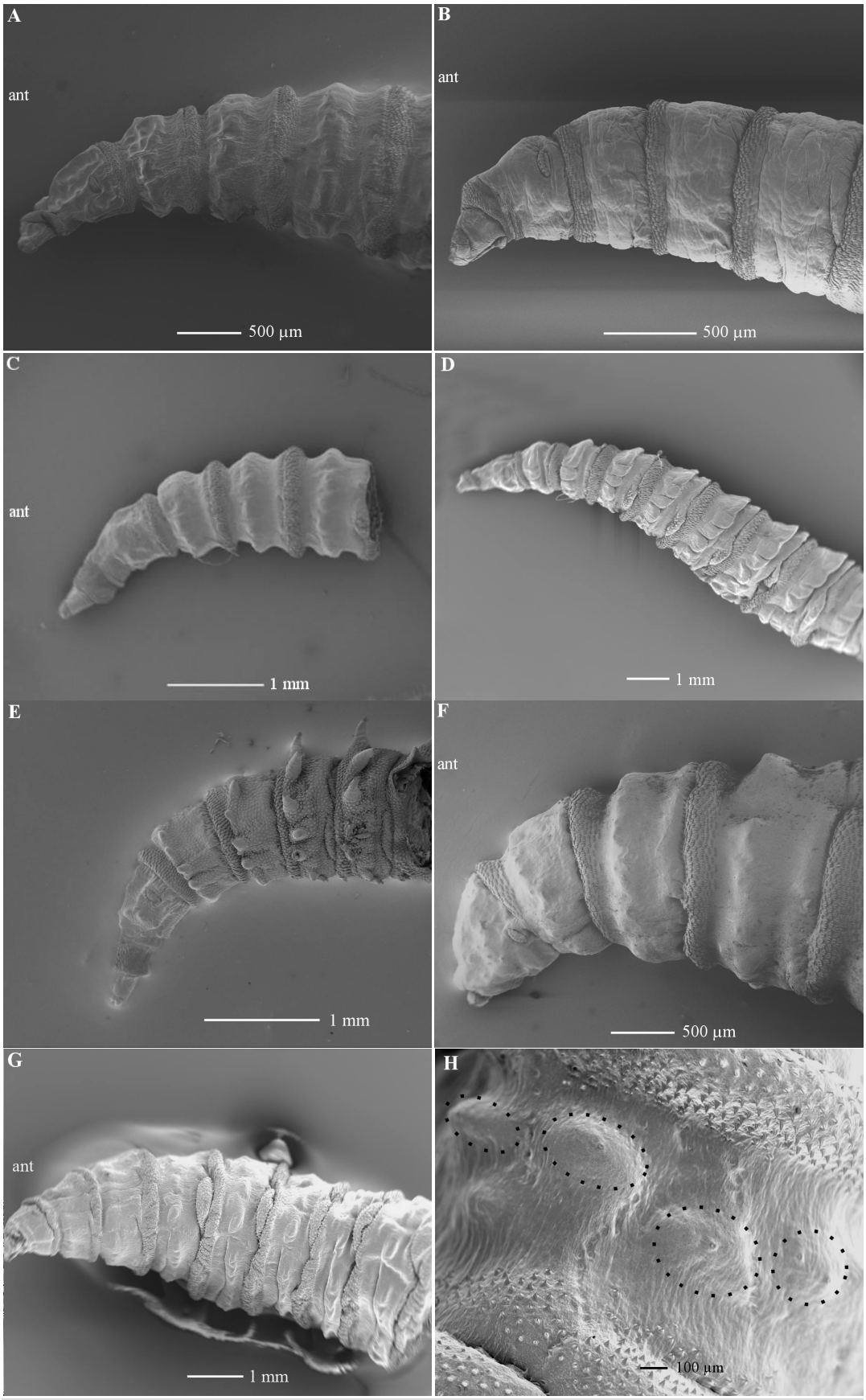


Fig. 59: Scanning electron micrographs of the anterior ends of third instar larvae of A: *Lucilia cuprina*, B: *Lucilia sericata*, C : *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*; F: *Calliphora vicina* and G: *Sarcophaga cruentata.* H: Detail of the integument of *Sarcophaga cruentata*.
Legend: encircled: pit sensillae on bulges; ant: anterior.

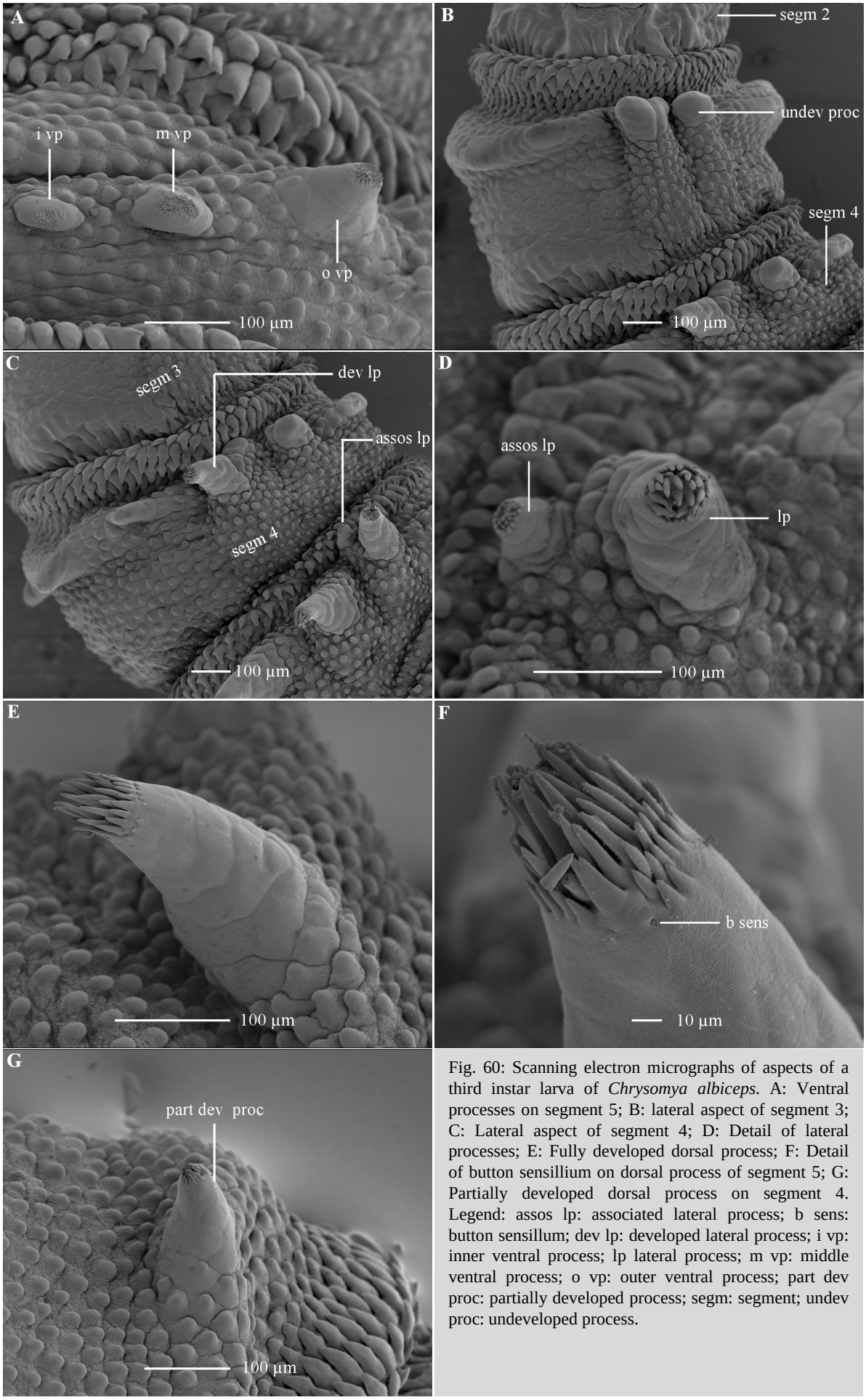


Fig. 60: Scanning electron micrographs of aspects of a third instar larva of *Chrysomya albiceps*. A: Ventral processes on segment 5; B: lateral aspect of segment 3; C: Lateral aspect of segment 4; D: Detail of lateral processes; E: Fully developed dorsal process; F: Detail of button sensillium on dorsal process of segment 5; G: Partially developed dorsal process on segment 4. Legend: assos lp: associated lateral process; b sens: button sensillum; dev lp: developed lateral process; i vp: inner ventral process; lp lateral process; m vp: middle ventral process; o vp: outer ventral process; part dev proc: partially developed process; segm: segment; undev proc: undeveloped process.

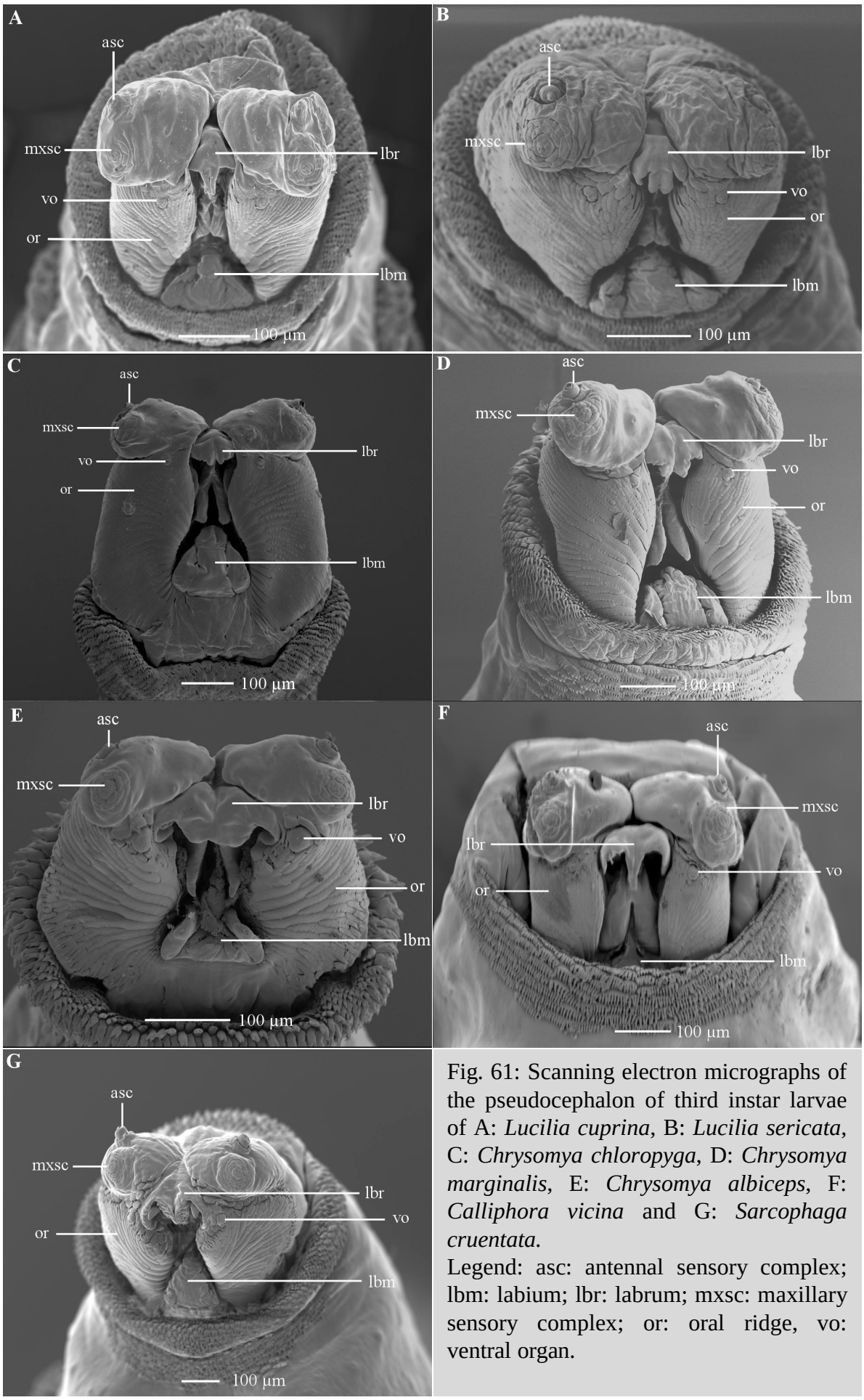


Fig. 61: Scanning electron micrographs of the pseudocephalon of third instar larvae of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina* and G: *Sarcophaga cruentata.*

Legend: asc: antennal sensory complex; lbm: labium; lbr: labrum; mxsc: maxillary sensory complex; or: oral ridge, vo: ventral organ.

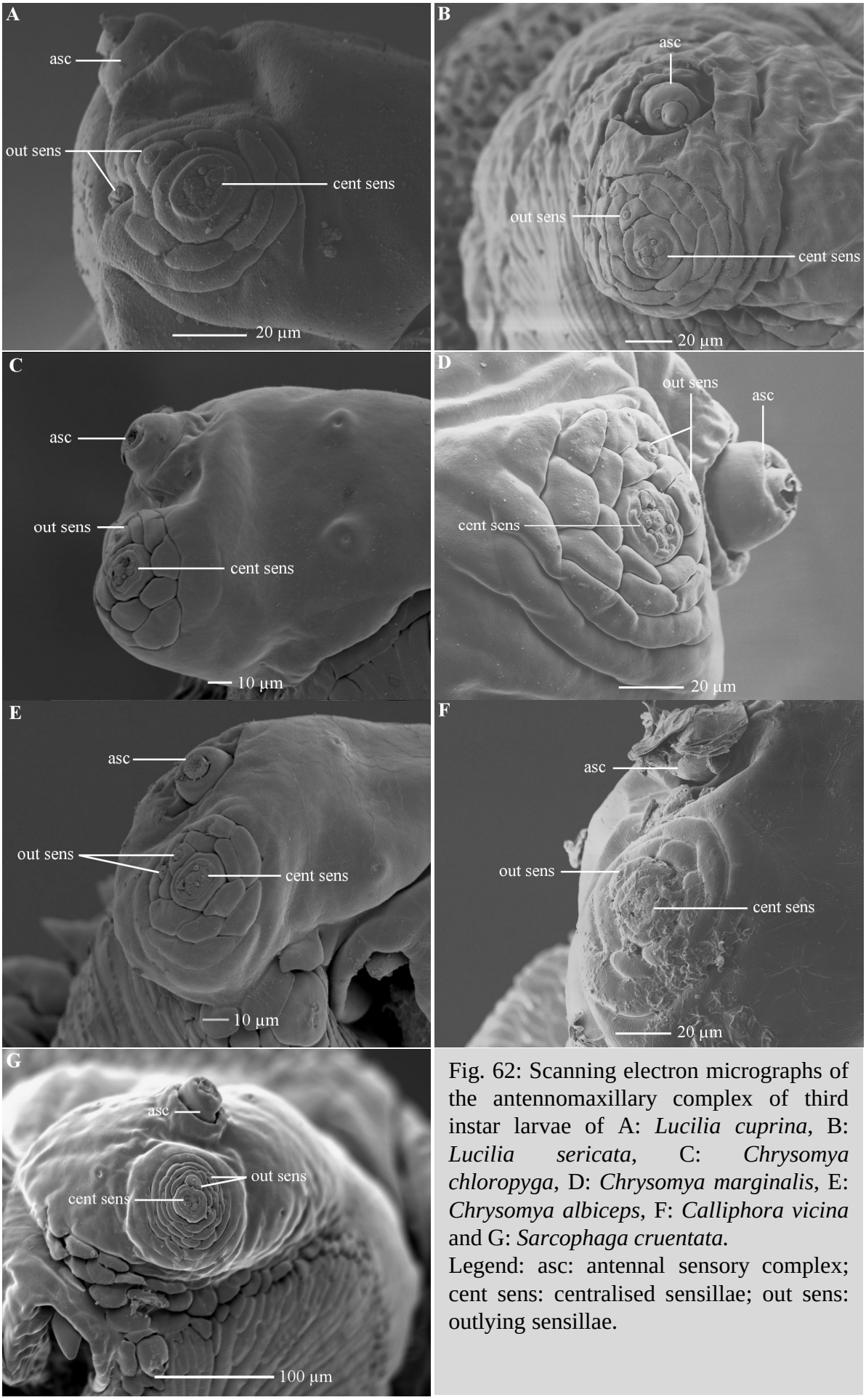


Fig. 62: Scanning electron micrographs of the antennomaxillary complex of third instar larvae of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina* and G: *Sarcophaga cruentata*.
Legend: asc: antennal sensory complex; cent sens: centralised sensillae; out sens: outlying sensillae.

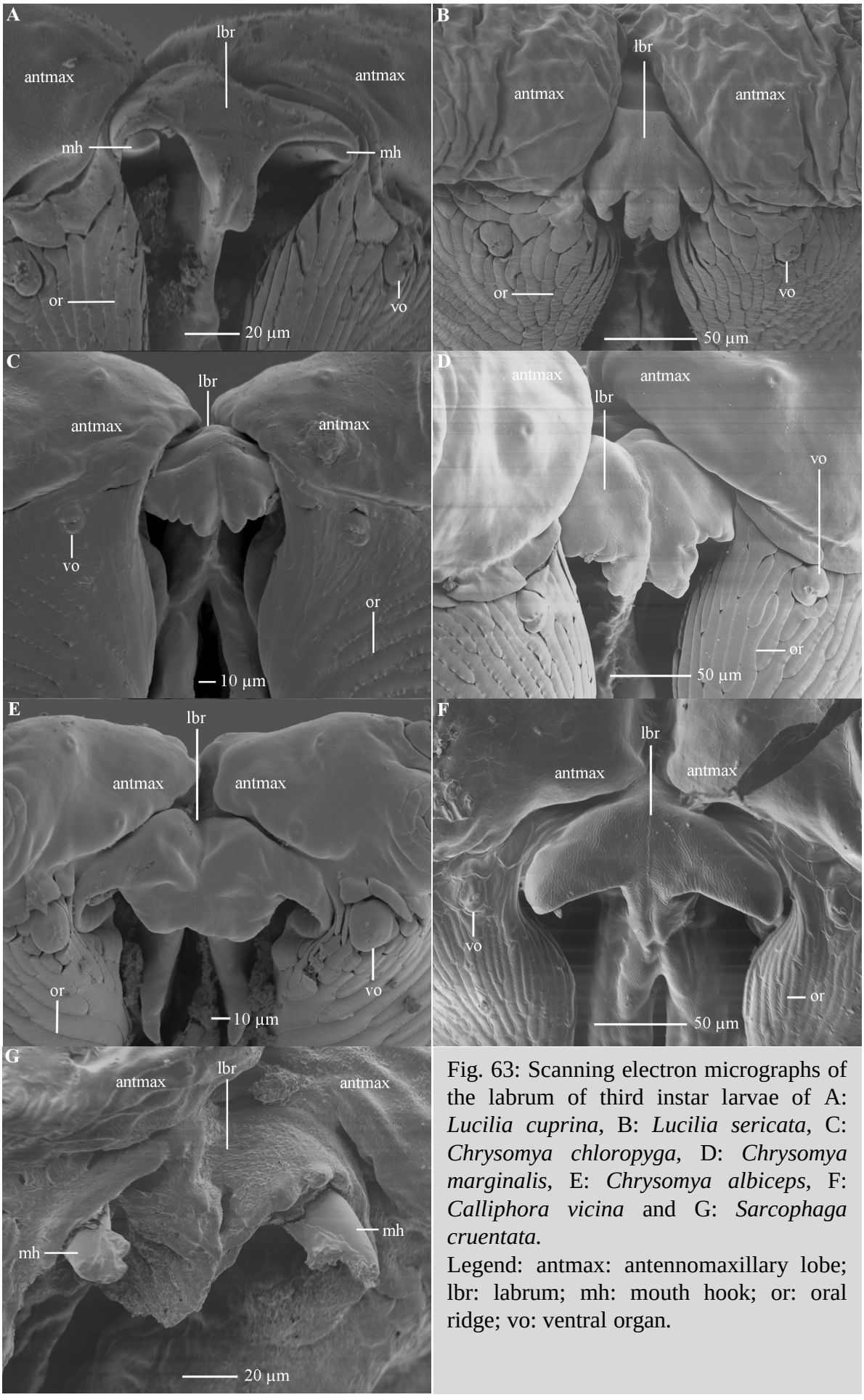


Fig. 63: Scanning electron micrographs of the labrum of third instar larvae of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina* and G: *Sarcophaga cruentata.*

Legend: antmax: antennomaxillary lobe; lbr: labrum; mh: mouth hook; or: oral ridge; vo: ventral organ.

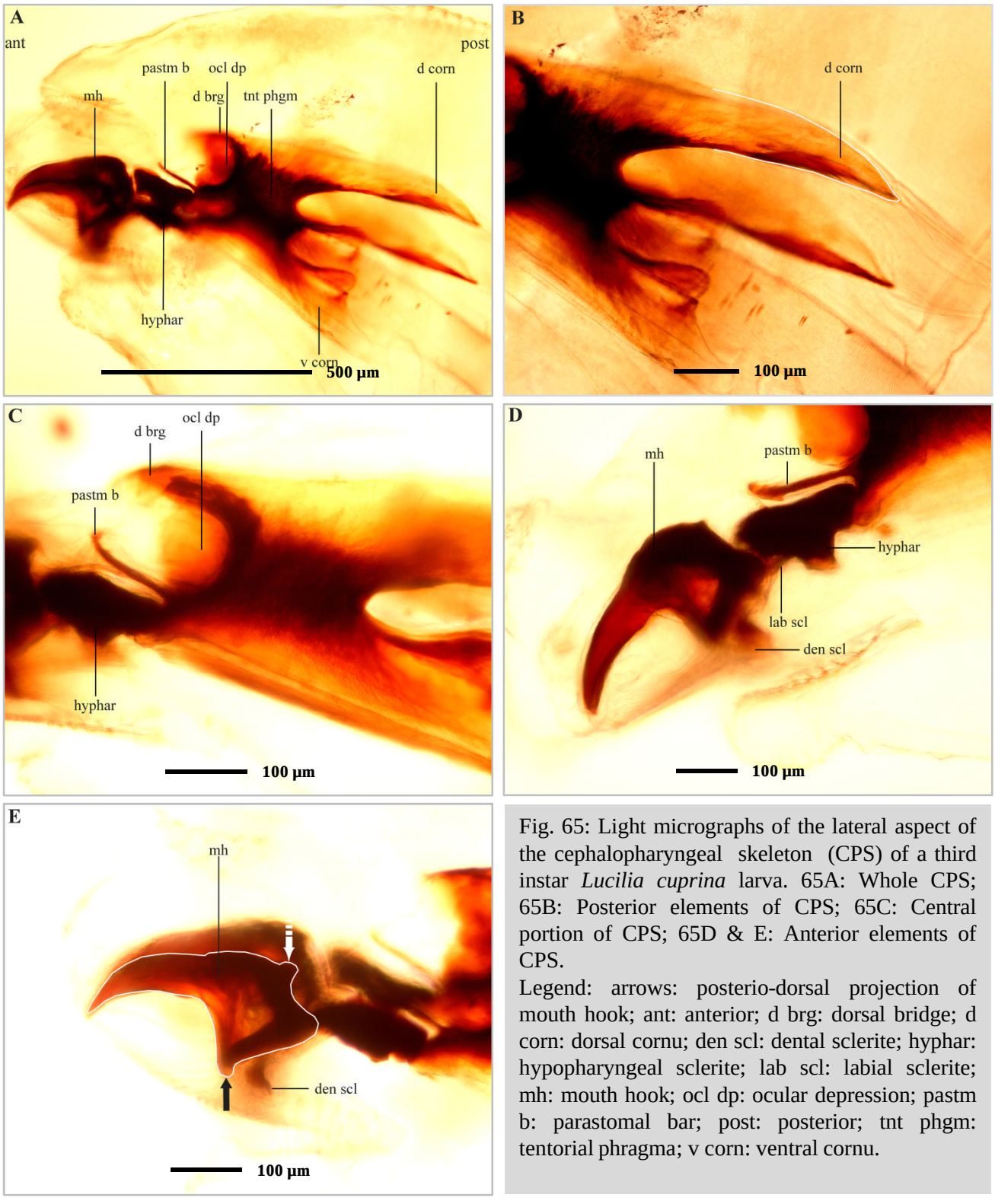


Fig. 65: Light micrographs of the lateral aspect of the cephalopharyngeal skeleton (CPS) of a third instar *Lucilia cuprina* larva. 65A: Whole CPS; 65B: Posterior elements of CPS; 65C: Central portion of CPS; 65D & E: Anterior elements of CPS.

Legend: arrows: posterio-dorsal projection of mouth hook; ant: anterior; d brg: dorsal bridge; d corn: dorsal cornu; den scl: dental sclerite; hyphar: hypopharyngeal sclerite; lab scl: labial sclerite; mh: mouth hook; ocl dp: ocular depression; pastm b: parastomal bar; post: posterior; tnt phgm: tentorial phragma; v corn: ventral cornu.

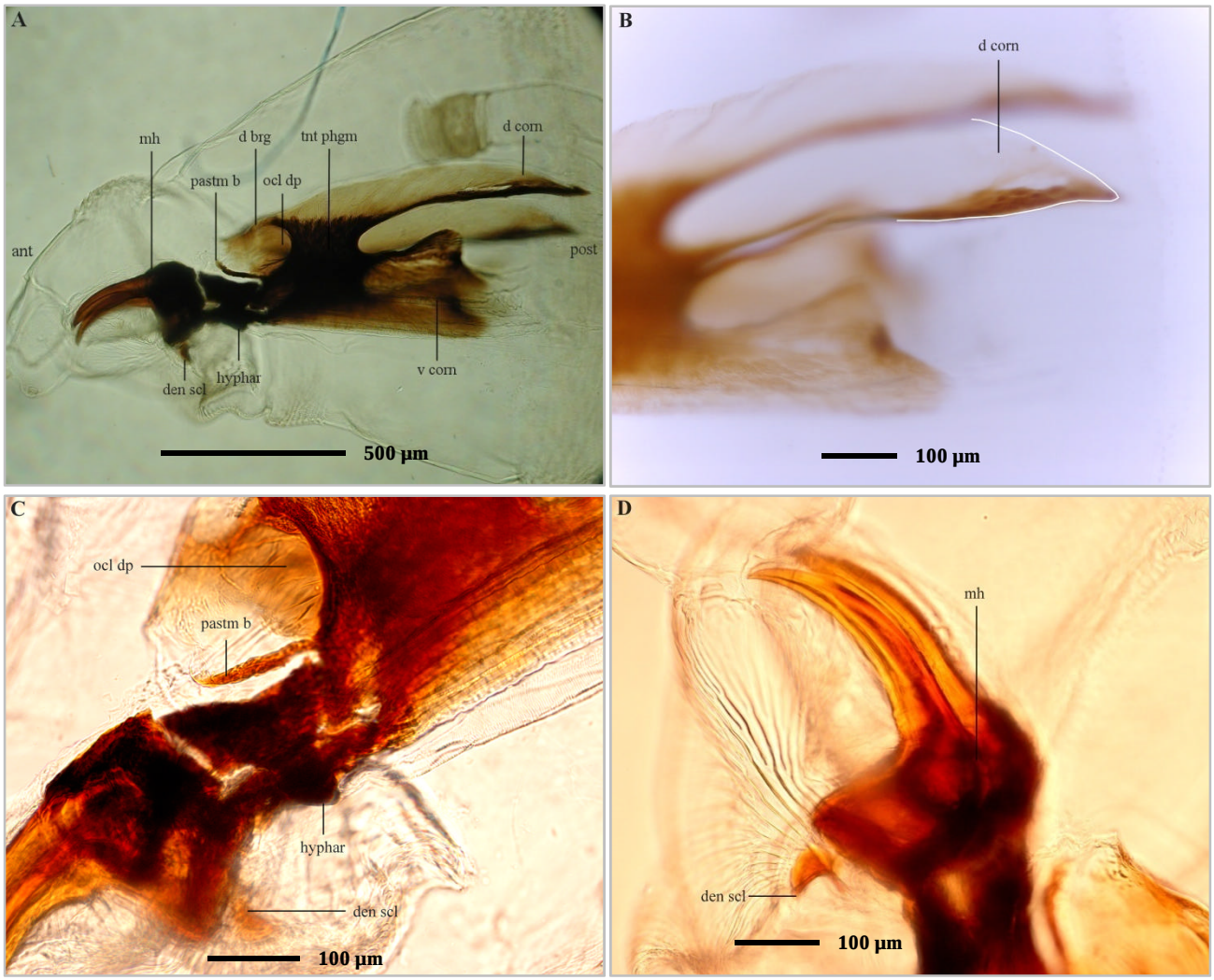


Fig. 66: Light micrographs of the lateral aspect of the cephalopharyngeal skeleton (CPS) of a third instar *Lucilia sericata* larva. 66A: Whole CPS; 66B: Posterior elements of CPS; 66C: Central portion of CPS; 66D: Anterior elements of CPS.
Legend: ant: anterior; d brg: dorsal bridge; d corn: dorsal cornu; den scl: dental sclerite; hyphar: hypopharyngeal sclerite; mh: mouth hook; ocl dp: ocular depression; pastm b: parastomal bar; post: posterior; tnt phgm: tentorial phragma; v corn: ventral cornu.

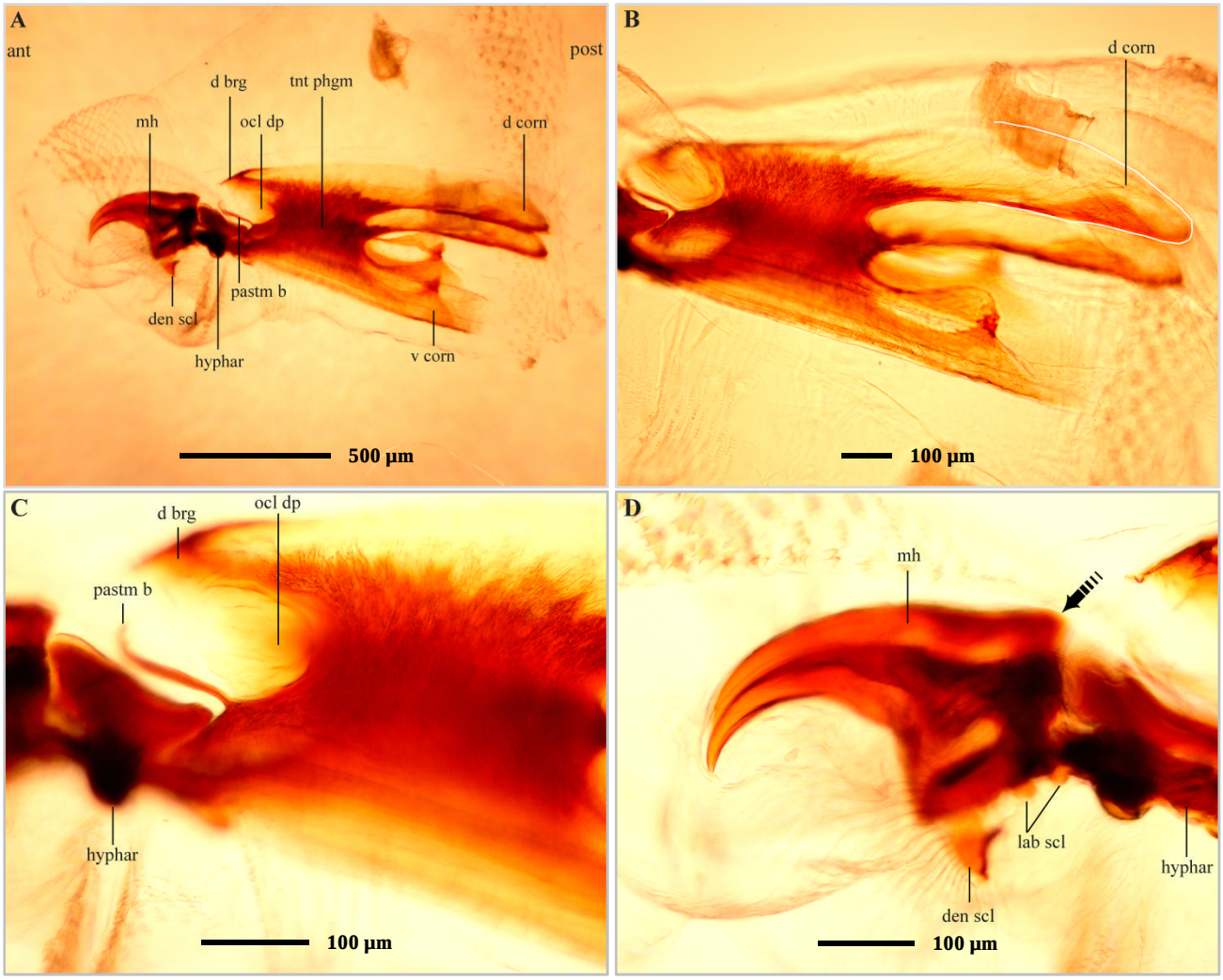


Fig. 67: Light micrographs of the lateral aspect of the cephalopharyngeal skeleton (CPS) of a third instar *Chrysomya chloropyga* larva. 67A: Whole CPS; 67B: Posterior elements of CPS; 67C: Central portion of CPS; 67D: Anterior elements of CPS.
Legend: arrow: posterio-dorsal projection of mouth hook; ant: anterior; d brg: dorsal bridge; d corn: dorsal cornu; den scl: dental sclerite; hyphar: hypopharyngeal sclerite; lab scl: labial sclerite; mh: mouth hook; ocl dp: ocular depression; pastm b: parastomal bar; post: posterior; tnt phgm: tentorial phragma; v corn: ventral cornu.

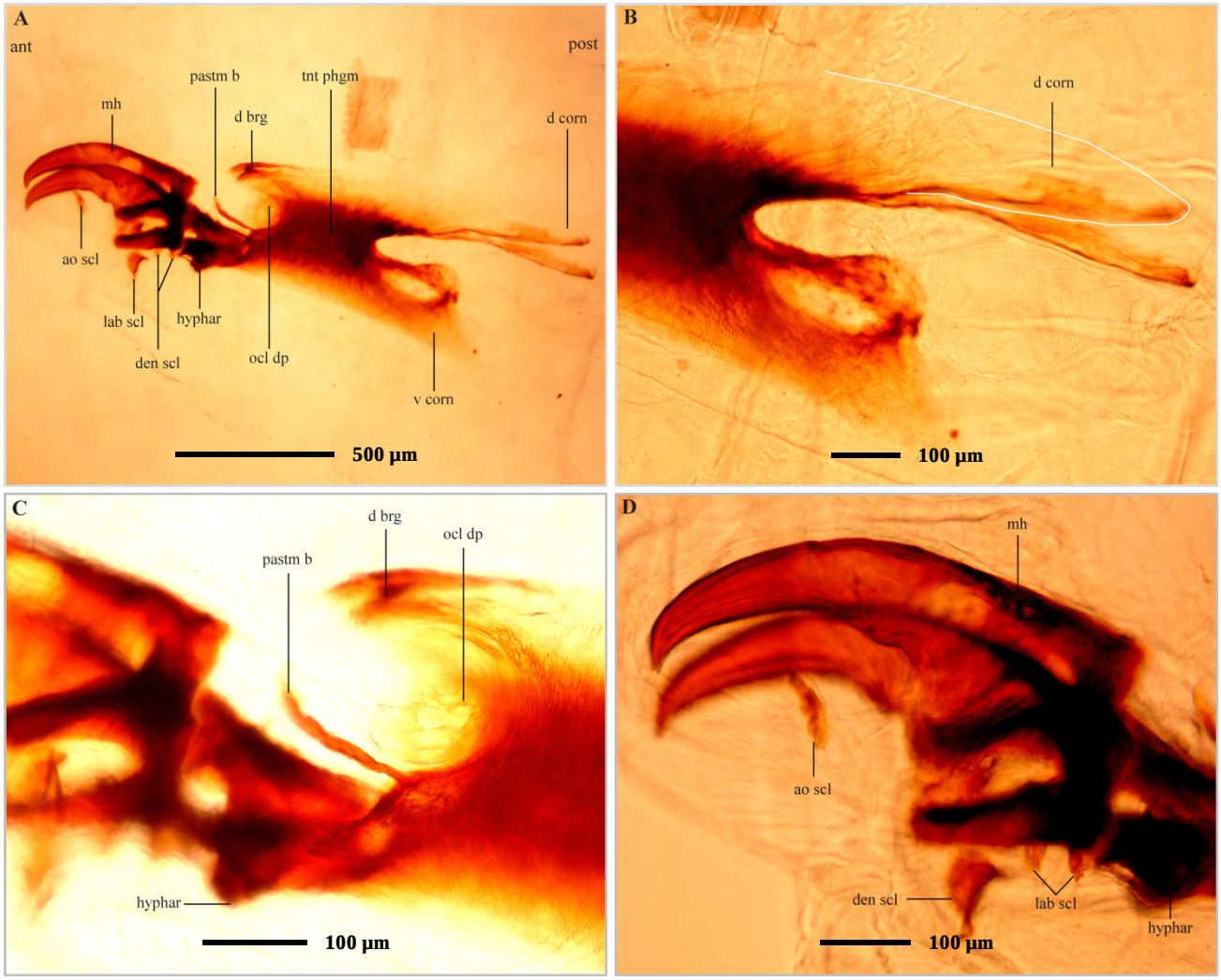


Fig. 68: Light micrographs of the lateral aspect of the cephalopharyngeal skeleton (CPS) of a third instar *Chrysomya marginalis* larva. 68A: Whole CPS; 68B: Posterior elements of CPS; 68C: Central portion of CPS; 68D: Anterior elements of CPS.
Legend: ant: anterior; ao scl: accessory oral sclerite; d brg: dorsal bridge; d corn: dorsal cornu; den scl: dental sclerite; hyphar: hypopharyngeal sclerite; lab scl: labial sclerite; mh: mouth hook; ocl dp: ocular depression; pastm b: parastomal bar; post: posterior; tnt phgm: tentorial phragma; v corn: ventral cornu.

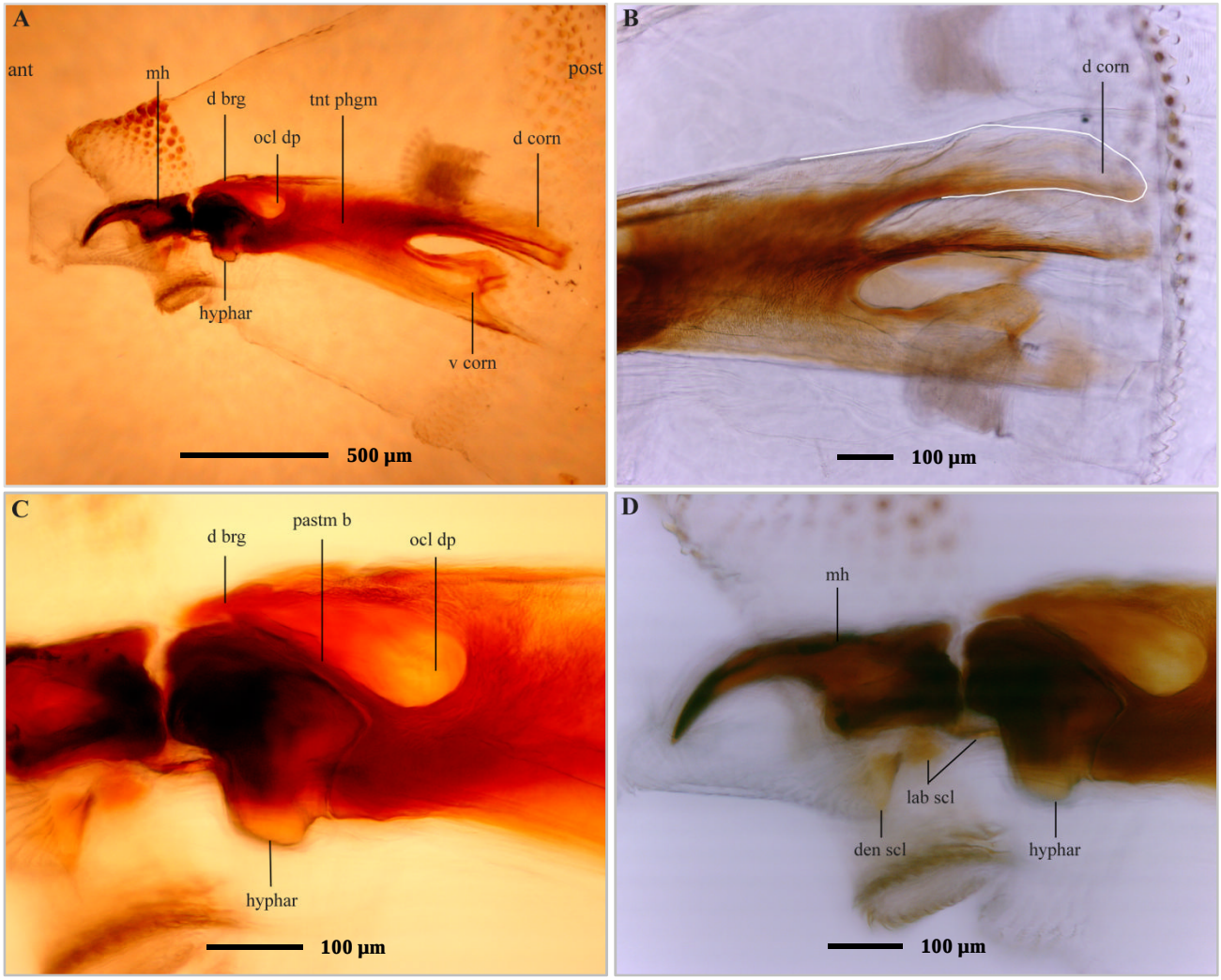


Fig. 69: Light micrographs of the lateral aspect of the cephalopharyngeal skeleton (CPS) of a third instar *Chrysomya albiceps* larva. 69A: Whole CPS; 69B: Posterior elements of CPS; 69C: Central portion of CPS; 69D: Anterior elements of CPS.
Legend: ant: anterior; d brg: dorsal bridge; d corn: dorsal cornu; den scl: dental sclerite; hyphar: hypopharyngeal sclerite; lab scl: labial sclerite; mh: mouth hook; ocl dp: ocular depression; pastm b: parastomal bar; post: posterior; tnt phgm: tentorial phragma; v corn: ventral cornu.

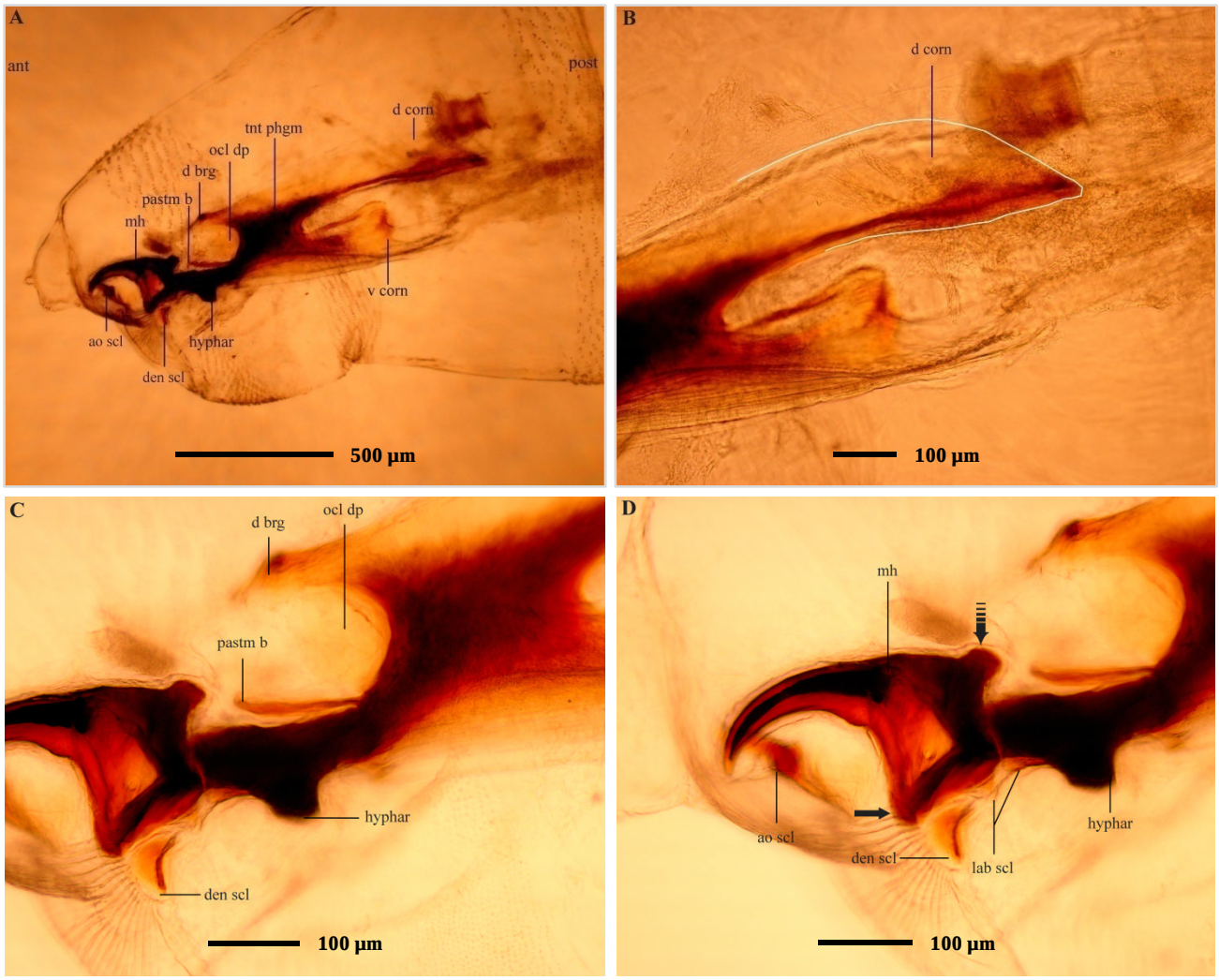


Fig. 70: Light micrographs of the lateral aspect of the cephalopharyngeal skeleton (CPS) of a third instar *Calliphora vicina* larva. 70A: Whole CPS; 70B: Posterior elements of CPS; 70C: Central portion of CPS; 70D: Anterior elements of CPS.
Legend: arrow: posterio-dorsal projection of mouth hook; ant: anterior; ao scl: accessory oral sclerite; d brg: dorsal bridge; d corn: dorsal cornu; den scl: dental sclerite; hyphar: hypopharyngeal sclerite; lab scl: labial sclerite; mh: mouth hook; ocl dp: ocular depression; pastm b: parastomal bar; post: posterior; tnt phgm: tentorial phragma; v corn: ventral cornu.

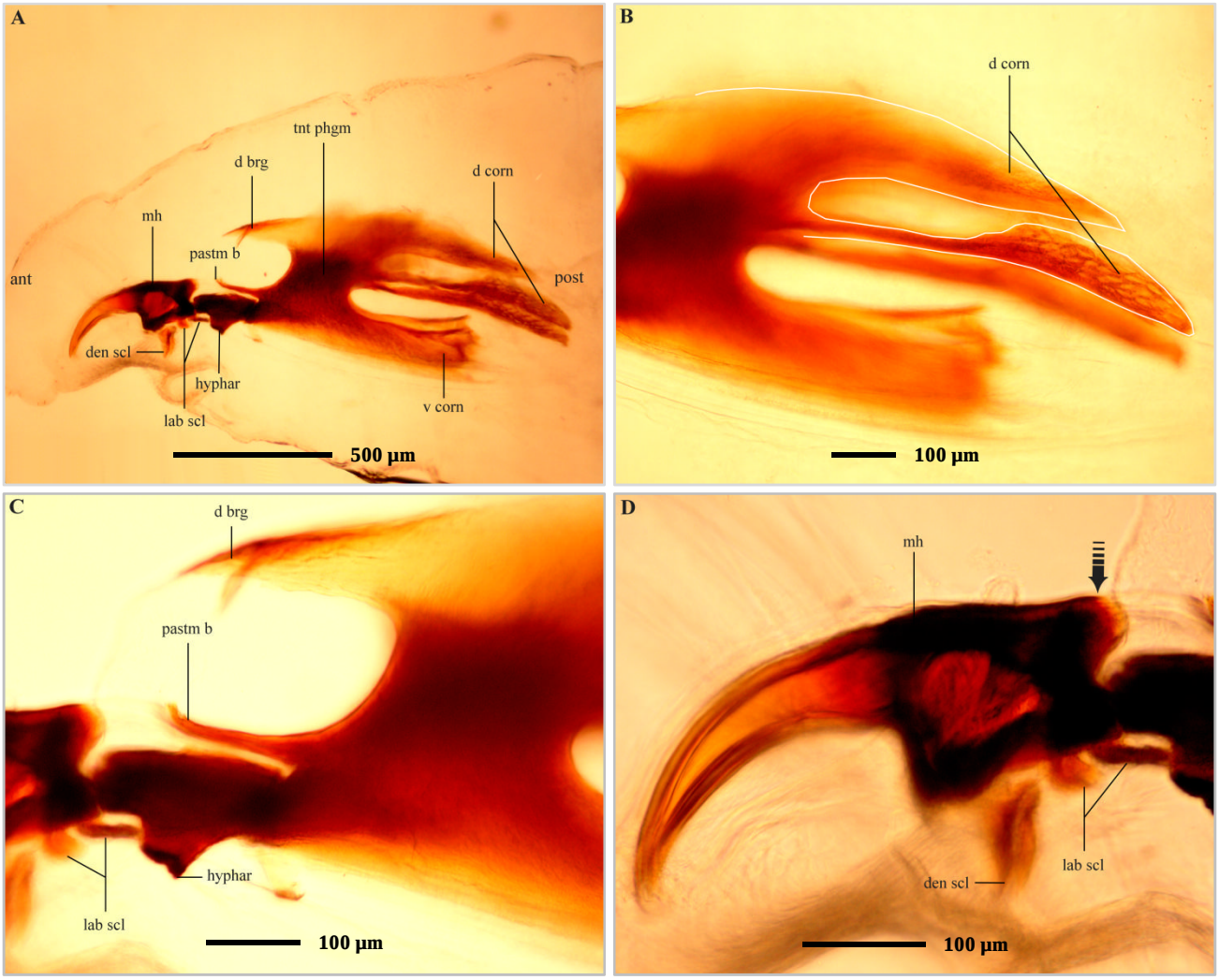


Fig. 71: Light micrographs of the lateral aspect of the cephalopharyngeal skeleton (CPS) of a third instar *Sarcophaga cruentata* larva. 71A: Whole CPS; 71B: Posterior elements of CPS; 71C: Central portion of CPS; 71D: Anterior elements of CPS.
Legend: arrow: posterio-dorsal projection of mouth hook; ant: anterior; d brg: dorsal bridge; d corn: dorsal cornu; den scl: dental sclerite; hyphar: hypopharyngeal sclerite; lab scl: labial sclerite; mh: mouth hook; pastm b: parastomal bar; post: posterior; tnt phgm: tentorial phragma; v corn: ventral cornu.

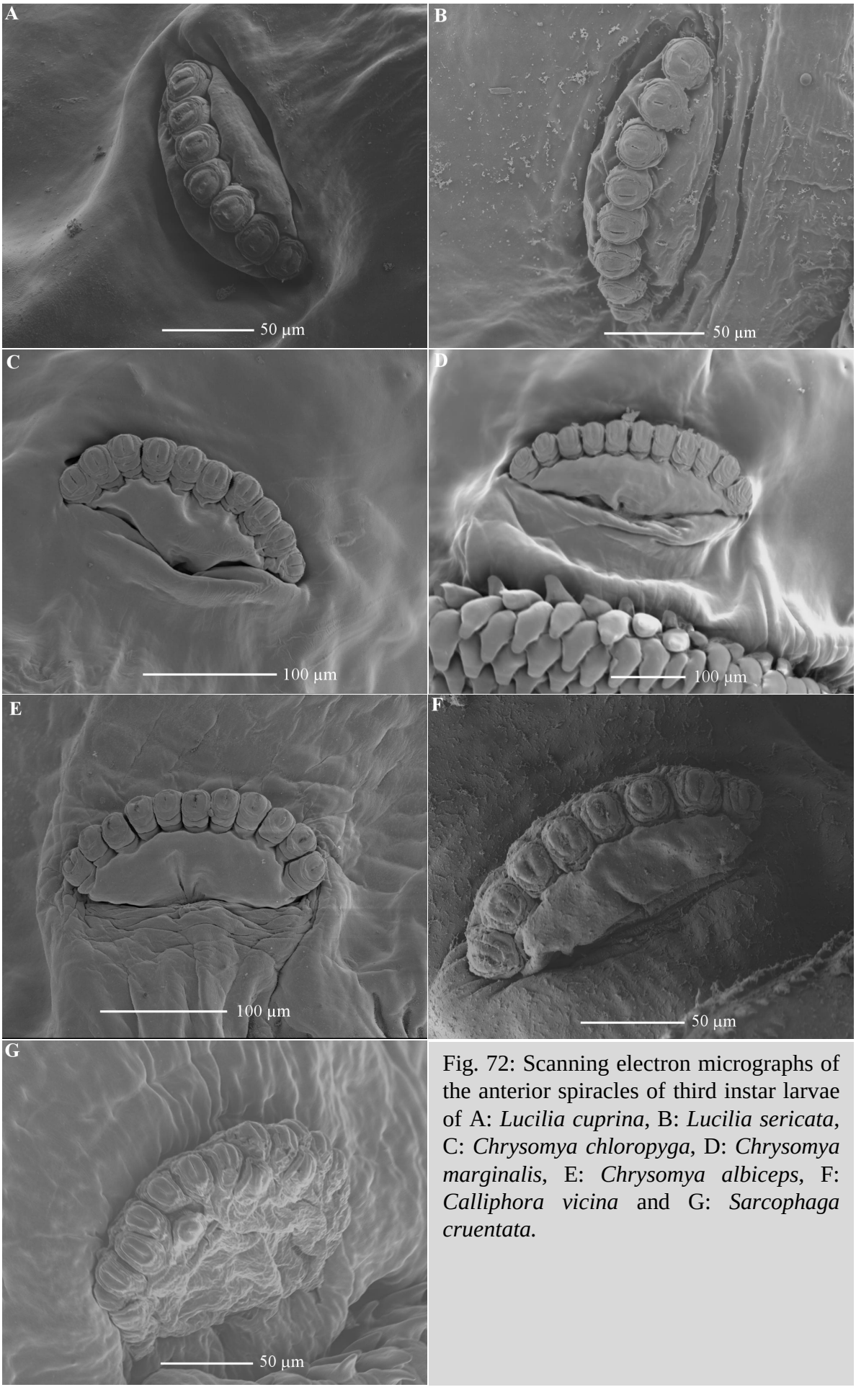


Fig. 72: Scanning electron micrographs of the anterior spiracles of third instar larvae of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina* and G: *Sarcophaga cruentata.*

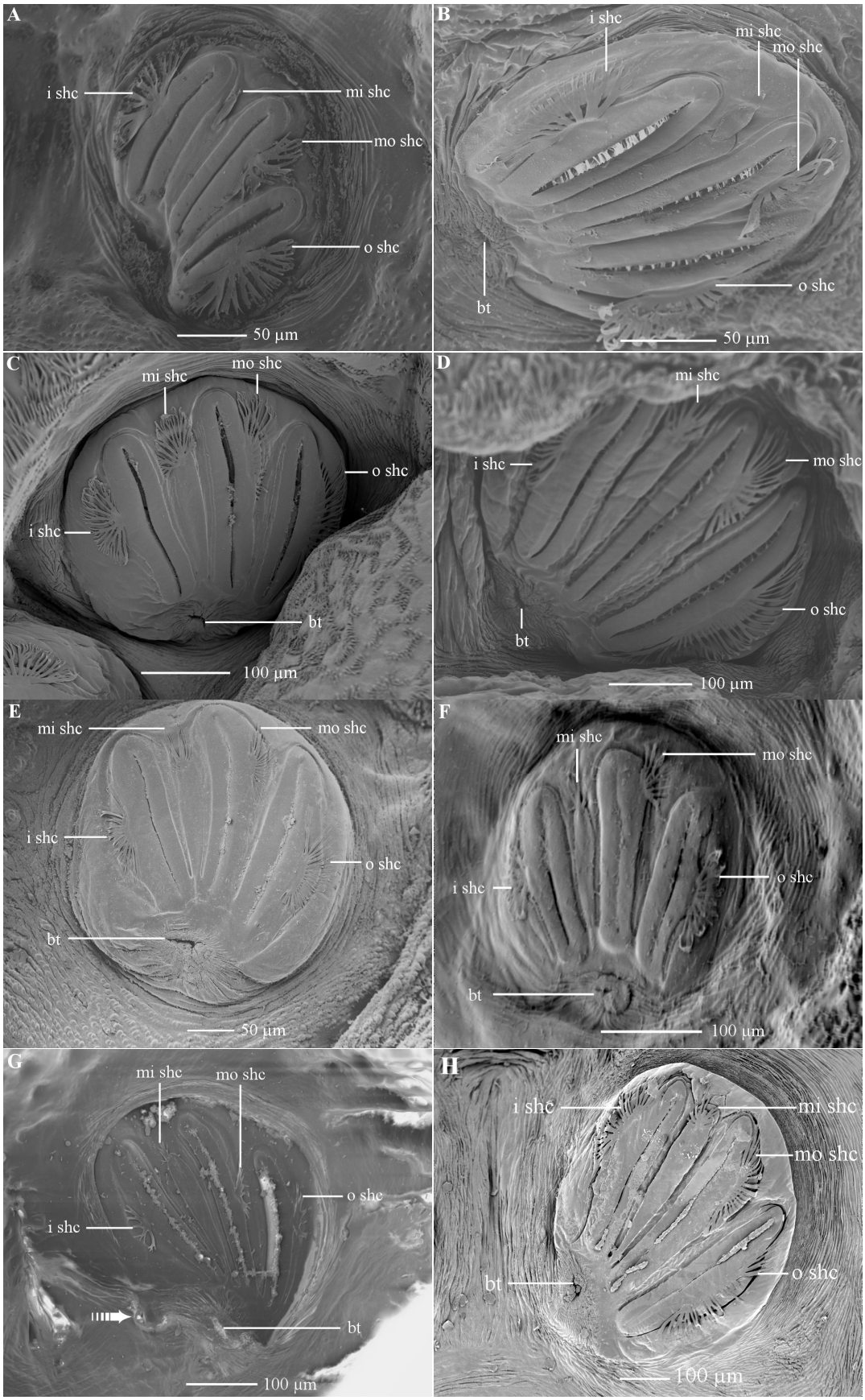


Fig. 74: Scanning electron micrographs of the posterior spiracles of third instar larvae of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina*, G: *Sarcophaga cruentata* and H: of the puparium of *C. marginalis*.
Legend: arrow: tear-shaped protrusion; bt: button; i shc: inner spiracular hair cluster; mi shc: middle-inner spiracular hair cluster, mo shc: middle-outer spiracular hair cluster, o shc: outer spiracular hair cluster.

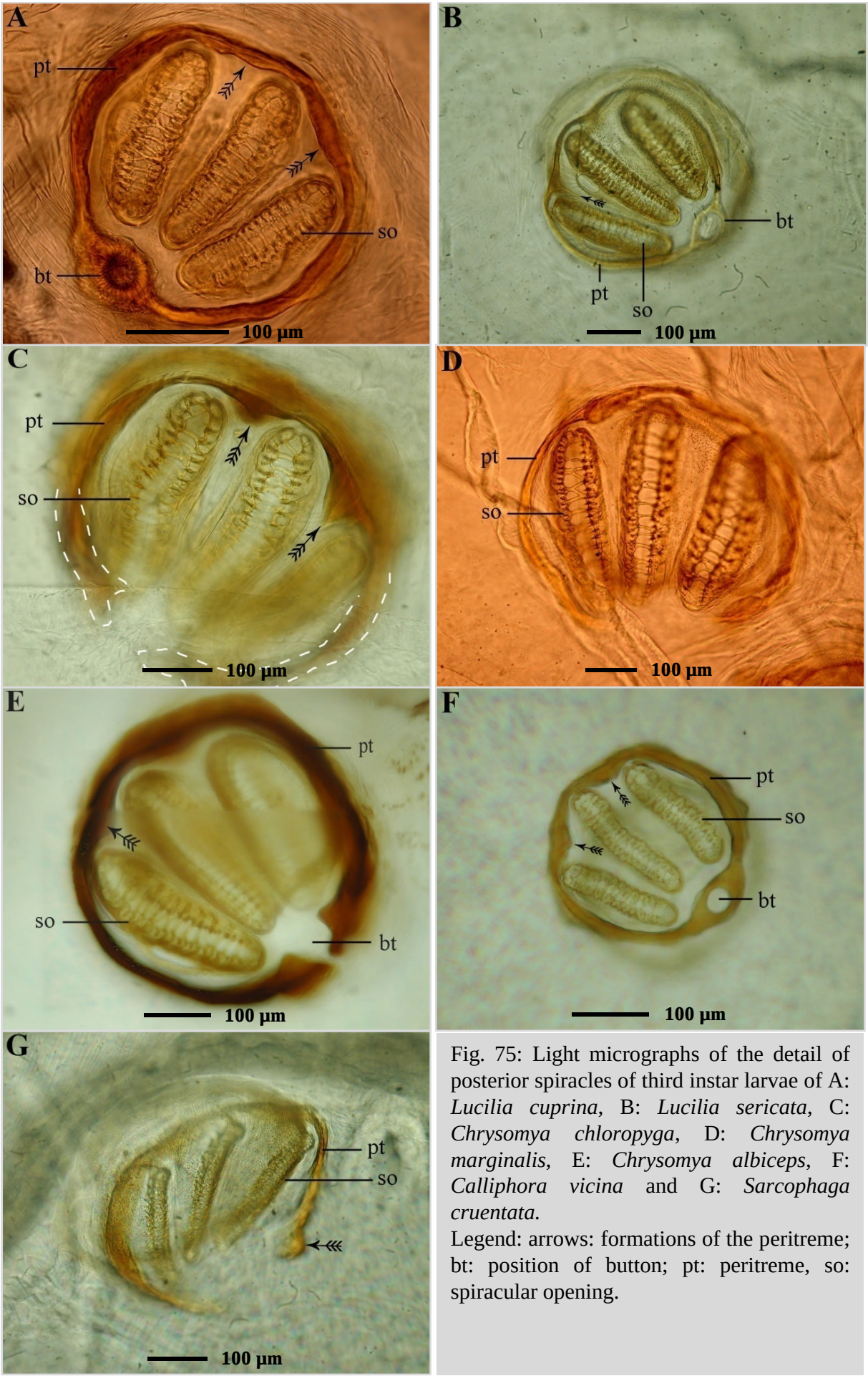


Fig. 75: Light micrographs of the detail of posterior spiracles of third instar larvae of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina* and G: *Sarcophaga cruentata.*

Legend: arrows: formations of the peritreme; bt: position of button; pt: peritreme, so: spiracular opening.

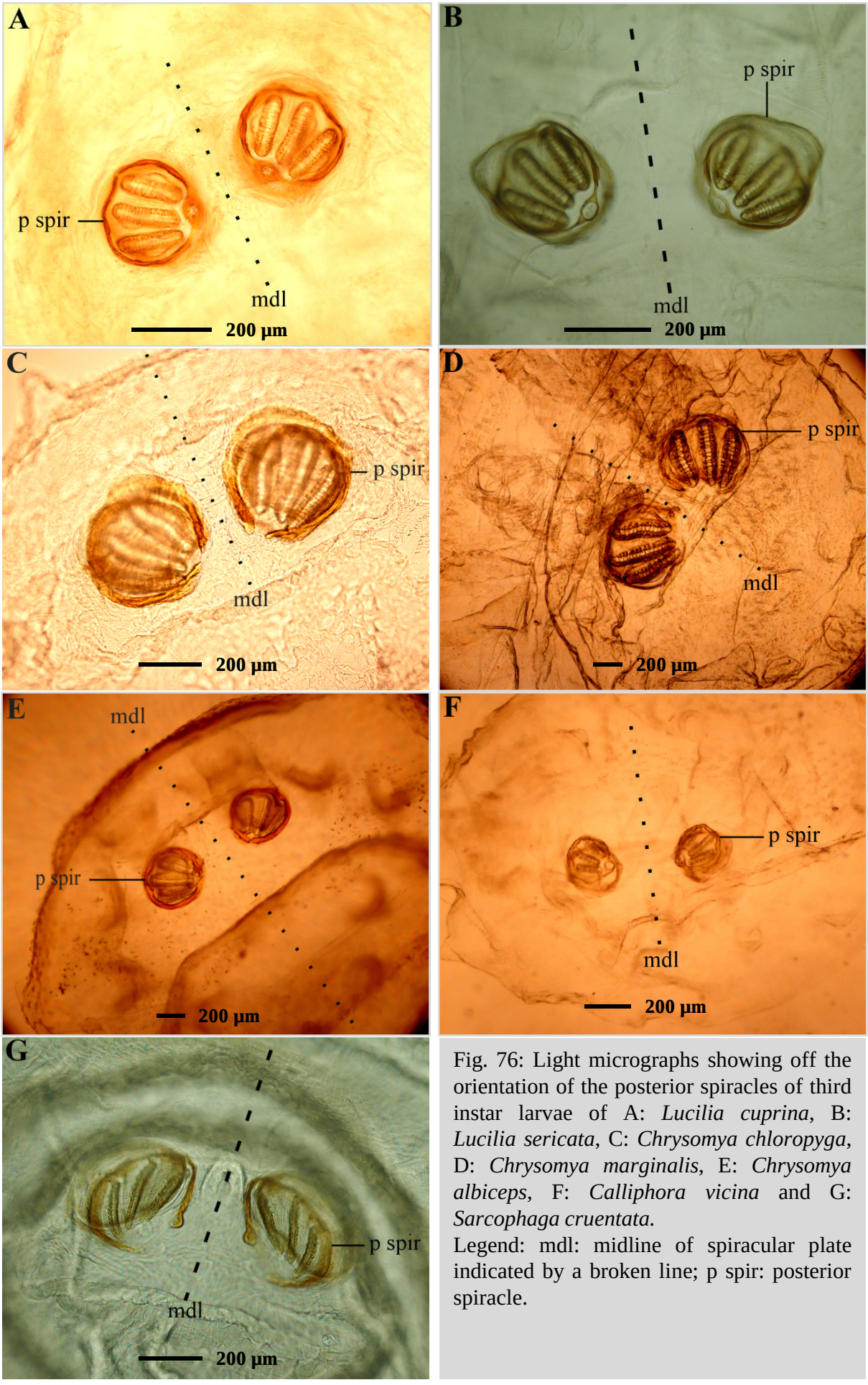


Fig. 76: Light micrographs showing off the orientation of the posterior spiracles of third instar larvae of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina* and G: *Sarcophaga cruentata.*

Legend: mdl: midline of spiracular plate indicated by a broken line; p spir: posterior spiracle.

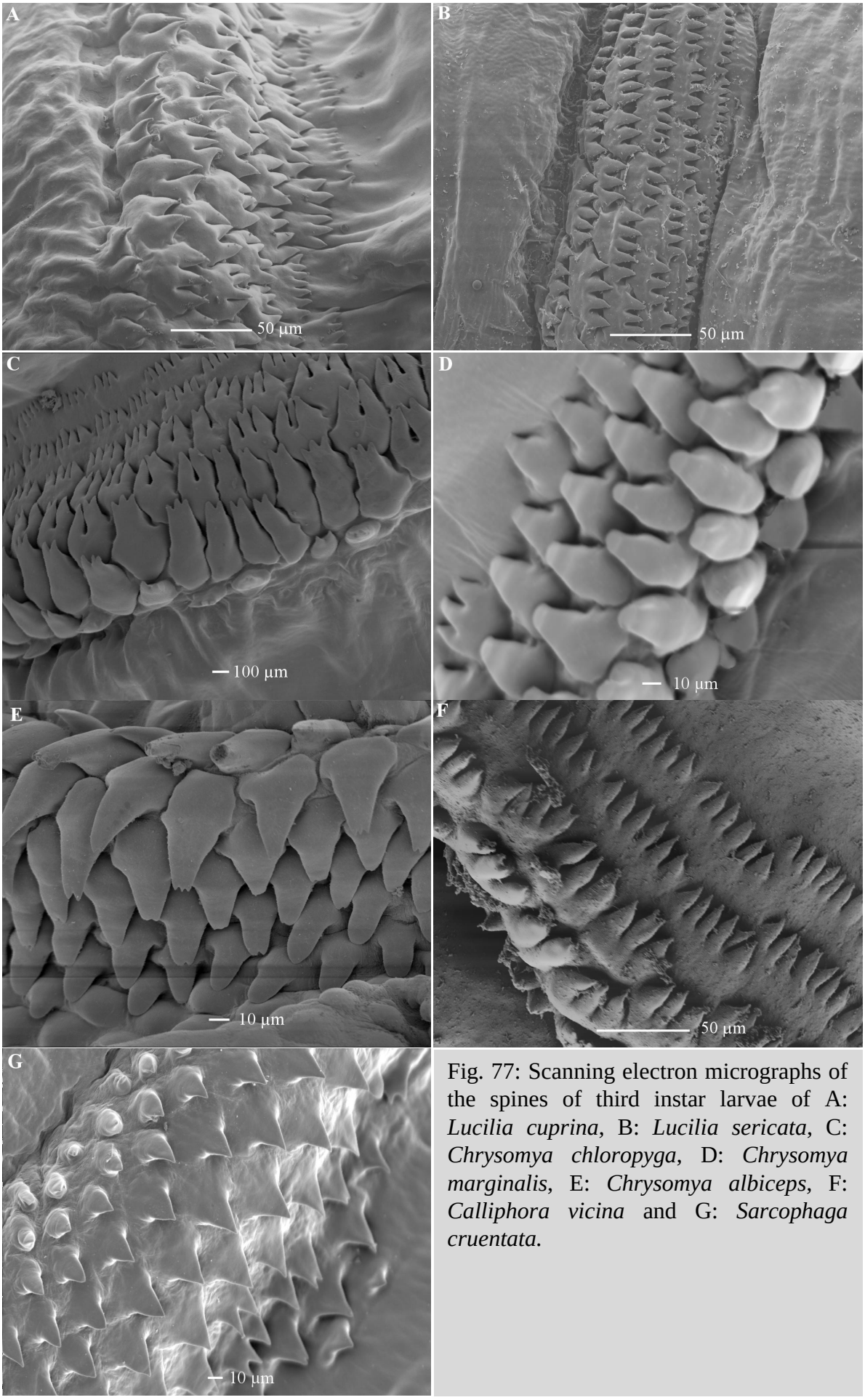


Fig. 77: Scanning electron micrographs of the spines of third instar larvae of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina* and G: *Sarcophaga cruentata.*

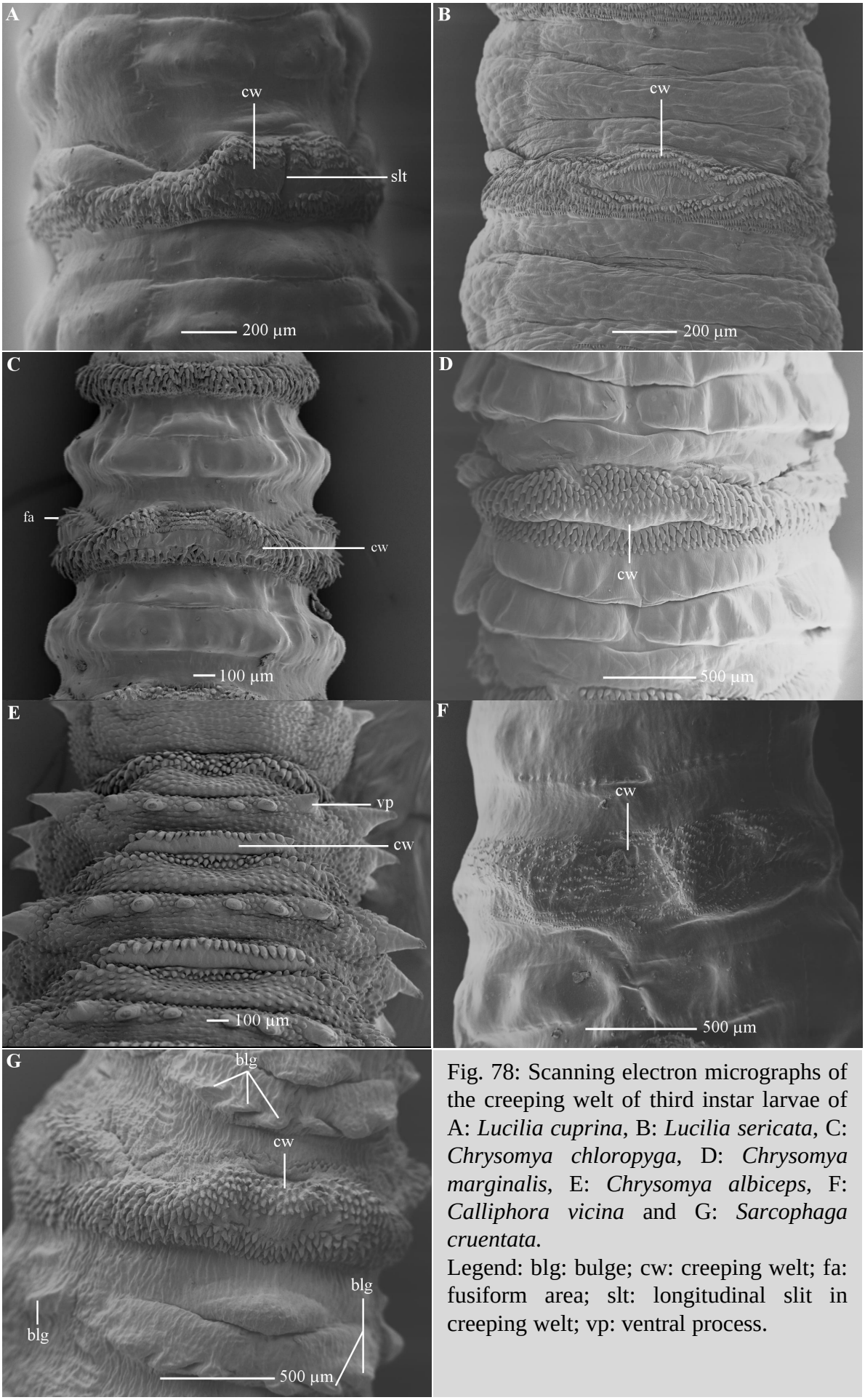


Fig. 78: Scanning electron micrographs of the creeping welt of third instar larvae of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina* and G: *Sarcophaga cruentata.*

Legend: blg: bulge; cw: creeping welt; fa: fusiform area; slt: longitudinal slit in creeping welt; vp: ventral process.

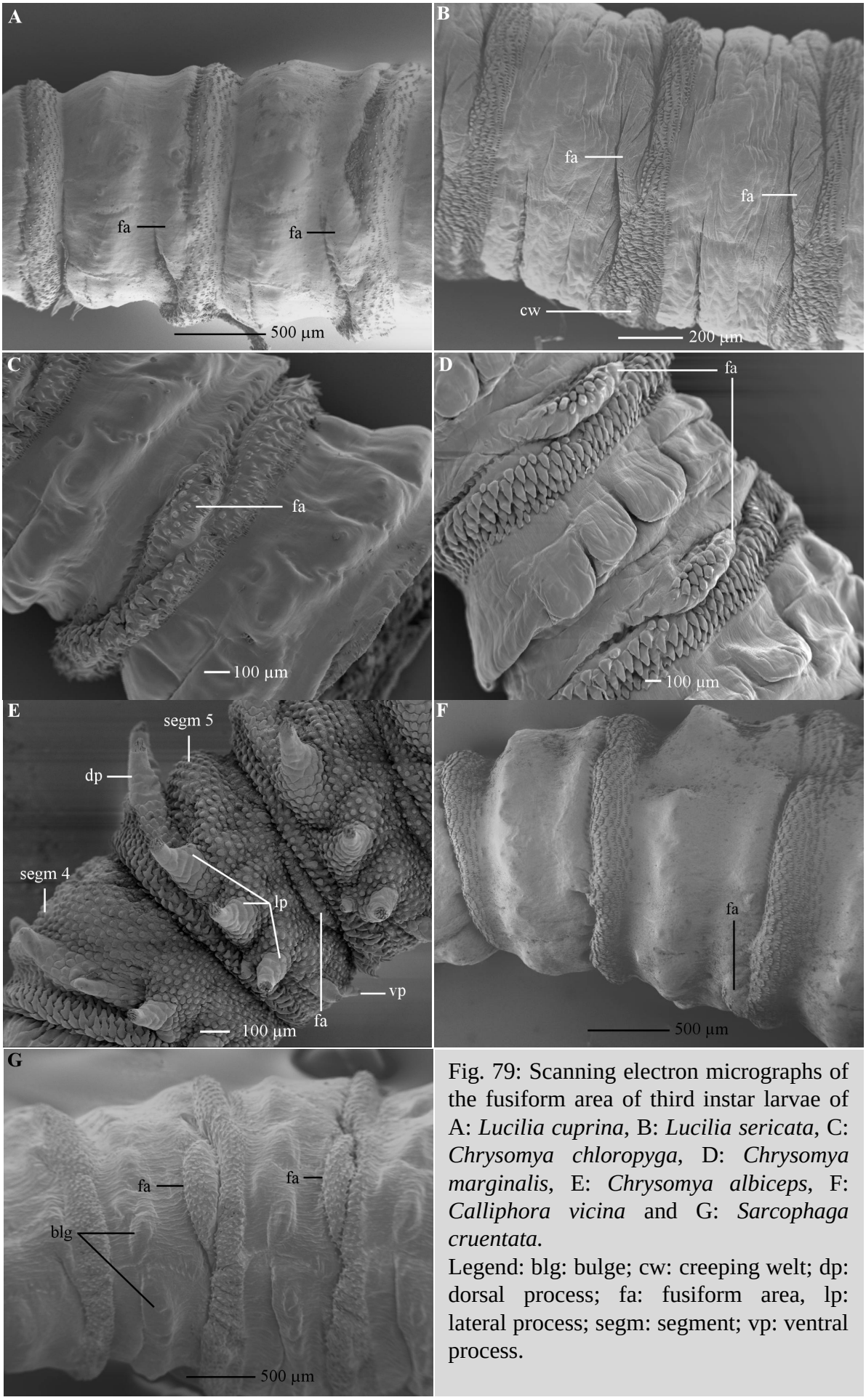


Fig. 79: Scanning electron micrographs of the fusiform area of third instar larvae of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina* and G: *Sarcophaga cruentata*.

Legend: blg: bulge; cw: creeping welt; dp: dorsal process; fa: fusiform area, lp: lateral process; segm: segment; vp: ventral process.

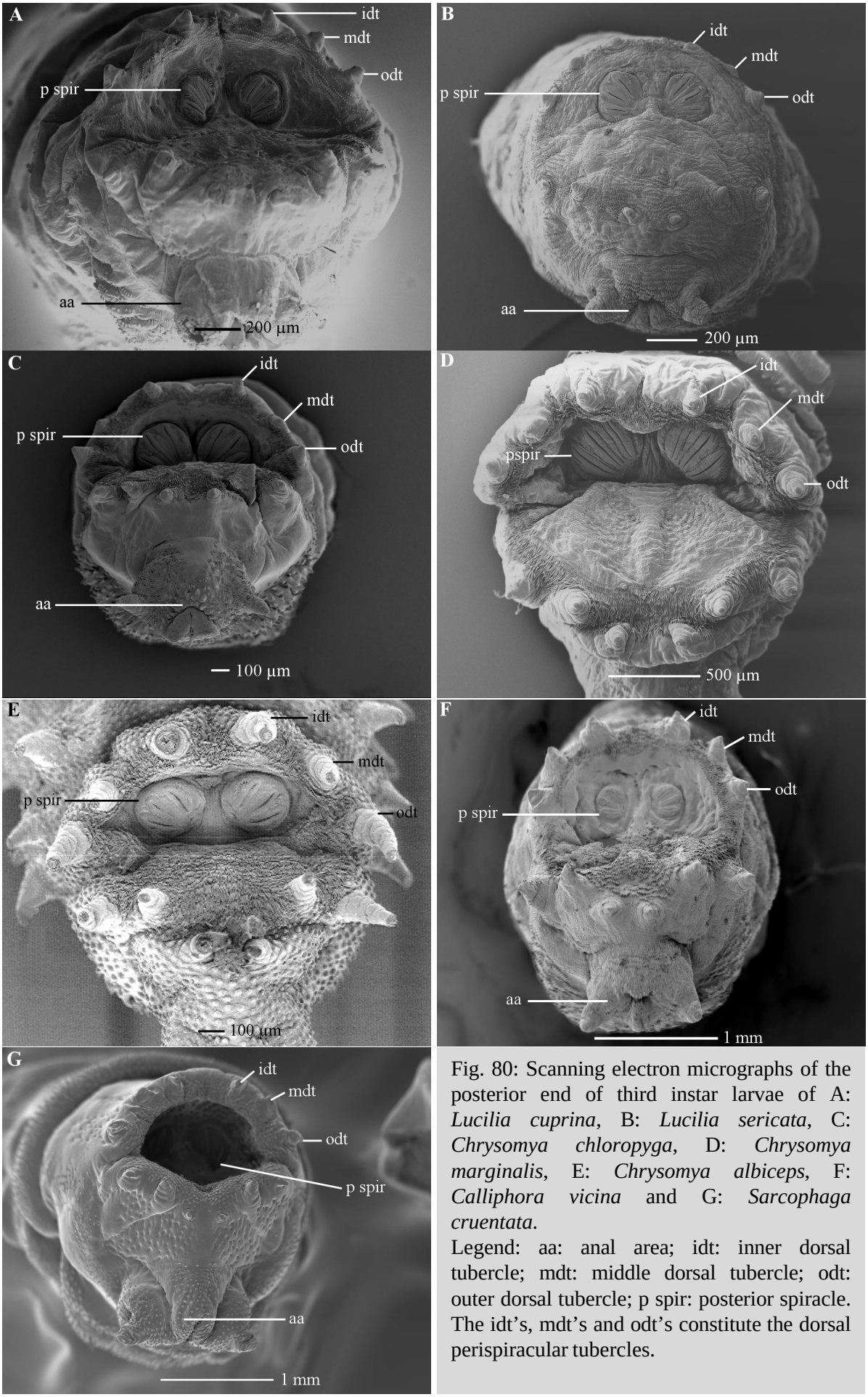


Fig. 80: Scanning electron micrographs of the posterior end of third instar larvae of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina* and G: *Sarcophaga cruentata*.
Legend: aa: anal area; idt: inner dorsal tubercle; mdt: middle dorsal tubercle; odt: outer dorsal tubercle; p spir: posterior spiracle. The idt's, mdt's and odt's constitute the dorsal perispiracular tubercles.

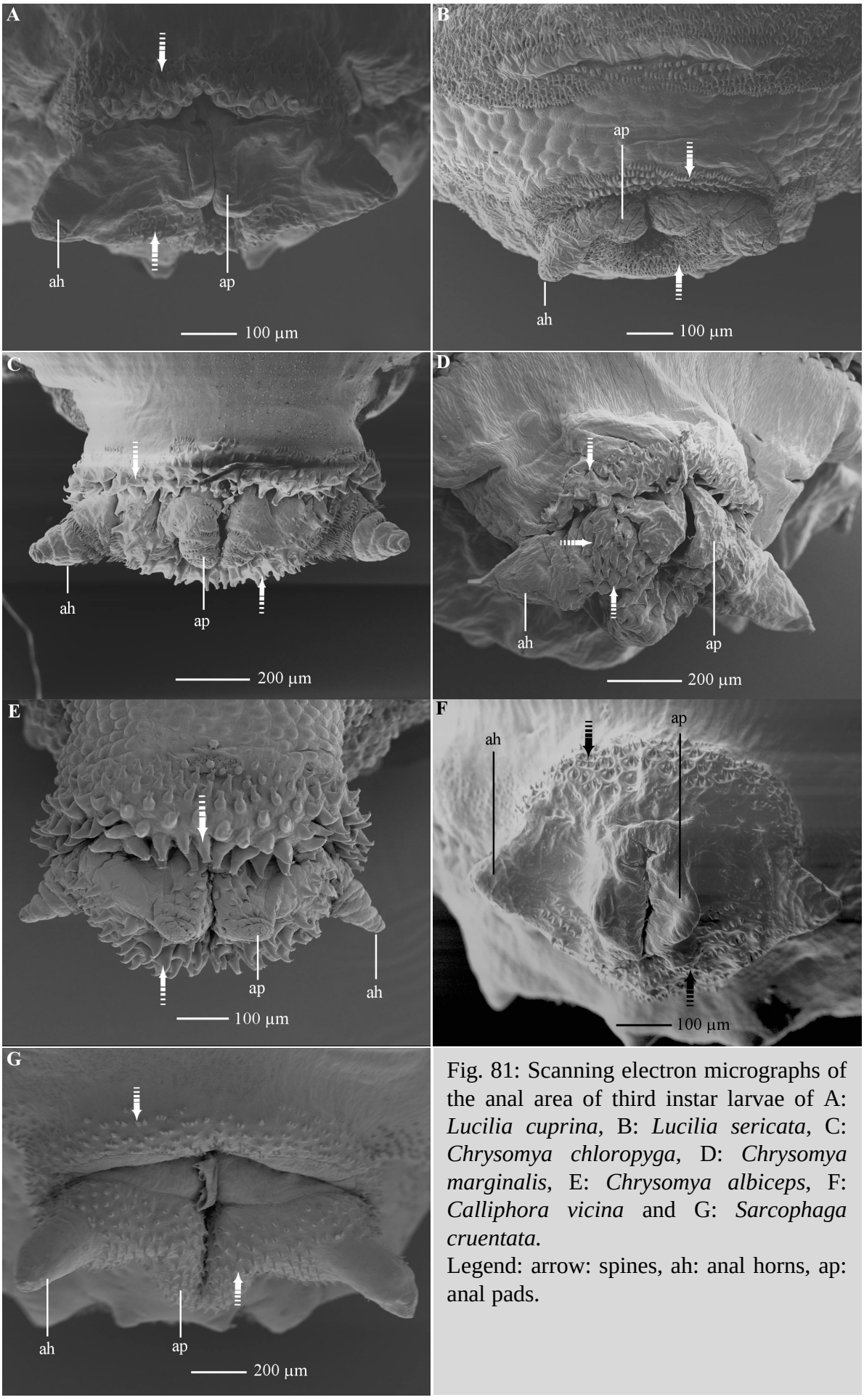


Fig. 81: Scanning electron micrographs of the anal area of third instar larvae of A: *Lucilia cuprina*, B: *Lucilia sericata*, C: *Chrysomya chloropyga*, D: *Chrysomya marginalis*, E: *Chrysomya albiceps*, F: *Calliphora vicina* and G: *Sarcophaga cruentata.*

Legend: arrow: spines, ah: anal horns, ap: anal pads.

3.6. MORPHOLOGY OF PUPARIA

Pupariation, the process whereby the post-feeding larvae shrinks and its integument becomes hardened, is initiated due to environmental stimuli and the resultant secretion of the hormone ecdysone (Greenberg & Kunich 2002). Given that the puparium is formed from the last larval instar integument, most third larval instar characteristics are still of diagnostic value for puparia. Unchanged third instar characteristics are (i) the cephalopharyngeal skeleton which is plastered against the inside ventral wall of the puparium, (ii) the respiratory structures of third instar larvae, namely the anterior and posterior spiracles and (iii) intersegmental spines. Since these characteristics were described in full as part of the third instar larval description, it will not be repeated here as part as the puparium description. Zdarek & Fraenkel (1972) listed shrinkage, water loss and the hardening of the integument as some of the changes that occur during the pupariation process. Due to these changes, the following third instar characteristics were altered: (i) the integument, (ii) the shape of the puparium and (iii) the extent and shape of the anal area. The three anterior segments retract during pupariation (Zdarek & Fraenkel 1972). Due to this change the definitive diagnostic characteristic of the first segment of third instar larvae, namely the labrum, was lost for diagnostic purposes in puparia. However, this retraction of the pseudocephalon resulted in unique folding patterns of the anterior field of the puparium in each of the species. Similar to the labrum of third instar larvae, the unique folding patterns of the anterior field in puparia was identified for its diagnostic potential for the first time during the current study. The lateral ridge and the respiratory structures of the puparium were also found to be differentiated in the puparium and will be evaluated for their diagnostic value.

3.6.1. SHAPE OF THE PUPARIUM (Figs. 82 to 84)

The puparia of the blow- and flesh flies under discussion were generally oblong-shaped. The oblong-shape was punctuated by variation in the termination pattern of its anterior and posterior ends forming part of the current study. Furthermore, in some of the species, a constriction was noted in the anterior third portion of the puparium.

Lucilia cuprina (Figs. 82A, 83A and 84A) and ***Lucilia sericata*** (Figs. 82B, 83B and 84B)

Lucilia cuprina (Figs. 82A, 83A and 84A) and *L. sericata* (Figs. 82B, 83B and 84B) puparia were similar in shape. The narrowing of the puparium to its anterior and posterior ends was gradual and the termination pattern at both ends was rounded (Figs. 82A and 83A for *L. cuprina* and Figs. 82B and 83B for *L. sericata*). Furthermore, the puparium was slightly constricted between segment 5 and 6 (Figs. 82A and 83A for *L. cuprina* and Figs. 82B and 83B for *L. sericata*). The shape of *L. cuprina* puparia examined from Thailand (Sukontason *et al.* 2006a, Sukontason *et al.* 2007) and Peru (Greenberg & Szyska 1984) were similar to that of *L. cuprina* puparia examined during the current study.

Chrysomya chloropyga (Figs. 82C, 83C and 84C)

There was a distinct narrowing of the puparium from segment 5 to the anterior end, resulting in a pointed anterior termination pattern (Figs. 82C, 83C and 84C). A slight constriction was noted between segments 5 and 6 (Figs. 82C and 83C). The narrowing of the puparium to its posterior end was gradual for the largest part, but the narrowing at the last segment was significant (Figs. 82C and 83C). The posterior terminated pattern was relatively narrow and truncated (Figs. 82C, 83C and 84C). The anterior termination pattern in the specimens of *C. putoria* puparia examined by Greenberg & Szyska (1984) was similar to that of *C. chloropyga* puparia examined during the present study. However, the posterior termination pattern of *C. putoria* puparia examined by Greenberg & Szyska (1984) differed from that of *C. chloropyga* puparia examined during the present study. The posterior end narrowed at the penultimate segment, before flaring out at its termination end in the puparia of *C. putoria* examined by Greenberg & Szyska (1984), opposed to the simplified form noted for *C. chloropyga* puparia (Figs. 82C and 83C).

Chrysomya marginalis (Figs. 82D, 83D and 84D)

There was a marked narrowing of the puparium from segment 5 to the anterior end, resulting in a pointed anterior termination pattern (Figs. 82D, 83D and 84D). The puparium was constricted between segments 5 and 6 (Figs. 82D and 83D). From segment 6 the puparium narrowed gradually to its posterior end to terminate broad

and truncated (Figs. 82D, 83D and 84D). All aspects of the puparium shape were similar to that illustrated by Prins (1982) for *C. marginalis* puparia examined in the Cape Province (South Africa).

Chrysomya albiceps (Figs. 82E, 83E and 84E)

There was a marked narrowing of the puparium from segment 5 to the anterior end, resulting in a pointed anterior termination pattern (Figs. 82E, 83E and 84E). The transitions between segments were not smooth in *C. albiceps* puparia (Figs. 82E and 83E). Consequently, the constriction of the puparium noted between segments 5 and 6 in the puparia of other species were not definitive in *C. albiceps* puparia. The narrowing to the posterior end was gradual and it terminated broadly truncated (Figs. 82E, 83E and 84E). The closely related *C. rufifacies* puparia examined in Australia by Kitching (1976a) and those examined in Thailand by Sukontason *et al*. (2006b) was similar in shape to *C. albiceps* puparia examined during the current study.

Calliphora vicina (Figs. 82F, 83F and 84F)

Due to the barely noticeable constriction between segments 5 and 6, the narrowing to the anterior end appeared to originate at segment 6 (Figs. 82F and 83F). Furthermore the narrowing to the anterior end was gradual, with segment 5 not markedly broader than segments 4. The narrowing at segment 3 was more severe, resulting in a pointed anterior termination pattern (Figs. 82F and 83F). The narrowing to the posterior end was gradual and ended rounded (Figs. 82F, 83F and 84F).

Sarcophaga cruentata (Figs. 82G, 83G and 84G)

No constriction was noted at segments 5 and 6 of the puparia (Figs. 82G and 83G). The anterior end narrowed gradually into a rounded shape (Figs. 82G, 83G and 84G). The narrowing to the posterior end was gradual for the largest part, but at the last segment the narrowing was abrupt, forming a narrow, truncated end (Figs. 82G, 83G and 84G). Similar anterior and posterior termination patterns were noted for the sarcophagid, *Liosarcophaga dux*, examined by Sukontason *et al*. (2006a).

The puparium shape as a diagnostic feature

Based on the anterior termination pattern the species were grouped into (i) those with broad, rounded anterior ends (*L. cuprina*, *L. sericata* and *S. cruentata*) and (ii) those

with pointed, narrow anterior ends (*C. chloropyga*, *C. marginalis*, *C. albiceps* and *C. vicina*). For those species grouped together based on their broad, rounded anterior ends, the posterior termination patterns and the constrictions at segments 5 and 6 revealed no distinct attributes to separate *L. cuprina* (Figs. 82A and 83A) from *L. sericata* (Figs. 82B and 83B). *Sarcophaga cruentata* (Figs. 82G and 83G) was clearly distinguishable from the puparia of the two *Lucilia* species based on the lack of any constriction at segments 5 and 6 and its narrow, truncated posterior termination pattern. For those species grouped by their narrow, pointed anterior ends, *C. albiceps* (Figs. 82E and 83E) was differentiated from the other species due to the distinct constrictions noted between segments, which were in contrast to the smooth transition between segments noted in the other species. The termination pattern of the posterior end allowed for a distinction among the remainder of these species. In *C. vicina* puparia (Figs. 82F and 83F) the posterior ends were rounded; broad and truncated in puparia of *C. marginalis* (Figs. 82D and 83D) and narrow and truncated in puparia of *C. chloropyga* (Figs. 82C and 83C).

As a diagnostic characteristic, distinguishing among the species based on the shape of the puparium was possible for almost all of the species. The two *Lucilia* species could not be distinguished from each other on the basis of this characteristic.

The shape of the puparia revealed no intraspecific variation as demonstrated by the similarities of the puparia in other populations (Greenberg & Szyska 1984, Sukontason *et al.* 2006a, Sukontason *et al.* 2007) of *L. cuprina*. Similarly, the shape of the puparia of *C. marginalis* examined by Prins (1982) did not show variation when compared to the *C. marginalis* puparia examined during the current study.

Based on the differences in the posterior termination forms, the shape of the puparium can be utilised to distinguish between the closely related *C. chloropyga* and *C. putoria*. However, it was not useful to distinguish between the closely related *C. albiceps* and *C. rufifacies*.

3.6.2. TEXTURE OF THE PUPARIUM SURFACE (Figs. 82 to 86)

The reflectivity of the puparium surface was evaluated by Amorim & Ribeiro (2001), but was not utilised in that publication to distinguish among the species. This might be because of the subjective nature of an assessment based on comparative reflectivity of the puparium surfaces in different species. Differences in reflectivity were difficult to define or measure. For the purpose of the current study, reflectivity was assessed by comparing the differences in reflectivity in the same puparium (i) between different segments and (ii) between the dorsal, lateral and ventral aspects of the puparium. The puparium surface was also assessed for (i) the nature of the transverse surface striations, (ii) indentations {*indent*}, (iii) pleating {*pleat*} and (iv) projections of the puparium surface.

Lucilia cuprina (Figs. 82A, 83A, 84A, 85A and 86A)

All surfaces were equally reflective (Figs. 82A, 83A and 84A). The puparium surface was smooth, with fine, superficial transverse striations (Fig. 85A). No marked indentations or pleating of the puparium surface was noticed (Figs. 82A, 83A and 84A). Small, narrow, barely visible ridges were located on the dorso-lateral and ventro-lateral aspects of most segments (Fig. 86A). The smoothness of the puparium surface was also noted in *L. cuprina* puparia examined by Sukontason *et al.* (2006a).

Lucilia sericata (Figs. 82B, 83B, 84B, 85B and 86B)

The ventral surface (Fig. 83B) was slightly less reflective than the dorsal (Fig. 82B) and lateral surfaces (Fig. 84B). All other aspects of the puparium surface was similar to that of *L. cuprina* puparia: (i) no marked indentations or pleating of the puparium surface (Figs. 82B, 83B and 84B) (ii) fine, superficial transverse striations on the puparium surface (Fig. 85B) and (iii) small, narrow, barely visible ridges on the dorso-lateral and ventro-lateral aspects of most segments (Fig. 86B). Similar to the findings of the current study, the puparium surface of *L. sericata* specimens examined by Liu & Greenberg (1989) were also described as smooth.

Chrysomya chloropyga (Figs. 82C, 83C, 84C, 85C and 86C)

The dorsal surface (Fig. 82C) was more reflective than the ventral - (Fig. 83C) and lateral surfaces (Fig. 84C). Distinct transverse striations marked the surface of the puparium (Fig. 85C). Indentations of varying depths marked the lateral surface on

some segments, with a resultant pleating of the area between them (Fig. 86C). Amorim & Ribeiro (2001) did not refer to the difference in the reflectivity between the dorsal surface and the other surfaces, but described the surface of *C. putoria* puparia as dull. The micrograph presented by Greenberg & Szyska (1984) of the puparium of *C. putoria* revealed its rough surface and the indentations on the lateral aspect of the puparium; findings comparable to that found for *C. chloropyga* puparia examined during the current study.

Chrysomya marginalis (Figs. 82D, 83D, 84D, 85D and 86D)

The dorsal surface (Fig. 82D) was generally more reflective than the ventral - (Fig. 83D) and lateral surfaces (Fig. 84D). From segments 3 to 5 the puparium surface was generally smooth with fine, superficial transverse striations and the surface was more reflective at this location than at the subsequent segments (Fig. 85D). Robust transverse striations covered the puparium surface from segments 6 to 12 with a resultant diminished reflectivity (Fig. 85D). Shallow indentations were noted on the lateral aspect of some segments (Fig. 86D). Prins (1982) did not refer to the difference in striation of the anterior and posterior segments, but described the coarse transverse striations of the puparium in general.

Chrysomya albiceps (Figs. 82E, 83E, 84E, 85E and 86E)

The puparium surface was adorned with dorsal and lateral processes (Figs. 82E, 83E, 84E and 86E) as well as numerous small papillae (Fig. 85E), similar to that found on the integument of third instar *C. albiceps* larvae. In *C. rufifacies* the puparium surface was similarly adorned with processes and papillae (Sukontason *et al*. 2006b). The micrographs presented by Sukontason *et al*. (2006b) indicated that the small papillae and the spines covering the tips of the processes, i.e. the same characteristics used to distinguish among the third instar larvae of the two species, can be utilised for distinction between the puparia of *C. albiceps* and *C. rufifacies*.

Calliphora vicina (Figs. 82F, 83F, 84F, 85F and 86F)

All surfaces of the puparium exhibited the same level of reflectivity (Figs. 82F, 83F and 84F). The puparium surface was smooth, with fine, superficial transverse striations (Fig. 85F). Small, narrow, slightly wavy ridges were noted on most segments on the lateral midline as well as in a dorso-lateral and a ventro-lateral

position (Figs. 84F and 86F). Liu & Greenberg (1989) described a smooth puparium surface for the specimens of *C. vicina* puparia examined by them. Similarly, Prins (1982) described the puparium surface as smooth, dull to slightly shiny with fine transverse striations for the closely related *C. croceipalpis* puparia.

Sarcophaga cruentata (Figs. 82G, 83G, 84G, 85G and 86G)

All surfaces of the puparium had the same level of reflectivity (Figs. 82G, 83G and 84G). The puparium surface was smooth, with fine, superficial, transverse striations and papillae (Fig. 85G). These papillae were only slightly raised from the surface of the puparia. Superficial, barely noticeable indentations were observed on the lateral aspects of various segments (Figs. 84G and 86G).

The puparium surface texture as a diagnostic feature

The species can be broadly divided into two groups, i.e. (i) those with smooth surfaces with fine, superficial transverse striations (*L. cuprina, L. sericata, C. vicina* and *S. cruentata*) and (ii) those with roughened surfaces with coarse transverse striations or with projections (*C. chloropyga, C. marginalis* and *C. albiceps*). Although *L. sericata* puparia exhibited somewhat differential reflectivity on some surfaces (Figs. 82B, 83B and 84B), this variation was so slight that it could not be utilised to separate it from the other species in that group, which all exhibited equal reflectivity on all surfaces. *Calliphora vicina* was distinct due to the small, but clearly visible narrow, wavy ridges seen on the midline of the lateral surface as well as on the dorso-lateral and dorso-ventral areas of the puparia (Figs. 84F and 86F). *Sarcophaga cruentata* was recognisable due to the delicate, superficial indentations on its lateral aspect (Figs. 84G and 86G). No indentations were noted and the ridges on the puparium surface were simple and barely noticeable for *L. cuprina* (Fig. 86A) and *L. sericata* (Fig. 86B). The differences in the puparium surface for these two species were too slight to differentiate between them. In the group with rough integuments, the processes and papillae on the puparium surface of *C. albiceps* (Figs. 82E, 83E, 84E and 85E) distinguished it from the rest of the species. *Chrysomya marginalis* puparia (Fig. 85D) were distinguished from *C. chloropyga* puparia based on the difference in reflectivity of its anterior segments compared to the posterior segments, whereas the puparium surface of *C. chloropyga* was uniform (Fig. 85C) in this regard.

As a diagnostic feature, the puparium surface can be utilised to distinguish among most of the species examined. Due to insufficient variation noted in the puparia surfaces, it was not possible to distinguish the two *Lucilia* species from each other.

Comparisons regarding the puparium surface texture in different populations were possible for *L. cuprina*, *L. sericata*, *C. marginalis* and *C. vicina*. The conclusions drawn from the relevant publications (Prins 1982, Liu & Greenberg 1989 and Sukontason *et al.* 2006a) showed an absence of intraspecific variation for these species.

The available information regarding the puparium surface texture of *C. putoria* puparia (Greenberg & Szyska 1984) did not reveal differences to distinguish it from *C. chloropyga* puparia examined during the current study. The same characteristics used to separate third instar *C. albiceps* larvae from third instar *C. rufifacies* larvae, can be utilised to distinguish the puparia of these two species from each other.

3.6.3. LATERAL RIDGE (Fig. 87)

The lateral ridge, as its name indicates, was located on the lateral aspect of the puparium, stretching across segments 3 and 4. The lateral ridge was evaluated in terms of its extent and prominence.

Lucilia cuprina (Fig. 87A)

The lateral ridge was narrow (Fig. 87A). In the majority of the cases, the lateral ridge was in a more dorsal position on segment 4 than on segment 3. It continued up to the posterior margin of segment 4. The lateral ridge was similar in *L. cuprina* puparia examined by Sukontason *et al.* (2006a) to those found during the current study.

Lucilia sericata (Fig. 87B)

The lateral ridge was narrow (Fig. 87B). In the majority of specimens examined the lateral ridge continued on segment 4 at approximately the same level as on segment 3. The lateral ridge continued up to the posterior margin of segment 4.

Chrysomya chloropyga (Fig. 87C)

The lateral ridge was contained in a prominent lateral ridge area (Fig. 87C). Generally the thin ridge could not be distinguished from the rest of the ridge area. The lateral ridge area was narrow at its origin on segment 3 and widened gradually to its termination end on segment 4 (Fig. 87C). Shallow transverse striations, similar to those noted on the rest of the puparium surface, were noted for the lateral ridge area.

Chrysomya marginalis (Fig. 87D)

The lateral ridge was contained in a prominent lateral ridge area (Fig. 87D). Generally, the thin ridge could not be distinguished from the rest of the ridge area. The lateral ridge area was narrow at its origin on segment 3 and widened gradually to its termination end at segment 4. Deep transverse striations, similar to those noted on the rest of the puparium surface, were observed for the lateral ridge area.

Chrysomya albiceps (Fig. 87E)

The lateral ridge was contained in a prominent lateral ridge area. Generally, the thin ridge could not be distinguished from the rest of the ridge area. The lateral ridge area was narrow at its origin on segment 3 and ended markedly wider at its termination end on segment 4 (Fig. 87E). Similar to the integument on the rest of the puparium, papillae adorned the surface of the ridge area. This overall structure of the lateral ridge area was similar to that of *C. rufifacies* puparia examined by Sukontason *et al*. (2006b).

Calliphora vicina (Fig. 87F)

The lateral ridge was narrow (Fig. 87F) and was contained within a slightly raised ridge area. This narrow, raised area was of equal width across the two segments. It continued on segment 4 on approximately the same level as on segment 3 in the majority of specimens examined. The lateral band continued to the spine band on the posterior aspect of segment 4.

Sarcophaga cruentata (Fig. 87G)

The lateral ridge was narrow (Fig. 87G). Its path on segment 4 was not at the same level as in segment 3. In most of the specimens examined, the lateral ridge extended

to the posterior aspect of segment 4. The puparia of the sarcophagid, *L. dux* presented with a similar narrow lateral ridge (Sukontason *et al*. 2006a).

The prominence of the lateral ridge as a diagnostic feature

Two categories can be defined, (i) where the puparia presented with a narrow lateral ridge (*L. cuprina*, *L. sericata*, *C. vicina* and *S. cruentata*) and (ii) where the puparia presented with a prominent lateral ridge area (*C. chloropyga*, *C. marginalis* and *C. albiceps*). For those species with a narrow lateral ridge area, *C. vicina* was distinguished from the other by the slightly raised ridge area (Fig. 87F). *Sarcophaga cruentata* was distinguished from the two *Lucilia* species by the lateral ridge extending into the spine band area of the fourth segment. Generally, the lateral ridge was in a slightly more dorsal position on the fourth segment than on the third segment in *L. cuprina* (Fig. 87A), compared to it being on approximately equal levels in *L. sericata* (Fig. 87B). In those species with a prominent lateral ridge area, characteristics of the puparium surface and not so much the ridge itself was utilised for diagnostic purposes. In *C. chloropyga* the striations were superficial, compared to the deep striations in *C. marginalis* (Fig. 87D) and the distinct papillae in *C. albiceps* (Fig. 87E). In conclusion, a complete identification based on the lateral ridge itself was not possible. The species under discussion could just be separated into two broad categories. For those species with narrow lateral ridges, the variability in the aspects examined rendered the lateral ridge unsuitable as a diagnostic feature. In those species with broad lateral ridge areas, the lateral ridge area itself was not adequately varied and characteristics of the puparium surface had to be considered in conjunction with it to distinguish the species from each other.

The lateral ridge area was not examined in detail in the literature. Consequently, an assessment of whether the lateral ridge could be utilised to distinguish the closely related *C. albiceps* from *C. rufifacies*, as well the closely related *C. putoria* from *C. chloropyga* could not be made.

3.6.4. FRONTAL FIELD (Fig. 88)

The first segment, the pseudocephalon, of the third instar larva will be withdrawn into the puparium during pupariation. Due to this, the structures of the pseudocephalon

were not visible in the puparium. The withdrawal of the pseudocephalon resulted in the formation of distinct folds of the frontal field of the puparia in the different species. An aperture, i.e. the mouth scar {*m scr*}, is formed due to the retraction of the pseudocephalon. The upper and lower frontal fields were demarcated by the position of the mouth scar. The unique folding patterns of the frontal field were identified as a diagnostic characteristic for the first time in the present study.

Lucilia cuprina (Fig. 88A)

Predominantly small, tightly wrinkled, deep-set folds were typical of the frontal field (Fig. 88A). The folds around the mouth scar were the largest and were arranged radial symmetrically around the mouth scar. On the upper frontal field, in a position between the anterior spiracles, the large folds that originated from the mouth scar transcended into smaller folds. These folds continued up to approximately a third from the periphery of the frontal field. The rest of the folds of the frontal field, i.e. those on the periphery of the frontal field and on the lateral and lower margins of the frontal field were the smallest of the folds. *Lucilia cuprina* puparia examined by Sukontason *et al*. (2006a) exhibited a similar folding pattern to that of *L. cuprina* examined during the current study.

Lucilia sericata (Fig. 88B)

Small, superficial folds were typical of the frontal field of *L. sericata* puparia (Fig. 88B). The folds around the mouth scar were the largest. The extent of these folds was not radially symmetrical around the mouth scar, extending further on the upper frontal field than on the lower frontal field. These large folds transcended into smaller folds in the area between the anterior spiracles and continued up to approximately a third from the periphery of the frontal field. The smallest folds were located on the periphery of the frontal field, as well as on the lateral and ventral aspects of the frontal field.

Chrysomya chloropyga (Fig. 88C)

A combination of large and small folds graced the surface of the frontal field of *C. chloropyga* puparia (Fig. 88C). The folds were wrinkled and deep-set. The large folds dominated the upper frontal field and were contained between the two anterior spiracles. It transcended into smaller folds on the periphery of this field. The folds of

the lower frontal field, in the area of the mouth scar, were similar in size to the large folds of the upper frontal field. These large folds were contained in a relatively small area around the mouth scar. The folds radiating from the fore-mentioned folds transcended into smaller folds. The folds on the periphery - and on the lateral aspect of the frontal field were the smallest folds in the frontal field.

Chrysomya marginalis (Fig. 88D)

Large folds dominated the frontal field of *C. marginalis* puparia (Fig. 88D). All folds were wrinkled and deep-set. The larger folds occupied the whole of the upper frontal field. These large folds were not contained in the area between the anterior spiracles, but were extended to the area below the anterior spiracles, radiating laterally. A small area around the mouth scar on the lower frontal field also contained large folds. The rest of the folds on the lower frontal field were small.

Chrysomya albiceps (Fig. 88E)

The folds of *C. albiceps* puparia were in the form of ridges and defined loops (Fig. 88E). Central in the frontal field a longitudinal, hairpin-shaped loop {*hrp l*} was noted. A large loop {*lrg l*} was formed on both sides of the hairpin-shaped fold. The loop intersected with the centralised hairpin fold at approximately its midpoint. On the upper frontal field, originating from the loops, on both side of the hairpin-shaped fold, bilateral symmetrical ridges {*lrg rdg*} originated, stretching to the margin of the upper frontal field. Between these ridges, above the hairpin fold, a smaller ridge {*sml rdg*} was formed centrally. From the lower margin of the large loop a smaller loop {*sml l*} originated, projecting ventrally. Central from these loops, unsymmetrical ridges {*rdg*} were noted.

Calliphora vicina (Fig. 88F)

Folds were small, deep-set and wrinkled in *C. vicina* puparia (Fig. 88F). The folds converging into the mouth scar that had smooth edges and were the largest folds in the frontal field. These larger folds were not arranged radially symmetrical around the mouth scar, with its lower and lateral aspects not extending as far as that of the upper frontal field. The large folds on the upper frontal field transcended into smaller folds that were contained between the anterior spiracles. On the periphery of the upper

frontal field it transcended into even smaller folds. The rest of the folds, i.e. those on the lateral and on the lower frontal fields were also small.

Sarcophaga cruentata (Fig. 88G)

The frontal field was characterised by large, structured, well-defined folds in *S. cruentata* puparia (Fig. 88G). All, but the central fold of the upper frontal field, converged to the position of the mouth scar. The middle central fold ended at a position halfway within the two adjacent folds. The medial ventral fold {*m v f*} were in the shape of a lip-like structure overlapping the lateral folds as they converged into the mouth scar. All folds were similar in size. The folds of *S. cruentata* puparia examined were similar to that presented by Sukontason *et al.* (2006a) for *L. dux* puparia.

The frontal field as a diagnostic feature

The puparia of the different species could be separated into three categories, (i) those with unwrinkled, structured folds or ridges (*C. albiceps* and *S. cruentata*), (ii) those with large folds (*C. chloropyga* and *C. marginalis*) and (iii) those with small folds (*L. cuprina*, *L. sericata* and *C. vicina*). Those puparia of the species that falls in the first category were markedly different from each other. *Chrysomya albiceps* puparia had distinct ridges and loops (Fig. 88E) and *S. cruentata* puparia had structured unwrinkled folds, with the middle lower one shaped as a lip-like structure (Fig. 88G). Those puparia of the species that were grouped in the second category were separated based on the containment area of the folds on the upper frontal field. In *C. marginalis* puparia the largest folds were not contained within the margins set by the anterior spiracles (Fig. 88D), opposed to the large folds of *C. chloropyga* puparia that were contained between the margins set by the anterior spiracles (Fig. 88C). Those puparia of the species grouped in the third category were separated due to superficial folds observed in *L. sericata* puparia (Fig. 88B), opposed to the deep folds observed in *C. vicina* (Fig. 88F) and *L. cuprina* puparia (Fig. 88A). The larger folds around the mouth scar were arranged radially symmetrical in *L. cuprina* puparia (Fig. 88A), opposed to it being arranged radially unsymmetrical around the mouth scar as in *C. vicina* puparia (Fig. 88F).

This characteristic was not discussed specifically anywhere else in the literature, consequently few micrographs or sketches were available for comparison. Sukontason *et al*. (2006a) showed this view of puparia to illustrate their discussion on the anterior spiracles. Subsequently a comparison with *L. cuprina* was possible. This showed that the characteristic was similar in *L. cuprina* puparia examined during the present study to that of *L. cuprina* puparia examined by Sukontason *et al*. (2006a). This indicates a lack of intraspecific variation regarding this feature. Since the sarcophagid examined by Sukontason *et al*. (2006a) were not the same species as that examined during the current study, comparisons made in this regard were generalised to the genus level. The similarities between *L. dux* puparia examined by Sukontason *et al*. (2006a) and *S. cruentata* puparia examined during the current study indicated that utilising this characteristic might be problematic when distinguishing sarcophagids from each other.

3.6.5. RESPIRATORY STRUCTURES (Fig. 89)

The respiratory structures of the puparium were located in a dorso-lateral position on the fifth segment of the puparium. The bubble membrane differed in the species under discussion in terms of (i) whether the bubble membrane was level with the puparium surface, was raised from the puparium surface or was located within a depression, (ii) the shape and size of bubbles and (iii) the arrangement of the bubbles. The bubble membrane was a temporary structure and was destroyed in older puparia by the emerging respiratory horn breaking through it. The morphology of the respiratory horn was not clear due to the debris from the bubble membrane obscuring it, and could thus not be assessed for its diagnostic value.

Lucilia cuprina (Fig. 89A)

A mix of smaller and larger sized bubbles, arranged in close proximity to each other, was contained in the bubble membrane (Fig. 89A). The bubbles were not organised in a structured pattern. The bubble membrane was level with the puparium surface. Similar to the findings of the present study, Sukontason *et al*. (2006a) showed a clustered bubble membrane for the puparia of *L. cuprina* examined.

Lucilia sericata (Fig. 89B)

All bubbles were approximately equal in size (Fig. 89B). The bubbles on the periphery of the bubble membrane were arranged in a circular ring, surrounding those bubbles in the centre. The bubbles were in close proximity to each other. Furthermore, the bubble membrane was level with the puparium surface. The bubble membrane of *L. sericata* puparia examined by Liu & Greenberg (1989) did not display the same orderly arrangement of bubbles as that of the puparia of *L. sericata* examined during the current study.

Chrysomya chloropyga (Fig. 89C)

A mix of small and large sized bubbles, arranged in close proximity to each other, constituted the bubble membrane (Fig. 89C). The smaller bubbles were located on the periphery of the bubble membrane, surrounding the centralised grouping of larger bubbles. The bubble membrane was level with the puparium surface.

Chrysomya marginalis (Fig. 89D)

Bubbles in the bubble membrane were small, elongated structures of similar size (Fig. 89D). The bubbles were arranged as a small centralised grouping and two outlying groups of bubbles arranged semicircularly around the centralised group of bubbles. Within a grouping, the bubbles were in close proximity to each other, but the separate groupings were detached from each other. The bubble membrane was located in a slight depression of the puparium surface.

Chrysomya albiceps (Fig. 89E)

Bubbles in the bubble membrane were similar in size (Fig. 89E). The bubble membrane was made up of two concentric rings of bubbles, semi-encircling a cluster of bubbles at its centre. The bubble membrane was raised from the puparium surface. The bubble membrane of *C. rufifacies* puparia presented by Liu & Greenberg (1989) was not adequately displayed and thus a comparison with the bubble membrane of *C. albiceps* puparia was not possible.

Calliphora vicina (Fig. 89F)

The bubble membrane (Fig. 89F) was made up of smaller bubbles surrounding the larger ones at the centre of the bubble membrane. All bubbles were in close proximity

to each other. The bubble membrane was located within a slight depression of the puparium surface.

Sarcophaga cruentata

No bubble membrane was present on the dorso-lateral surface of the fifth segment. Sukontason *et al.* (2006a) also commented on the absence of a bubble membrane for *L. dux* puparia.

The bubble membrane as a diagnostic feature

Sarcophaga cruentata was unique due to the lack of a bubble membrane. The rest of the species were grouped into two groups, based on whether (i) the bubble membrane was located in a depression or (ii) not. In the first group were those species where the bubble membrane was located on a flat or raised area (*L. cuprina*, *L. sericata*, *C. chloropyga* and *C. albiceps*) and the second group where the bubble membrane was located in a depression (*C. marginalis* and *C. vicina*). For those on a flat or raised surface, the puparium of *C. albiceps* was clearly distinguished by the two concentric rings of bubbles encompassing the centralised bubbles (Fig. 89E). In *L. sericata* puparia, all bubbles were of similar size and were arranged as a concentric ring of bubbles surrounding the centralised bubbles (Fig. 89B). The bubbles were the same size, but arranged in an unstructured manner in *L. cuprina* puparia (Fig. 89A). In *C. chloropyga* puparia, the smaller bubbles surrounded the centralised larger bubbles (Fig. 89C). In the second grouping of species, the bubbles were small and elongated in *C. marginalis* puparia (Fig. 89D). Furthermore, the groupings of bubbles in this species were widely spaced from each other and were arranged in two disrupted concentric rings around the centralised bubbles (Fig. 89D). In *C. vicina* puparia, the closely grouped bubbles were arranged with the smaller bubbles on the periphery surrounding the larger centralised bubbles (Fig. 89F).

The bubble membrane exhibited enough unique characteristics to allow for it to be utilised distinguishing between the puparia of the various species examined during the present study. Unfortunately the bubble membrane was available for only a very brief period of time, due to it being destroyed by the emergent respiratory horn.

The evidence at hand suggested that intraspecific variation might be an issue, as the bubble membranes of *L. sericata* puparia examined during the current study, differed from that of the New World population examined by Liu & Greenberg (1989).

3.6.6. FEATURES OF THE TERMINAL SEGMENT (Figs. 90 to 93)

The terminal segment accommodates the spiracular plate with its perispiracular tubercles {*p tub*}, a pair of posterior spiracles {*p spir*) as well as the anal area. The spiracular plate was assessed for whether it followed an approximate convex (bulged) or concave (recessed) profile. The perispiracular tubercles, located on the rim of the spiracular plate, were assessed for their prominence. The posterior spiracles were raised from the surface of the spiracular plate. The degree to which the posterior spiracle was raised varied in the different species. The anal area, located on the ventral aspect of the terminal segment, shrunk significantly during pupariation. The anal area was assessed for the prominence of its various components, i.e. the anal pads {*ap*} and the anal horns {*ah*}.

Lucilia cuprina (Figs. 90A, 91A, 92A and 93A)

The posterior surface of the puparium was an open structure (Figs. 90A, 91A and 93A). The slightly outward bulging of the spiracular plate was revealed when viewed dorsally (Fig. 90A); the entire spiracular plate was visible when viewed from this angle. The posterior spiracles were slightly raised from the surface of the spiracular plate (Fig. 90A). The perispiracular tubercles were small and indistinct (Figs. 90A, 91A and 93A). The anal area was slightly raised from the surface of the puparium (Fig. 92A). The anal horns and anal pads were similar in size (Fig. 92A). The micrographs of *L. cuprina* puparia presented by Greenberg & Szyska (1984) and Sukontason *et al.* (2006a) revealed small perispiracular tubercles and an open posterior surface, similar to that observed for *L cuprina* puparia examined during the present study.

Lucilia sericata (Figs. 90B, 91B, 92B and 93B)

The posterior surface of the puparium was an open structure (Figs. 90B, 91B and 93B). The slightly outward bulging of the spiracular plate was revealed when viewed dorsally (Fig. 90B); the entire spiracular plate was visible when viewed from this

angle. The posterior spiracles were raised slightly from the surface of the spiracular plate (Fig. 90B). Small, indistinct perispiracular tubercles were noted on the rim of the spiracular plate (Figs. 90B and 93B). The anal area was not distinct with relatively small anal horns compared to the anal pads (Fig. 92B).

Chrysomya chloropyga (Figs. 90C, 91C, 92C and 93C)

The whole of the spiracular plate was not visible when viewed from the dorsal surface of the puparium (Fig. 90C). Some of the ventral perispiracular tubercles were observed from this view, but the posterior spiracles were hidden from view, due to it being sunken in with the rest of the spiracular plate (Fig. 90C). The lateral (Fig. 91C) and posterior view (Fig. 93C) of the spiracular plate revealed its slightly hinged / angled nature. The protrusive nature of the posterior spiracles was only noticeable from the posterior views (Fig. 93C). Small, but clearly discernible perispiracular tubercles were arranged on the rim of the spiracular plate (Figs. 90C, 91C and 93C). The anal horns were more pronounced than the anal pads (Fig. 92C). Features of the terminal segment were adequately differentiated to distinguish the closely related *C. putoria* from *C. chloropyga* on two levels. The first distinguishing feature is the spiracular plate that bulged outwards and the posterior spiracles being visible when viewed from the dorsal surface in *C. putoria* puparia (Greenberg & Szyska 1984, Amorim & Ribeiro 2001) opposed to the situation in *C. chloropyga* puparia where the posterior spiracles were not visible from the dorsal surface of the puparium. Secondly, the perispiracular tubercles were small in the puparia of *C. chloropyga* examined during the current study compared to the prominent perispiracular tubercles noted in the puparia of *C. putoria* examined by Amorim & Ribeiro (2001).

Chrysomya marginalis (Figs. 90D, 91D, 92D and 93D)

The spiracular plate was slightly recessed when viewed from the dorsal surface of the puparium (Fig. 90D). Although the spiracular plate was slightly recessed, the plate generally had a flat, open surface (Fig. 93D). The posterior spiracles bulged from the surface of the spiracular plate (Figs. 90D and 91D). Small, but discernible perispiracular tubercles were located on the rim of the spiracular plate (Figs. 91D and 93D). The anal pads and anal horns were similar in size (Fig. 92D). Similar to *C. marginalis* puparia examined during the current study, Prins (1982) described the features of the caudal segment of *C. marginalis* puparia as having (i) small

perispiracular tubercles, (ii) protruding posterior spiracles, (iii) a truncate posterior face and (iv) the short projections of the anal tubercles.

Chrysomya albiceps (Figs. 90E, 91E, 92E and 93E)

The whole spiracular plate was not visible when viewed from the dorsal surface of the puparium (Fig. 90E), due to it being markedly sunken in. Posterior (Fig. 93E) and lateral views (Fig. 91E) revealed the hinged / angled nature of the spiracular plate. Distinct perispiracular tubercles were located on the rim of the spiracular plate (Figs. 90E, 91E and 93E). Prominent spines in the form of a horse-shoe surrounded the anal area, overshadowing the anal pads and anal tubercles of this area (Fig. 92E). The only possible difference between *C. albiceps* and *C. rufifacies* puparia was found in the perispiracular tubercles which appeared longer and not as swollen in *C. albiceps* puparia as that of *C. rufifacies* puparia examined by Sukontason *et al*. (2006b).

Calliphora vicina (Figs. 90F, 91F, 92F and 93F)

The whole spiracular plate was visible when viewed from the dorsal surface of the puparium (Fig. 90F). Lateral (Fig. 91F) and posterior (Fig. 93F) views of the puparium revealed the open structure of the spiracular plate. The posterior spiracles were slightly raised from the surface of the spiracular plate (Figs. 90F and 91F). Small, indistinct perispiracular tubercles were located on the rim of the spiracular plate (Figs. 90F, 91F and 93F). The anal area was not a distinct structure on the ventral aspect of the puparium surface (Fig. 92F). The small anal tubercles and anal pads were similar in size (Fig. 92F).

Sarcophaga cruentata (Figs. 90G, 91G, 92G and 93G)

The spiracular plate was not visible when viewed from the dorsal surface of the atrium (Fig. 90G). This was due to the spiracular atrium being a cavity (Fig. 93G), a characteristic unique to sarcophagids in general. The circumference of the atrium was circular compared to the spindle-shaped atrium rim of third instar larvae (Fig. 93G). Small, indistinct perispiracular tubercles were located on the rim of the spiracular atrium (Figs. 90G, 91G and 93G). The degree to which the posterior spiracles were raised from the surface in the spiracular atrium could not be assessed. The anal pads and anal horns were indistinct structures on the puparium surface but appeared to be similar in size (Fig. 92G). The only discernible difference noted in *L. dux* puparia

examined by Sukontason *et al*. (2006a) was that the margins of the spiracular atrium rim was angular in this species compared to the circular circumference noted for the atrium rim in *S. cruentata* puparia examined during the current study.

Features of the terminal segment as a diagnostic feature

Spiracular field

Sarcophaga cruentata differed from the other species due to the deeply recessed nature of the spiracular atrium (Fig. 93G), whereas the spiracular plate was an open structure in the calliphorid species (Figs. 93A to 93F). The large perispiracular tubercles of *Chrysomya albiceps* (Fig. 93E) distinguished this species from the other calliphorid species with their indistinct or small perispiracular tubercles (Figs. 93A to 93D and 93F). These species were grouped as those with a bulging spiracular plate (*L. cuprina, L. sericata* and *C. vicina*) and those with a somewhat sunken spiracular plate (*C. chloropyga* and *C. marginalis*). This aspect of the spiracular plate was clearly discernable when viewed laterally (Fig. 91). Those species with a slightly bulging spiracular plate exhibited no further distinguishing characteristics that could be used to uniquely identify them. For those species with a slightly sunken spiracular plate, the posterior spiracles were visible on the spiracular plate when viewed from dorsally and laterally in *C. marginalis* (Figs. 90D and 91D) compared to it being hidden from view when viewed from these angles in *C. chloropyga* (Figs. 90C and 91C).

Anal area

The anal pads and anal horns were indistinct structures on the ventral aspect of the terminal segment in most of the species examined (Figs. 92A, 92B and 92D to 92G). The exception in this regard was *C. chloropyga* (Fig. 92C), where the anal pads and horns were distinct structures on the puparium surface. The only other species that could be uniquely identified through the features of the anal area was *C. albiceps*. *Chrysomya albiceps* was distinctly recognisable due to the spines forming a prominent semi-circular ridge (Fig. 92E) around the anal area.

The above analysis of the spiracular field and the anal area showed that these components of the terminal segment were not sufficiently varied for it to be used to uniquely identify all the species examined.

Due to the terminal segment not being assessed from all angles in the available publications on the topic, intraspecific variation could not be evaluated for most of the species examined. From the comparisons made with regard to *L. cuprina* (as reviewed by Greenberg & Szyska (1984) and Sukontason *et al*. (2006a)) and *C. marginalis* (as reviewed by Prins (1982)), those aspects that could be compared showed no intraspecific variation.

The terminal segment showed sufficient variation with regards to the perispiracular tubercles and the visibility of the posterior spiracles when viewed dorsally for the closely related *C. chloropyga* and *C. putoria* to be distinguished from each other. A distinction between the closely related *C. albiceps* and *C. rufifacies* is also possible utilising features of the perispiracular tubercles.

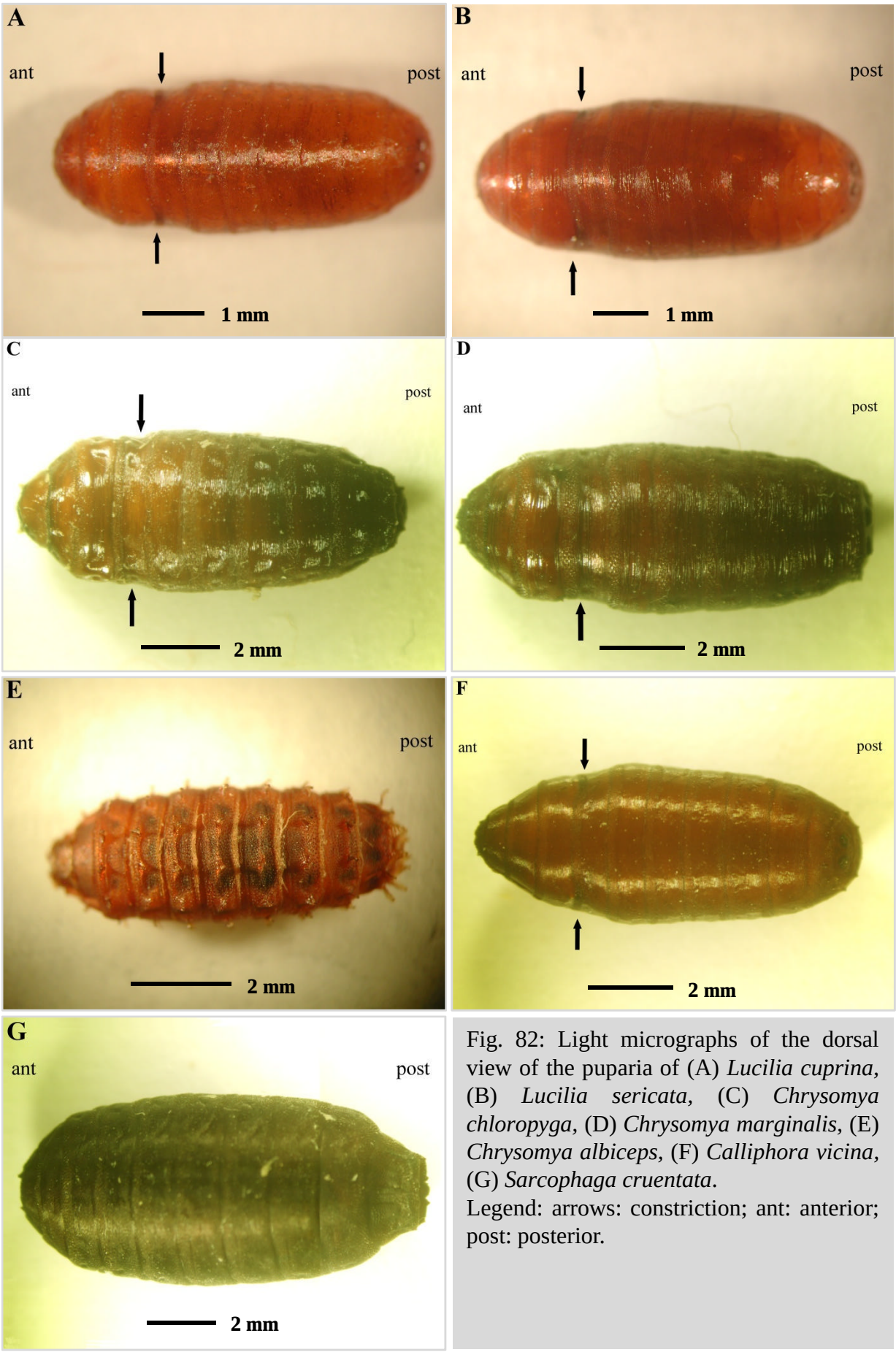


Fig. 82: Light micrographs of the dorsal view of the puparia of (A) *Lucilia cuprina*, (B) *Lucilia sericata*, (C) *Chrysomya chloropyga*, (D) *Chrysomya marginalis*, (E) *Chrysomya albiceps*, (F) *Calliphora vicina*, (G) *Sarcophaga cruentata*.
Legend: arrows: constriction; ant: anterior; post: posterior.

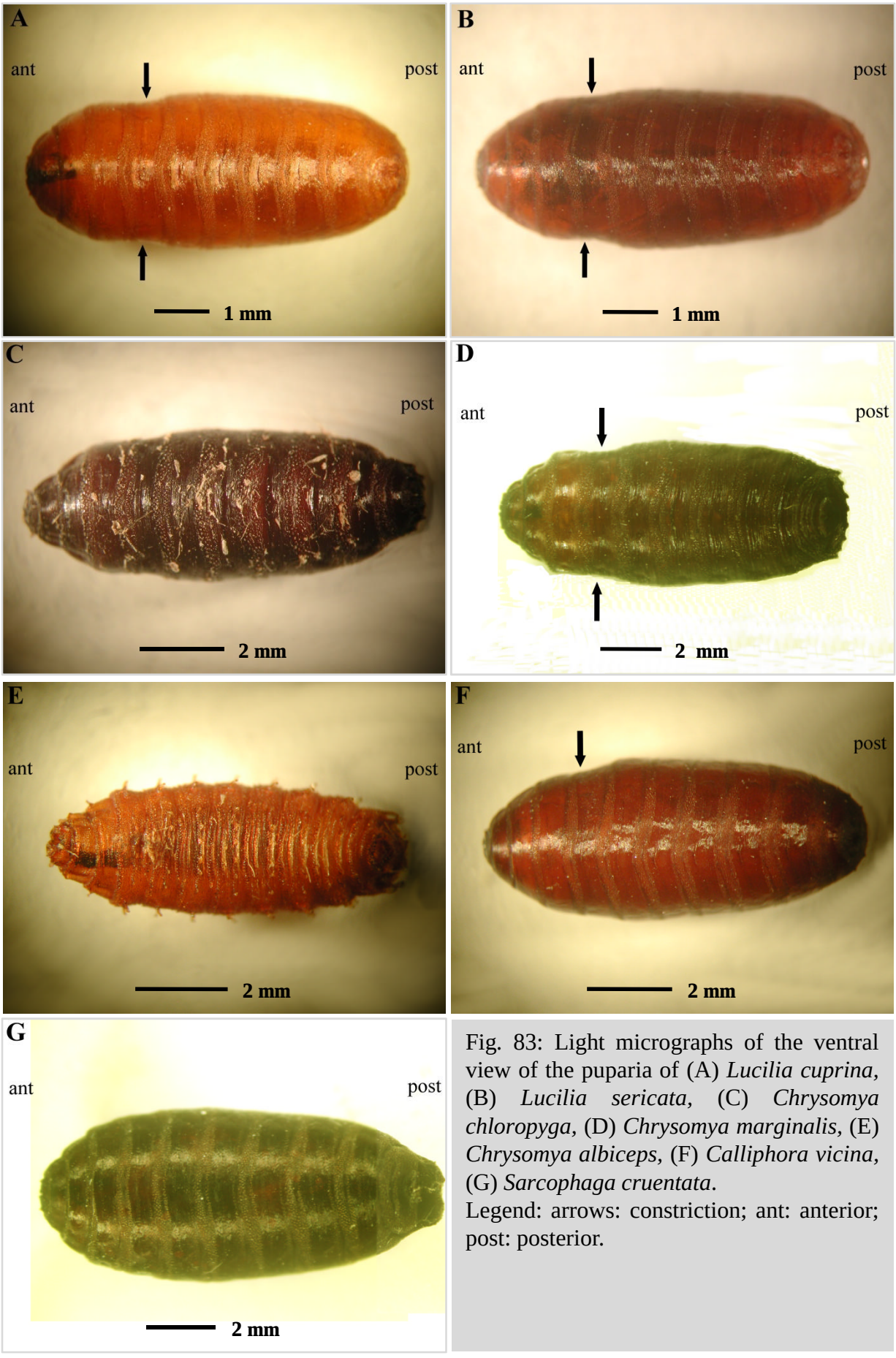


Fig. 83: Light micrographs of the ventral view of the puparia of (A) *Lucilia cuprina*, (B) *Lucilia sericata*, (C) *Chrysomya chloropyga*, (D) *Chrysomya marginalis*, (E) *Chrysomya albiceps*, (F) *Calliphora vicina*, (G) *Sarcophaga cruentata*.
Legend: arrows: constriction; ant: anterior; post: posterior.

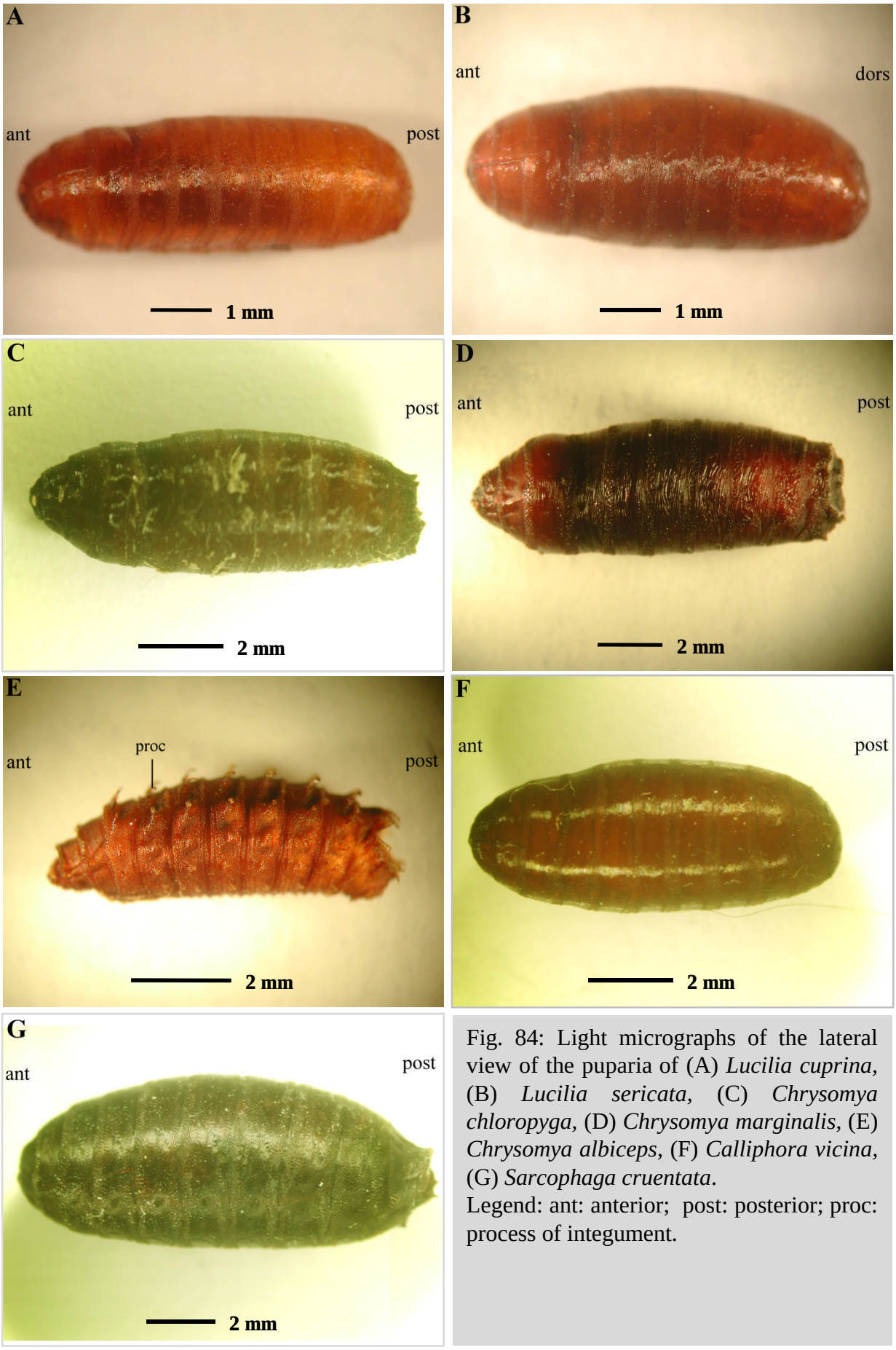


Fig. 84: Light micrographs of the lateral view of the puparia of (A) *Lucilia cuprina*, (B) *Lucilia sericata*, (C) *Chrysomya chloropyga*, (D) *Chrysomya marginalis*, (E) *Chrysomya albiceps*, (F) *Calliphora vicina*, (G) *Sarcophaga cruentata*.
Legend: ant: anterior; post: posterior; proc: process of integument.

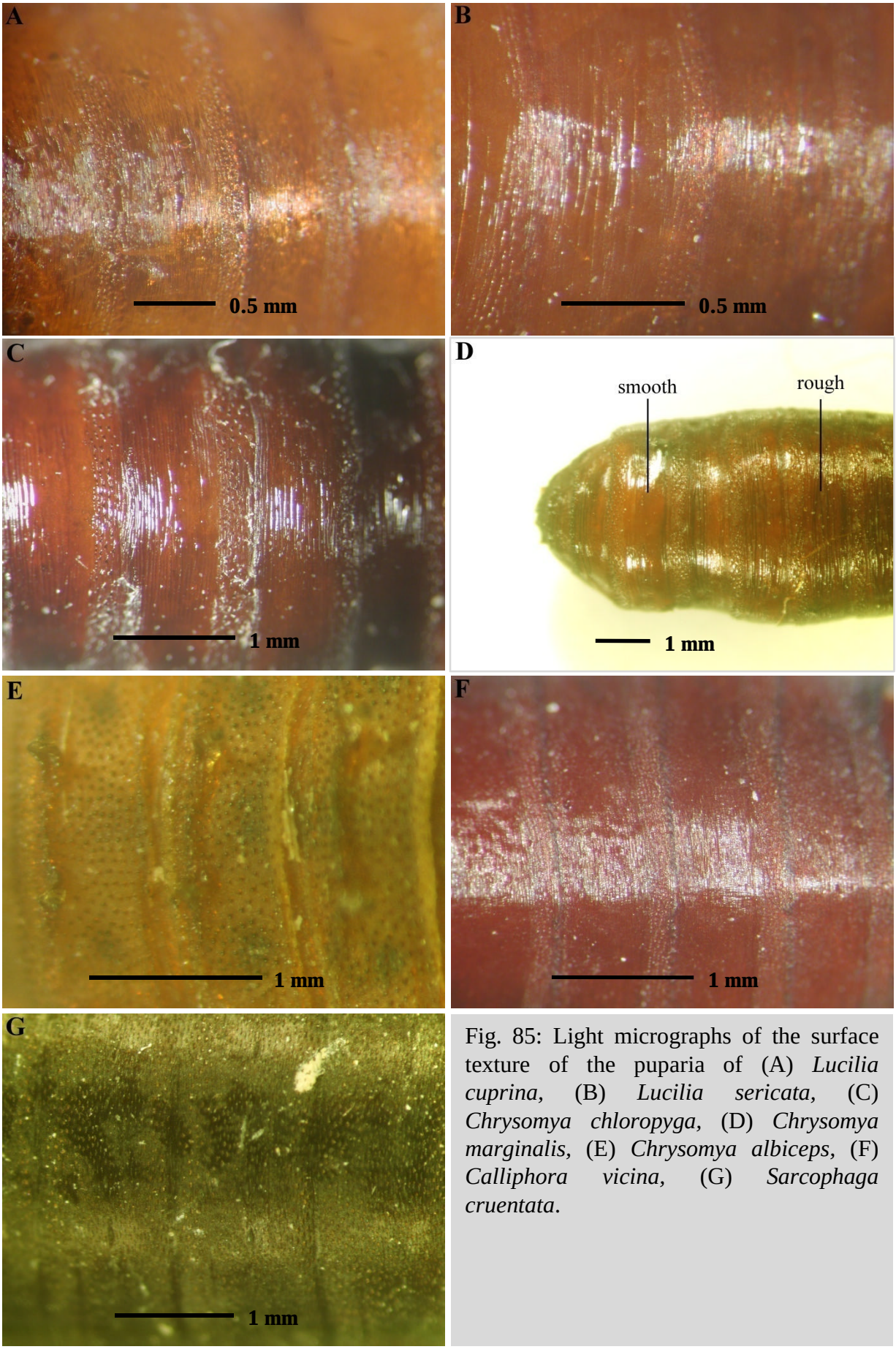


Fig. 85: Light micrographs of the surface texture of the puparia of (A) *Lucilia cuprina*, (B) *Lucilia sericata*, (C) *Chrysomya chloropyga*, (D) *Chrysomya marginalis*, (E) *Chrysomya albiceps*, (F) *Calliphora vicina*, (G) *Sarcophaga cruentata*.

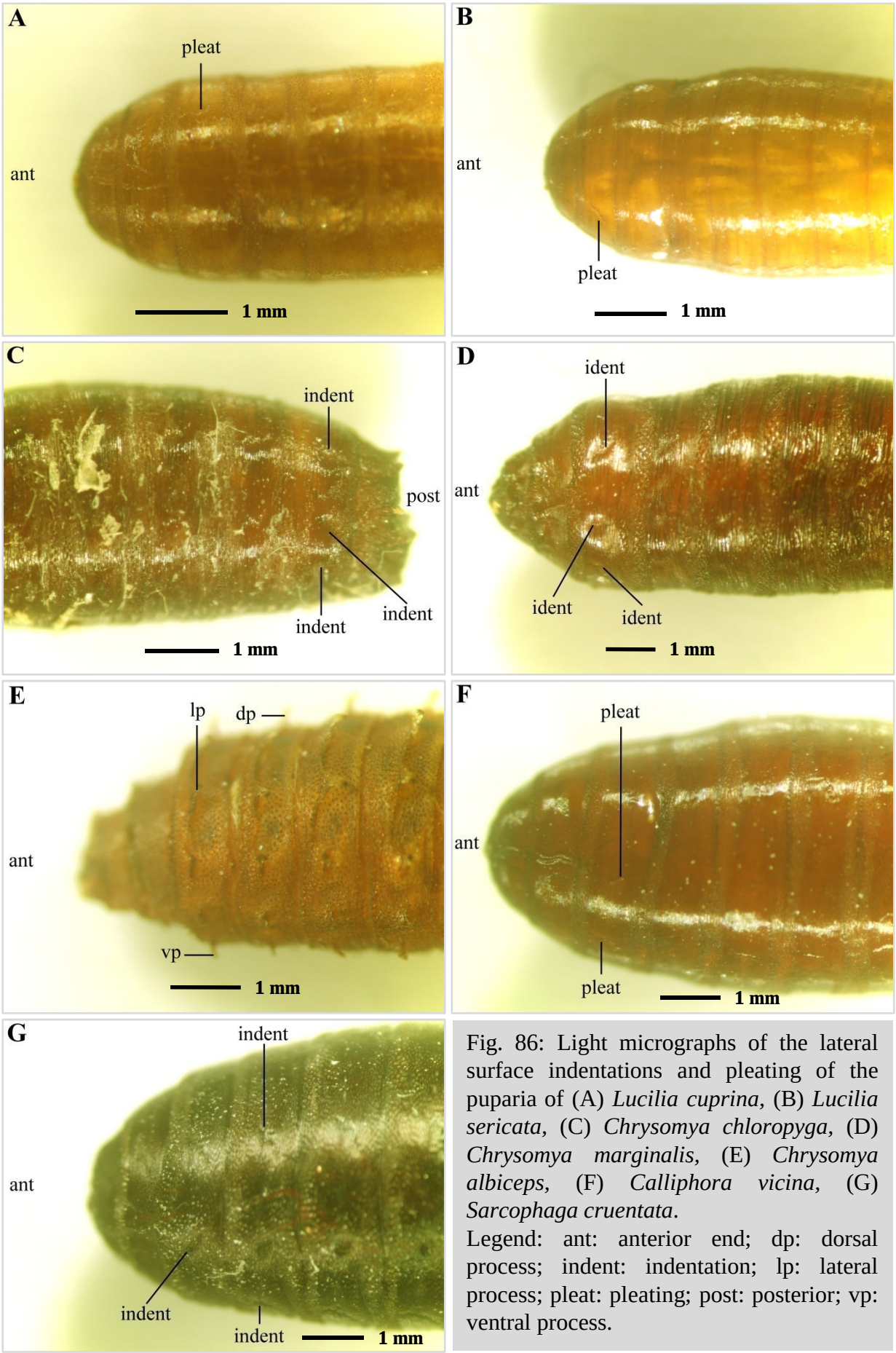


Fig. 86: Light micrographs of the lateral surface indentations and pleating of the puparia of (A) *Lucilia cuprina*, (B) *Lucilia sericata*, (C) *Chrysomya chloropyga*, (D) *Chrysomya marginalis*, (E) *Chrysomya albiceps*, (F) *Calliphora vicina*, (G) *Sarcophaga cruentata*.
Legend: ant: anterior end; dp: dorsal process; indent: indentation; lp: lateral process; pleat: pleating; post: posterior; vp: ventral process.

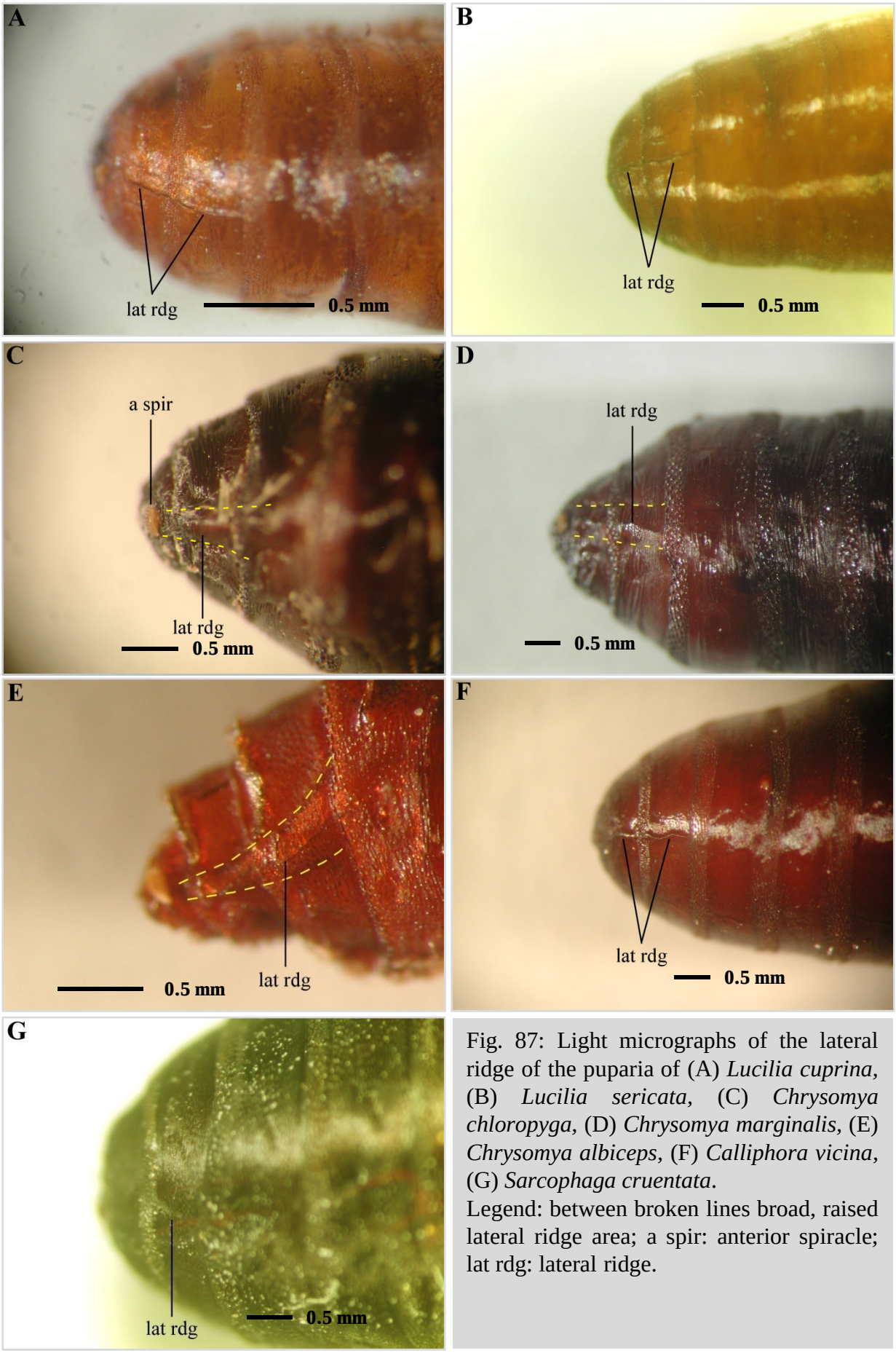


Fig. 87: Light micrographs of the lateral ridge of the puparia of (A) *Lucilia cuprina*, (B) *Lucilia sericata*, (C) *Chrysomya chloropyga*, (D) *Chrysomya marginalis*, (E) *Chrysomya albiceps*, (F) *Calliphora vicina*, (G) *Sarcophaga cruentata*.
Legend: between broken lines broad, raised lateral ridge area; a spir: anterior spiracle; lat rdg: lateral ridge.

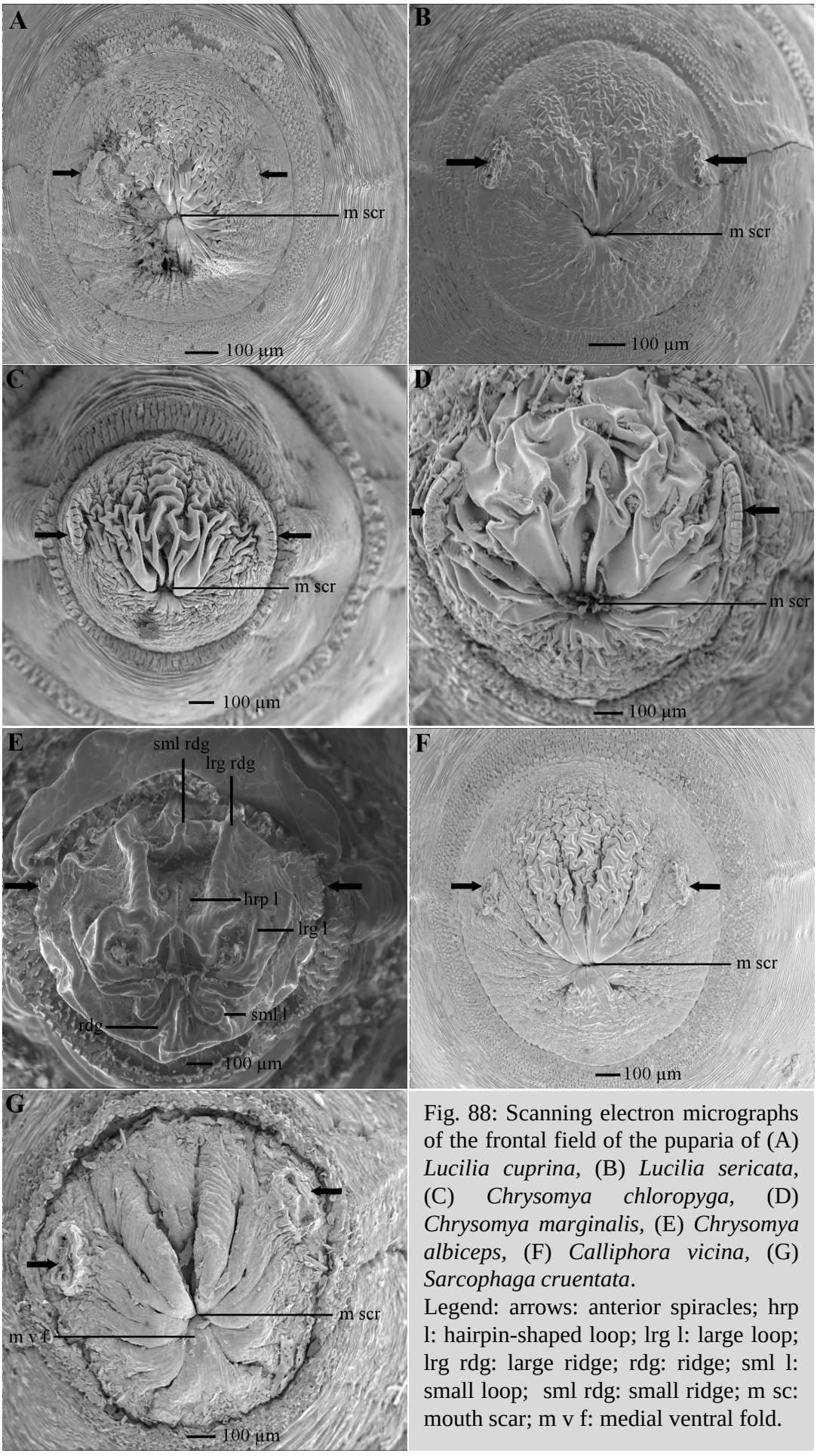


Fig. 88: Scanning electron micrographs of the frontal field of the puparia of (A) *Lucilia cuprina,* (B) *Lucilia sericata,* (C) *Chrysomya chloropyga,* (D) *Chrysomya marginalis,* (E) *Chrysomya albiceps,* (F) *Calliphora vicina,* (G) *Sarcophaga cruentata*.

Legend: arrows: anterior spiracles; hrp l: hairpin-shaped loop; lrg l: large loop; lrg rdg: large ridge; rdg: ridge; sml l: small loop; sml rdg: small ridge; m sc: mouth scar; m v f: medial ventral fold.

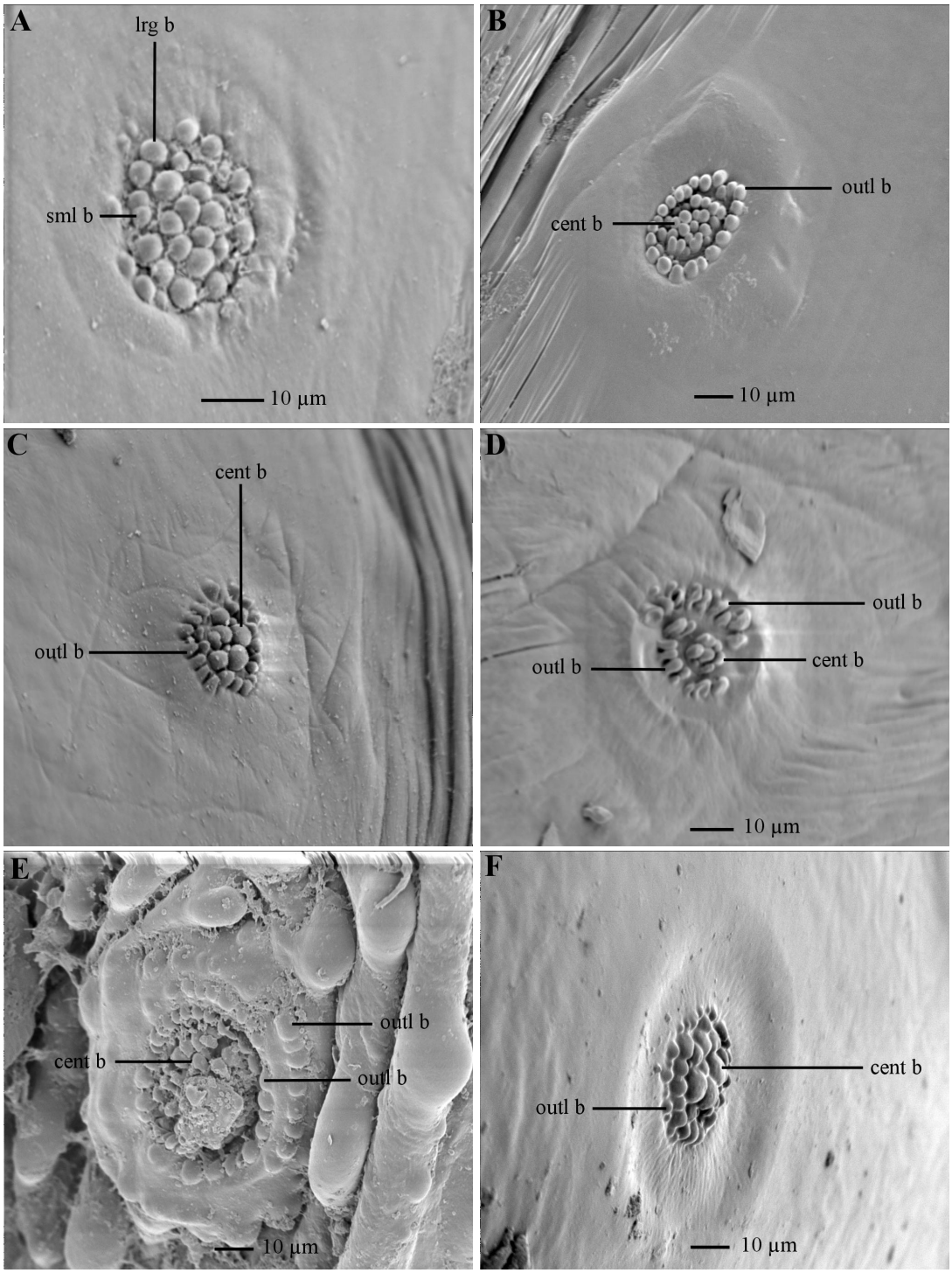


Fig. 89: Scanning electron micrographs of the bubble membrane of the puparia of (A) *Lucilia cuprina,* (B) *Lucilia sericata,* (C) *Chrysomya chloropyga,* (D) *Chrysomya marginalis,* (E) *Chrysomya albiceps,* (F) *Calliphora vicina.*
Legend: cent b: centralised bubbles; lrg b: large bubble; outl b: outlying bubbles; sml b: small bubble.

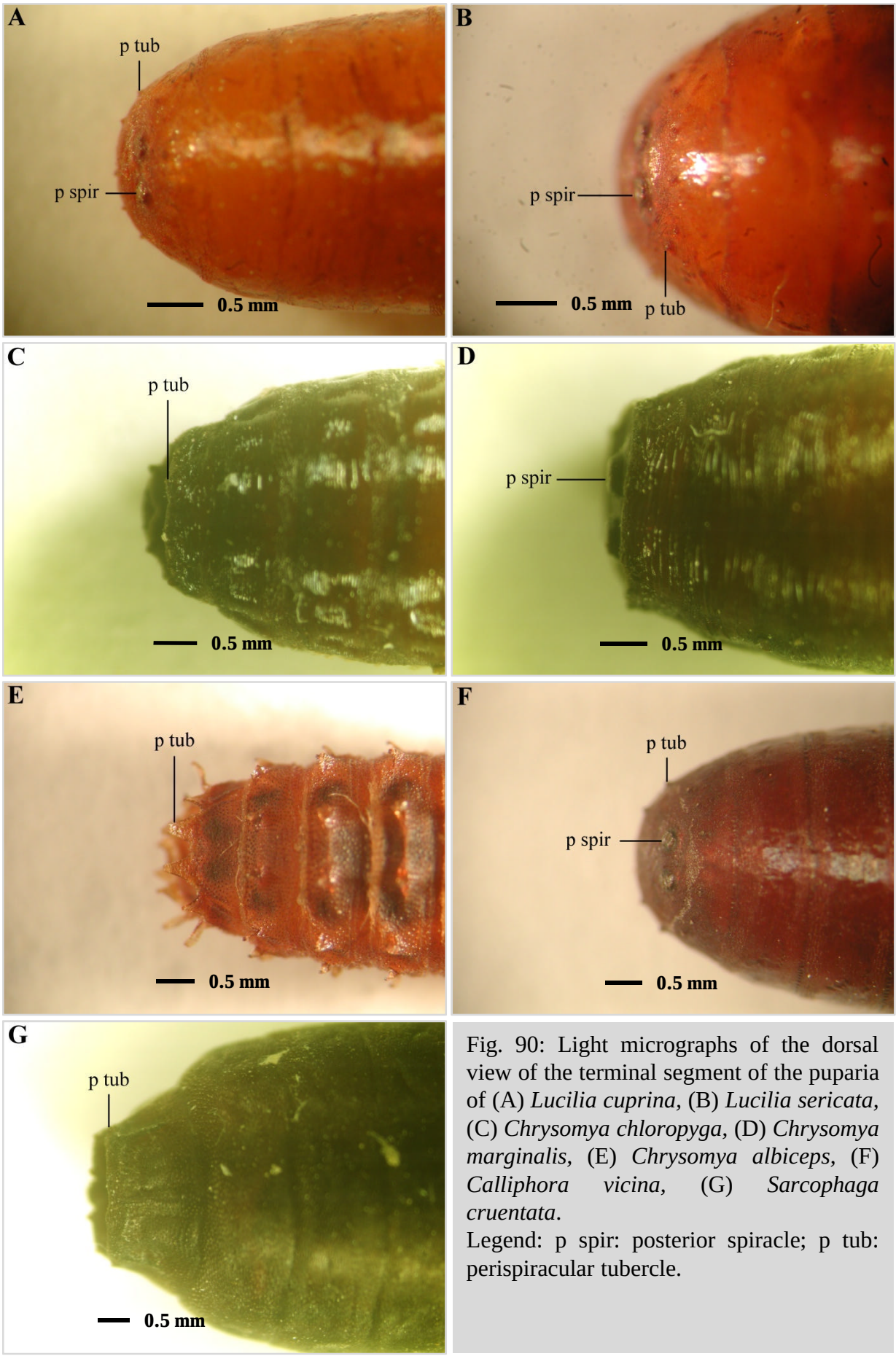


Fig. 90: Light micrographs of the dorsal view of the terminal segment of the puparia of (A) *Lucilia cuprina*, (B) *Lucilia sericata*, (C) *Chrysomya chloropyga*, (D) *Chrysomya marginalis*, (E) *Chrysomya albiceps*, (F) *Calliphora vicina*, (G) *Sarcophaga cruentata*.
Legend: p spir: posterior spiracle; p tub: perispiracular tubercle.

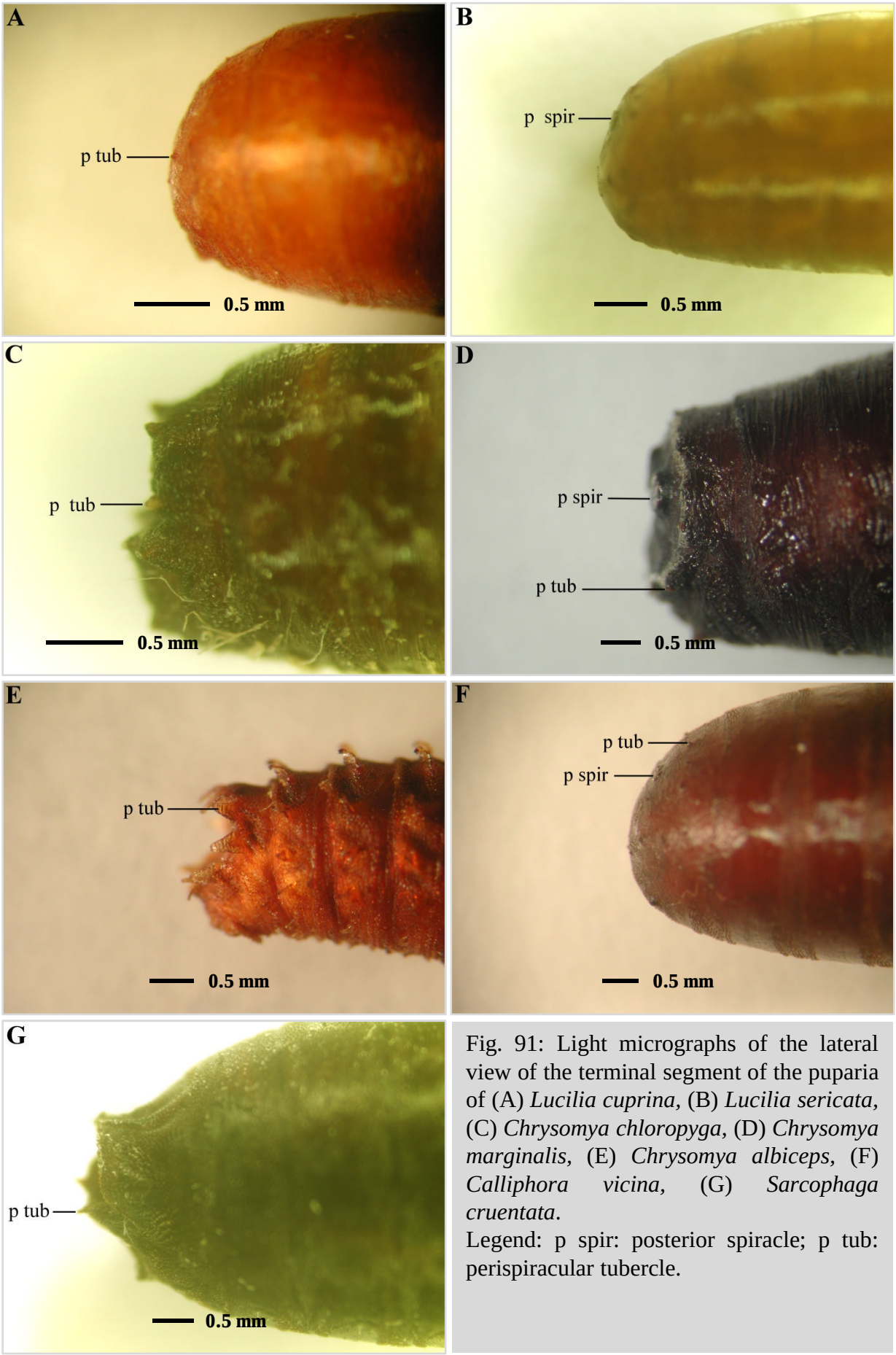


Fig. 91: Light micrographs of the lateral view of the terminal segment of the puparia of (A) *Lucilia cuprina*, (B) *Lucilia sericata*, (C) *Chrysomya chloropyga*, (D) *Chrysomya marginalis*, (E) *Chrysomya albiceps*, (F) *Calliphora vicina*, (G) *Sarcophaga cruentata*.
Legend: p spir: posterior spiracle; p tub: perispiracular tubercle.

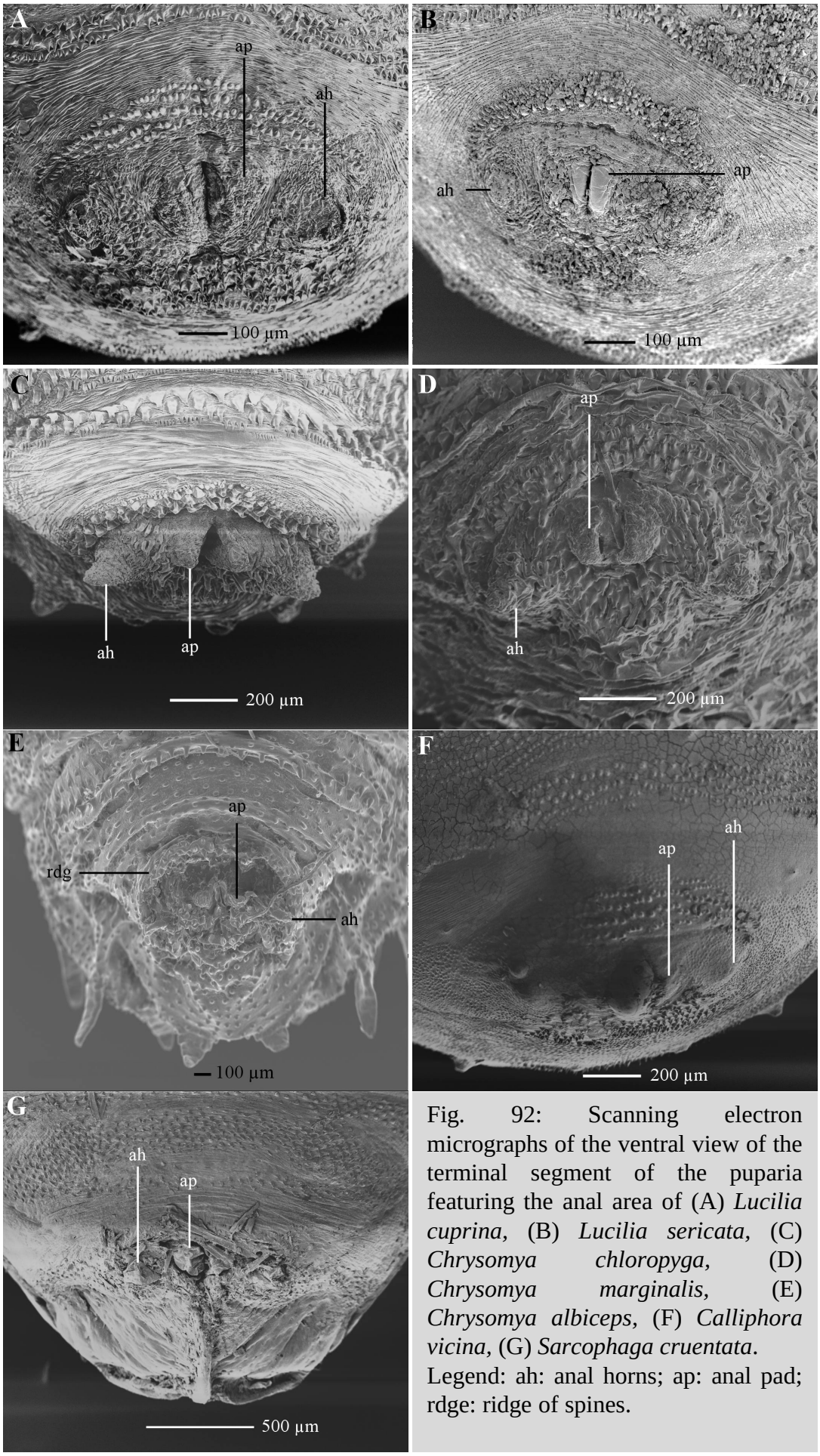


Fig. 92: Scanning electron micrographs of the ventral view of the terminal segment of the puparia featuring the anal area of (A) *Lucilia cuprina*, (B) *Lucilia sericata*, (C) *Chrysomya chloropyga*, (D) *Chrysomya marginalis*, (E) *Chrysomya albiceps*, (F) *Calliphora vicina*, (G) *Sarcophaga cruentata*.
Legend: ah: anal horns; ap: anal pad; rdge: ridge of spines.

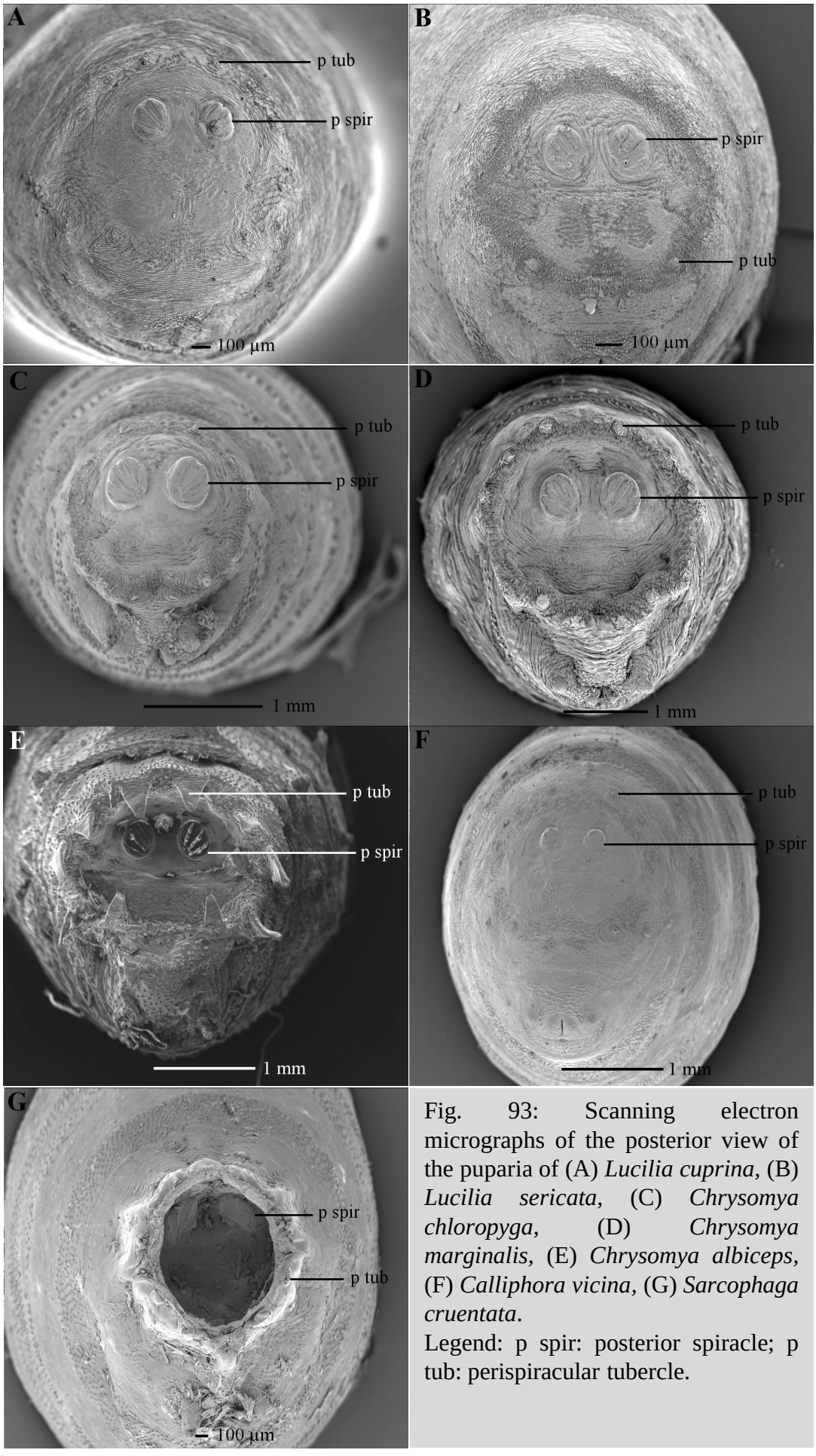


Fig. 93: Scanning electron micrographs of the posterior view of the puparia of (A) *Lucilia cuprina*, (B) *Lucilia sericata*, (C) *Chrysomya chloropyga*, (D) *Chrysomya marginalis*, (E) *Chrysomya albiceps*, (F) *Calliphora vicina*, (G) *Sarcophaga cruentata*.

Legend: p spir: posterior spiracle; p tub: perispiracular tubercle.

CHAPTER 4

CONCLUSION

4. CONCLUSION

The objective of this study was to identify the key diagnostic characteristics of the immature stages of forensically important calliphorids and sarcophagids of central South Africa. Ultimately these diagnostic characteristics were to be organised as keys that will assist the forensic entomologist to identify calliphorid and sarcophagid eggs, larvae and puparia should they be found at the scene of a homicide.

The diagnostic characteristics of the calliphorid and sarcophagid immature stages were described in full in the previous chapter. In this chapter these characteristics will be assembled and presented as keys. All characteristics described will be briefly evaluated for their individual strengths or weaknesses and a conclusion will be made as to whether the specific characteristic could be utilised on its own for identification purposes or not. A characteristic that could be utilised on its own to base identification on is considered the strongest type there could be. It will allow for rapid identification based on a single specimen. Those characteristics that could not be utilised on a stand-alone basis, formed the building blocks of keys where more than one characteristic was used to complete the key. An approach followed in constructing the keys was to pool characteristics visible from a specific view. An identification made from this type of key will take longer than those based on a single characteristic, but have the added benefit of the identification being based on more than one characteristic. With this type of key, it might still be possible to make an identification based on a single specimen that was examined. However, practical experience showed that identification was based on more than one specimen examined and by combining various characteristics of different keys. Finally, a combined key that will include all usable characteristics, irrespective of the angle it was viewed from or whether this information was gained from scanning electron microscopy or light microscopy will be presented. Practical experience gained in the present study, demonstrated that this type of key did not facilitate identification. The first two types of keys and combining the results of these keys were found to be the best approach to follow when attempting identification. The combined key should not

be seen as a tool for identification, but as an abridged description of those characteristics typical of a species.

4.1. KEYS FOR EGGS

Sarcophaga cruentata normally does not deposit eggs and this species should be excluded from an analysis due to this. However, for the sake of completeness, and to cater for the rare occasion where eggs by this species are deposited, the eggs of *S. cruentata* were included in the keys presented. However, the main area of distinction in the keys presented will be for calliphorid eggs.

Nine characteristics were evaluated for their possible diagnostic value. Of these nine characteristics, six were of no or very limited diagnostic use. The first of these characteristics were the shape of the eggs. The shape of calliphorid eggs were not sufficiently or distinctly varied from each other to be used for identification purposes. However, calliphorid eggs with pointed, narrow anterior ends and broad, rounded posterior ends were distinctly different from the barrel-shaped sarcophagid eggs where both ends were pointed. The second feature not used for diagnostic purposes was the chorionic structure. Although some differences were noted for the different species, these differences where difficult to grade quantitatively. Consequently, this feature was excluded as a diagnostic characteristic. The four other characteristics considered of no or very little diagnostic value, i.e. the median area length extent, the flange width, the egg length and the egg width, were eliminated due to the large overlap noted in the ranges of these measurements in the different species examined.

Of the nine characteristics evaluated for their diagnostic value, three were found to be of value, i.e. the median area structure, the median area width and the anterior termination pattern of the median area. However, as will be illustrated in the following keys, none of these characteristics could be used in isolation for diagnostic purposes.

Eggs; Key 1a: Utilising the median area structure (considering whether the struts were merged or individualised as an initial approach) as a diagnostic marker. The dorsal aspect was the best vantage point from which to evaluate eggs in this instance. This aspect was examined by means of scanning electron microscopy. Only some of the species were identified uniquely with this characteristic.

1. No median area present.....*Sarcophaga cruentata*
   Median area present.....2

2. Struts in median area largely merged.....*Chrysomya marginalis*
   Struts in median area largely individualised.....3

3. Numerous, small indentation on surface of struts.....unresolved for *Lucilia cuprina* and *Calliphora vicina*
   One to three deep indentations on surface of struts.....unresolved for *Lucilia sericata*, *Chrysomya chloropyga* and *Chrysomya albiceps*

Another approach can be followed when using this characteristic. The person doing the identification should use the approach she / he would be most comfortable with.

Eggs; Key 1b: Utilising the median area structure (considering the indentations on the surface of the struts as an initial approach). The dorsal aspect was the best vantage point from which to evaluate eggs in this instance. This aspect was examined by means of scanning electron microscopy. Only some of the species were identified uniquely with this characteristic.

1. No median area present.....*Sarcophaga cruentata*
   Median area present.....2

2. Numerous, small indentations on surface of struts.....unresolved for *Lucilia cuprina* and *Calliphora vicina*
   One to three deep indentations on surface of struts.....3

3. Struts in median area largely merged.....*Chrysomya marginalis*
   Struts in median area largely individualised.....unresolved for *Lucilia sericata*, *Chrysomya chloropyga* and *Chrysomya albiceps*

Eggs; Key 2: Utilising the median area width as a diagnostic marker. The dorsal aspect was the best vantage point from which to evaluate eggs in this instance. This aspect was examined by means of scanning electron microscopy. Only some of the species were identified uniquely with this characteristic.

1. No median area present.....*Sarcophaga cruentata*
   Median area present.....2

2. Median area narrow (<3% of egg width).....unresolved for *Chrysomya chloropyga, Chrysomya marginalis* and *Chrysomya albiceps*
   Median area broad (>6% of egg width).....unresolved for *Calliphora vicina, Lucilia cuprina* and *Lucilia sericata*

It should be noted that some *L. cuprina, C. marginalis* and *C. vicina* specimens presented with an intermediate median area width (i.e. 4 – 5% of the egg width).

Eggs; Key 3: Utilising the anterior termination pattern of the median area as a diagnostic marker. The anterior aspect was the best vantage point from which to evaluate eggs in this instance. This aspect was examined by means of scanning electron microscopy. Only some of the species were identified uniquely with this characteristic.

1. No median area or micropyle present.....*Sarcophaga cruentata*
   Median area and micropyle present.....2

2. Median area end bluntly at micropylar region.....*Calliphora vicina*
   Median area bifurcate at micropylar region.....3

3. Slight bifurcation.....*Lucilia sericata*
   Distinct bifurcation.....4

4. Bifurcation of median area not reaching level of micropyle / not symmetrical.....*Chrysomya marginalis*
   Bifurcation of median area reaching level of micropyle and symmetrical.....unresolved for *Lucilia cuprina*, *Chrysomya chloropyga* and *Chrysomya albiceps*

The bifurcation to the anterior termination pattern of *L. sericata* eggs are sometimes so restricted that the median area appeared to end bluntly at the micropylar region, similar to the situation found for *C. vicina*. In these instances, additional features should be considered to base identification on.

The next step was to consider specific views and which of the characteristics could be combined when an egg was viewed from a specific angle. For eggs, only the dorsal and anterior views were considered. None of the useful diagnostic features were located on the ventral surface of the eggs and consequently, this view was excluded from analyses. When an egg was placed to expose the dorsal surface, all characteristics considered being of diagnostic value could be utilised. Although the exact extent of the anterior termination pattern of the median area around the micropyle could not be assessed, an assessment could be made as to whether it ended bluntly at the micropyle or whether it bifurcated. This analysis was complicated since three scenarios could be encountered regarding the width of the median area with regard to the egg width. The median area width was classified as either broad (>6% of the egg width) or narrow (<3% of the egg width). However, some specimens of some species (*L. cuprina*, *C. marginalis* and *C. vicina*) also presented with a median area width of 4 to 5% of the egg width. Should an egg be found with an intermediate median area width, it should be identified by means of a separate key.

Eggs; Key 4: A combined key utilising the characteristics exposed when viewing the dorsal surface of eggs (i.e. the median area extent, the median area structure as well as the anterior bifurcation pattern of the median area). These aspects were examined by means of scanning electron microscopy. Only some of the species were identified uniquely with this characteristic.

1. No median area present.....*Sarcophaga cruentata*
   Median area present.....2

2. Median area narrow (<3% of egg width).....3
   Median area broad (>6% of egg width).....5

3. One to three deep-set indentations on surface of struts.....*Lucilia sericata*
   Numerous, small indentations on surface of struts.....4

4. Median area bifurcated at micropylar region.....*Lucilia cuprina*
   Median area end bluntly at micropylar region.....*Calliphora vicina*

5. Struts in median area merged.....*Chrysomya marginalis*
   Struts in median area largely individualised.....unresolved for *Chrysomya chloropyga* and *Chrysomya albiceps*

An egg with an intermediate median area will either be *L. cuprina*, *C. marginalis* or *C. vicina*. Two approaches can be followed when an egg is encountered with an intermediate median area width.

Eggs; Key 5a: Utilising the characteristics exposed when viewing the dorsal surface of eggs (i.e. utilising the median area structure as well as the anterior bifurcation pattern of the median area) for eggs with an intermediate median area width (considering the anterior termination pattern of the median area as an initial approach to identify calliphorid eggs). This aspect was examined by means of scanning electron microscopy. Only some of the species were identified uniquely with this characteristic.

1. Median area end bluntly at micropylar region.....*Calliphora vicina*
   Median area bifurcate at micropylar region.....2

2. Struts merged with one to three deep-set indentations on surface of struts.....*Chrysomya marginalis*
   Struts individualised with numerous, small indentations on surface of struts.....*Lucilia cuprina*

Eggs; Key 5b: Utilising the characteristics exposed when viewing the dorsal surface of eggs (i.e. utilising the median area structure as well as the anterior bifurcation pattern of the median area) for eggs with an intermediate median area width (considering the surface indentation of the struts of the median area as an initial approach to identify calliphorid eggs). This aspect was examined by means of scanning electron microscopy. Only some of the species were identified uniquely with this characteristic.

1. Struts merged with one to three deep-set indentations on surface of struts.....*Chrysomya marginalis*
   Struts individualised with numerous, small indentations on surface of struts.....2

2. Median area end bluntly at micropylar region.....*Calliphora vicina*
   Median area bifurcate at micropylar region.....*Lucilia cuprina*

Eggs; Key 6: Utilising the characteristics exposed when viewing the anterior end of eggs (i.e. utilising the median area structure as well as the anterior bifurcation pattern of the median area). These aspects were examined by means of scanning electron microscopy. Only some of the species were identified uniquely with this characteristic.

1. No median area or micropyle present.....*Sarcophaga cruentata*
   Median area and micropyle present.....2

2. Median area bifurcate at micropylar region.....3
   Median area end bluntly at micropylar region.....*Calliphora vicina*

3. Slight bifurcation.....*Lucilia sericata*
   Distinct bifurcation.....4

4. Bifurcation of median area not reaching level of micropyle / not symmetrical.....*Chrysomya marginalis*
   Bifurcation of median area reach level of micropyle and symmetrical.....5

5. Numerous, small indentations on surface of struts.....*Lucilia cuprina*
   One to three deep-set indentations on surface of struts.....unresolved for .....*Chrysomya chloropyga* and *Chrysomya albiceps*

Cognisance should be taken of a few aspects when making an identification based on the anterior view of eggs. The anterior termination pattern of the median area was not distinctly bifurcated in a few *L. sericata* specimens examined. Due to the very slight bifurcation of the median area noted in these specimens it appeared to be similar to that found in *C. vicina*. Reliance in these instances is on the surface structure of the struts. However, only a small area of plastron network was available for analysis when assessing the eggs from this view. Due to the fact that eggs were deposited on an unsterile medium, the median area structure can be obscured from view by the debris covering the egg. With such a small area exposed, the chances are that the area available for viewing would not be adequately cleaned to enable analysis using the median area structure. Two other pieces of information can be employed during the identification of these species. Since *C. vicina* is active predominantly in the colder winter months, this species can be excluded from an analysis where eggs were found in the warmer summer months. However, should eggs be found in the season where both of these species were reproductively active, egg size could be considered in an attempt to make an identification. The two species produced eggs of largely separate egg size ranges during the current study. However, it was noted when comparing the results of the current study with that found by others, that egg size was variable in different populations of the same species.

Eggs; Key 7: Abridged description key utilising all characteristics, irrespective of the angle the egg was viewed from. All aspects were examined by means of scanning electron microscopy. Two of the species were very similar to each other and a uniquely description could not be offered for them.

1a. No median area or micropyle present. (Barrel-shaped eggs with both ends pointed.).....*Sarcophaga cruentata*

1b. Median area and micropyle present.....2

2a. Median area broad. (>6% of egg width).....3

2b. Median area narrow. (<3% of egg width).....5

3a. Median area bifurcates at the micropylar region. (Arms of median area symmetrical, reaching the level of micropyle. Median area of most specimens broad, some specimens present with median area of intermediate width of 4 – 5%. Struts of median area individualised and surface marked with numerous, small indentations.).....*Lucilia cuprina*

3b. Median area end bluntly or slightly bifurcate at micropylar region.....4

4a. Numerous, small indentations on surface of struts. (Median area end bluntly at micropylar region. Median area of most specimens broad, some specimens with median area of intermediate width of 4 – 5%. Struts of median area individualised).....*Calliphora vicina*

4b. One to three deep-set indentations on surface of struts. (Anterior termination pattern ambiguous; slight bifurcation in most specimens, appear to be blunt ending. Struts of plastron network in broad median area individualised.).....*Lucilia sericata*

5a. Struts in the median area merged. (Median area bifurcate at micropylar region. Median area of most specimens broad, some specimens present with median area of intermediate width of 4 – 5%. Merged struts of plastron network contained one to three deep set indentations on surface.).....*Chrysomya marginalis*

5b. Struts in median area largely individualised. (Some struts merged. Median area bifurcate at micropylar region, reach in most instances level of micropyle. Narrow median area. Individualised struts of plastron network contained one to three deep-set indentations on surface.).....unresolved for *Chrysomya chloropyga* and *Chrysomya albiceps*

Based on the information gathered for *C. chloropyga* and *C. albiceps* in the current study, the opinion was that the eggs of these two species were indistinguishable from each other. This sentiment was also echoed by Greenberg & Singh (1995). Meskin (1991) distinguished these two species based on the median area length extent in relation to the egg length and the flange width extent in relation to the median area width. Both of these characteristics were not considered for their diagnostic value

during the current study due to large overlaps found for these measurements in the different species evaluated.

Greenberg & Singh (1995) expressed concern about the variability noted in the characteristics used for identification for certain species. Variation in the characteristics used during the current study was not significant. However, variability was noted when published descriptions were compared to the results obtained during the current study. The reasons for this variation should be investigated in future research before any key for eggs can be utilised with confidence.

4.2. KEYS FOR FIRST INSTAR LARVAE

Twelve aspects of first instar larvae were evaluated for their diagnostic value. Five of these aspects (the integument, the oral ridges, the ventral organ, the labium and the anterior spiracles) showed no significant variation in the different species examined and were thus of no diagnostic value. The antennomaxillary sensory complex and the labrum were useful to distinguish the calliphorids from the sarcophagid. The cuticular ridges surrounding the centralised group of sensillae in the maxillary sensory complex was made up of a single row of individualised ridges in the calliphorids. In the sarcophagid, a few rows of interlaced cuticular folds surrounded the centralised grouping of sensillae on the maxillary sensory complex. The labrum of the calliphorids was two tube-like structures, furnished with spinules on its distal margin. The distal margin of the labrum of the sarcophagid was simplified, i.e. not extended or furnished with spinules at its distal margin. Four of the aspects (the posterior spiracles, the spines, the spiracular fields and the anal area) were useful to uniquely identify some of the species. Only one of the features, i.e. the cephalopharyngeal skeleton, could be used to uniquely identify all the species.

First Instar Larvae; Key 1: Utilising the posterior spiracles as a diagnostic marker. This aspect was examined by means of scanning electron microscopy. Only some of the species were identified uniquely with this characteristic.

1. Posterior spiracles located in cavity.....*Sarcophaga cruentata*
   Posterior spiracles located on open spiracular plate.....2

2. Middle-inner spiracular hair cluster compacted.....3
   Middle-inner spiracular hair cluster branched.....unresolved for *Chrysomya chloropyga, Chrysomya marginalis, Chrysomya albiceps* and *Calliphora vicina*

3. Hair of spiracular clusters fine- textured.....*Lucilia sericata*
   Hair of spiracular clusters more coarsely-textured.....*Lucilia cuprina*

First Instar Larvae; Key 2: Utilising the spines as seen laterally as a diagnostic marker. This aspect was examined by means of scanning electron microscopy. Only some of the species were identified uniquely with this characteristic. The creeping welt was not utilised since it was not distinctly variable in the different species examined. Although the fusiform areas of *C. albiceps* and *S. cruentata* were more distinctly visible than that of the other species examined, this characteristic was not utilised in this specific key, since it was not necessary to use it to distinguish these species from the others.

1. Hair-like spines in spine bands of segments 6 to 12.....*Sarcophaga cruentata*
   Normal spines in spine band areas.....2

2. Spines on anterior and posterior margins of segments.....*Calliphora vicina*
   Spines predominately only on anterior margins of segments.....3

3. Spine bands complete for segments 2 to 6.....*Chrysomya albiceps*
   Complete spine bands stretched further posterior than the above.....4

4. Spine bands complete for segments 2 to 7.....unresolved for *Lucilia cuprina* and *Lucilia sericata*
   Spine bands complete for segments 2 to 9.....5

5. Few spines in spine bands on lateral aspect of larvae.....*Chrysomya chloropyga*
   Spines abundant in spine bands on lateral aspect of larvae.....*Chrysomya marginalis*

First Instar Larvae; Key 3: Utilising the spiracular field as a diagnostic marker. This aspect was examined by means of scanning electron microscopy. Only some of the species were identified uniquely with this characteristic.

1. Spiracular field deep-set atrium.....*Sarcophaga cruentata*
   Spiracular field open area.....2

2. Medium coverage of hair on rim of spiracular plate.....*Calliphora vicina*
   Sparse covering of hair on rim of spiracular plate.....3

3. Hair on the entire rim of spiracular plate.....unresolved for *Lucilia cuprina* and *Lucilia sericata*
   Hair absent from rim on dorsal aspect of the spiracular plate.....unresolved for *Chrysomya chloropyga, Chrysomya marginalis* and *Chrysomya albiceps*

First Instar Larvae; Key 4: Utilising the anal area as a diagnostic marker. This aspect was examined by means of scanning electron microscopy. Only some of the species were identified uniquely with this characteristic.

1. Anal pads similar in size to anal horns.....2
   Anal pads smaller than anal horns.....unresolved for *Lucilia sericata, Chrysomya marginalis, Calliphora vicina* and *Sarcophaga cruentata*

2. Robust spines around anal area.....*Chrysomya albiceps*
   Small spines around anal area.....unresolved for *Lucilia cuprina* and *Chrysomya chloropyga*

First Instar Larvae; Key 5a: Utilising the cephalopharyngeal skeleton as a diagnostic marker. This aspect was examined by means of light microscopy. All the species were identified uniquely with this characteristic.

1. No median tooth.....*Sarcophaga cruentata*
   Median tooth.....2

2. Tentorial phragma narrow (width of tentorial phragma <10% of length of cephalopharyngeal skeleton) 3
   Tentorial phragma broad (width of tentorial phragma >10% of length of cephalopharyngeal skeleton) 5

3. Median tooth had distinct posterio-dorsal notch.....*Calliphora vicina*
No distinct posterior-dorsal notch of median tooth noted.....4

4. Dorsal bridge extended.....*Lucilia sericata*
Dorsal bridge short.....*Lucilia cuprina*

5. Posterior aspect of base of mouth hook rounded; base narrowed significantly anteriorly
.....*Chrysomya albiceps*
Base of mouth hook of equal thickness.....6

6. Few hooks present as part of mouth hook.....*Chrysomya chloropyga*
Single hook present as part of mouth hook.....*Chrysomya marginalis*

First Instar Larvae; Key 5b: Separation among *Chrysomya* species can also be achieved through considering the shape of the hypopharyngeal sclerite. Step 5 and 6 can then be resolved as follow:

5. Posterior end of hypopharyngeal sclerite C-shaped.....*Chrysomya albiceps*
Posterior end of hypopharyngeal sclerite not C-shaped.....6

6. Hypopharyngeal sclerite narrowed significantly towards distal end.....*Chrysomya chloropyga*
Slight narrowing of the hypopharyngeal sclerite to distal end.....*Chrysomya marginalis*

The keys that follow use a combination of features as seen when the larvae were viewed from a specific angle. No keys will be presented for the dorsal and the lateral views since only features of the spines (which were covered as part of Key 2) could be assessed from these views.

First Instar Larvae; Key 6: A combined key utilising those features visible from the posterior end of the larva. Features of the posterior spiracles and the spiracular field were considered. This aspect was examined by means of scanning electron microscopy. Only some of the species were identified uniquely when viewed from this angle.

1. Spiracular field deep-set atrium.....*Sarcophaga cruentata*
Spiracular field open area.....2

2. Middle-inner spiracular hair cluster compacted.....3
Middle-inner spiracular hair cluster branched.....4

3. Hair of spiracular clusters finely- textured.....*Lucilia sericata*
Hair of spiracular clusters more coarsely-textured.....*Lucilia cuprina*

4. Medium coverage of hair on rim of spiracular plate.....*Calliphora vicina*
Sparse covering of hair on rim of spiracular plate.....unresolved for
Chrysomya chloropyga, Chrysomya marginalis, Chrysomya albiceps

First Instar Larvae; Key 7: A combined key utilising characteristics prominent of the ventral aspect of the larva. Features of the anal area, the creeping welts and the labrum could be considered here. These aspects were examined by means of scanning electron microscopy. The creeping welt was undifferentiated in various species and was of no value in a key based on this view. In Key 4, *S. cruentata* could not be distinguished from some of the calliphorids based on the characteristics of the anal area. However, since the labrum can be evaluated when viewed from this angle, *S. cruentata* could be distinguished from these species. A partial separation could be achieved using the characteristics of this view.

1. Anal pads similar in size to anal horns.....2
Anal pads smaller than anal horns.....3

2. Robust spines around anal area.....*Chrysomya albiceps*
Small spines around anal area.....unresolved for
Lucilia cuprina and *Chrysomya chloropyga*

3. Labrum with simplified margins without spinules on distal margin.....*Sarcophaga cruentata*

Labrum a tube-like structure, adorned with spinules on distal margin .....unresolved for
Lucilia sericata, Chrysomya marginalis and *Calliphora vicina*

First Instar Larvae; Key 8: An abridged descriptive key utilising all characteristics, irrespective of the angle the first instar larvae was viewed from. Aspects were examined by means of light and scanning electron microscopy. All species were uniquely described with these characteristic.

1a. Spiracular field deep set atrium. (Margins of labrum simplified, without spinules on distal margin. Cephalopharyngeal skeleton without median tooth. Posterior spiracles hidden from view in atrium. Spines on anterior margins of posterior segments hair-like; fusiform area distinct. Dense covering of hair on rim of spiracular plate. Anal horns larger than anal pads.).....*Sarcophaga cruentata*

1b. Spiracular field open area.....2

2a. Anal pads similar in size to anal horns.....3

2b. Anal pads smaller than anal horns.....5

3a. Middle-inner spiracular hair cluster compacted. (Labrum tube-like with spinules on distal margin. Cephalopharyngeal skeleton with short dorsal bridge, narrow tentorial phragma; no distinct notches on posterio-dorsal margin of median tooth. Finely textured hair in spiracular hair clusters. Single-pointed spines on anterior margin of segments; indistinct fusiform area. Open spiracular plate. Sparsely distributed hair on rim of entire spiracular field. Anal pads and horns similar in size; few spines posterior from anal pads.).....*Lucilia cuprina*

3b. Middle-inner spiracular hair cluster branched.....4

4a. Fusiform area distinct structure. (Labrum tube-like with spinules on distal margin. Cephalopharyngeal skeleton with broad tentorial phragma; posterior end of hypopharyngeal sclerite C-shaped; base of mouth hook rounded, narrowed considerably anteriorly; numerous distally located hooks. All spiracular hair clusters branched; finely-textured hair. Single-pointed spines on anterior margins of segments. Open spiracular plate. Sparsely distributed hair on rim of spiracular plate, absent from dorsal aspect of rim. Anal pads and horns similar in size; stubby spines around anal area.).....*Chrysomya albiceps*

4b. Fusiform area indistinct structure. (Labrum tube-like with spinules on distal margin. Cephalopharyngeal skeleton with broad tentorial phragma; defined posterior-dorsal notch to median tooth; hypopharyngeal sclerite associated to parastomal sclerite with pedicle. All spiracular hairs clusters branched; finely-textured hair. Single-pointed spines on anterior margins of segments. Open spiracular plate. Sparsely distributed hair on rim of spiracular plate, absent from dorsal aspect of rim. Anal pads smaller than anal horns; few spines posterior from anal pads.).....*Chrysomya chloropyga*

5a. Middle-inner spiracular hair cluster compacted. (Labrum tube-like with spinules on distal margin. Cephalopharyngeal skeleton with extended dorsal bridge; narrow tentorial phragma; no distinct notch to posterio-dorsal margin of median tooth. Robust hair in spiracular hair clusters. Single-pointed spines on anterior margins of segments; indistinct fusiform area. Open spiracular plate. Sparsely distributed hair on entire rim of spiracular plate. Anal pads smaller than anal horns; few spines posterior from anal pads.).....*Lucilia sericata*

5b. Middle-inner spiracular hair cluster branched.....6

6a. Sparse coverage of hair on rim of spiracular plate. (Labrum tube-like with spinules on distal margin. Cephalopharyngeal skeleton with broad tentorial phragma; no distinct notches on posterior-dorsal margin of median tooth; base of mouth hook uniform in thickness; single-tipped hook; hypopharyngeal sclerite slightly broader posteriorly, tapered slightly anteriorly. All spiracular hairs clusters branched; finely-textured hair. Single-pointed spines on anterior margins of segments; indistinct fusiform area. Open spiracular plate. Hair on rim of spiracular plate, absent from dorsal aspect of rim. Anal pads and horns similar in size; few spines posterior from anal pads.).....*Chrysomya marginalis*

6b. Dense coverage of hair on rim of spiracular plate. (Labrum tube-like with spinules on distal margin. Cephalopharyngeal skeleton with narrow tentorial phragma; defined posterio-dorsal notch to median tooth. All spiracular hairs clusters branched; finely-textured hair. Single-pointed spines on anterior and posterior margins of segments; indistinct fusiform area. Open spiracular plate. Dense coverage of hair on rim of spiracular plate. Anal pads smaller than anal horns; few spines posterior from anal pads.).....*Calliphora vicina*

4.3. KEYS FOR SECOND INSTAR LARVAE

As with first instar larvae twelve features were evaluated for their diagnostic value. Four features were found to be of no diagnostic use. These features were; (i) the oral ridges, (ii) the ventral organ, (iii) the labium and the (iv) the anterior spiracles. The first three features were discarded due to these showing little variations among the different species, whereas the last feature was discarded due to the large overlap noted in the ranges of the number of buttons present on an anterior spiracle for the different species. As with first instar larvae, the antennomaxillary lobes were useful to distinguish the sarcophagid from the calliphorids. The antennal sensory complex was morphologically similar in all of the species examined. Not more than two rings of interlaced cuticular folds surrounded the centralised grouping of sensillae of the maxillary sensory complex in calliphorids, while more than two rings were found in the sarcophagid. A partial separation could be achieved through six of the features i.e. (i) the integument, (ii) the labrum, (iii) the posterior spiracles, (iv) the spines, (v) the spiracular plate and the (vi) the anal area. All species could be uniquely identified through one of the features i.e. the cephalopharyngeal skeleton.

Second Instar Larvae; Key 1: Utilising the integument as a diagnostic marker. This aspect was examined by means of scanning electron microscopy. Only some of the species were identified uniquely with this characteristic.

1. Integument with prominent processes and papillae.....*Chrysomya albiceps*
   Relatively smooth integument, without prominent processes and papillae.....2

2. Hair on dorsum of posterior segments.....*Chrysomya chloropyga*
   No hair on dorsum of posterior segments.....3

3. Rounded protrusions of integument.....*Chrysomya marginalis*
   No rounded protrusions of integument.....unresolved for *Lucilia cuprina, Lucilia sericata, Calliphora vicina* and *Sarcophaga cruentata*

Cognisance should be taken that although hair was noted on the posterior dorsal aspect of most of the specimens of *C. chloropyga* examined this was not always the case in all specimens observed.

Second Instar Larvae; Key 2: Utilising the labrum as a diagnostic marker. This aspect was examined by means of scanning electron microscopy. Only some of the species were identified uniquely with this characteristic.

1. Labrum a distinct structure.....2
   Labrum indistinct from the margin of pre-oral cavity.....unresolved for *Lucilia cuprina, Lucilia sericata, Chrysomya chloropyga, Chrysomya marginalis* and *Calliphora vicina*

2. Four distinct lobes, broad distal margins.....*Chrysomya albiceps*
   Two curved lobes, curved to lateral position, edges rounded.....*Sarcophaga cruentata*

Second Instar Larvae; Key 3: Utilising the cephalopharyngeal skeleton as a diagnostic marker. This aspect was examined by means of light microscopy. All the species were identified uniquely with this characteristic.

1. Dorsal cornu split.....*Sarcophaga cruentata*
   Dorsal cornu solid.....2

2. Protuberances on ventral margin of hook of mouth hooks.....*Chrysomya albiceps*
   No protuberances on ventral margin of hook of mouth hooks.....3

3. Sickle-shaped mouth hook.....*Chrysomya marginalis*
Mouth hooks shaped differently.....4

4. Acute-angled mouth hook.....*Calliphora vicina*
Gradually curving mouth hooks.....5

5. Height of anterior margins of hypopharyngeal sclerite > 50% of height of posterior margin of mouth hooks.....*Chrysomya chloropyga*
Height of anterior margins of hypopharyngeal sclerite narrow in relation to height of posterior margin of mouth hooks.....6

6. Clearly visible, robust labial sclerite.....*Lucilia cuprina*
Less distinct labial sclerite.....*Lucilia sericata*

Although the protuberances on the ventral margin of the hooks of the mouth hooks were used at step 2 in the key to distinguish *C. albiceps* from the rest of the species, two other features were also distinctly different in the cephalopharyngeal skeleton of *C. albiceps* and could have been used at this point in the key. One of the features was the robust nature of the hypopharyngeal skeleton and the other one was the upward orientation of the parastomal bar in the cephalopharyngeal skeleton of *C. albiceps.*

Second Instar Larvae; Key 4: Utilising the posterior spiracles as a diagnostic marker. This aspect was examined by means of scanning electron microscopy. Only some of the species were identified uniquely with this characteristic.

1. Posterior spiracles hidden from view in spiracular cavity.....*Sarcophaga cruentata*
Posterior spiracles clearly visible on spiracular plate.....2

2. Middle-inner spiracular hair cluster compacted.....*Lucilia cuprina*
Middle-inner spiracular hair cluster branched.....3

3. Inner spiracular hair cluster attached to middle of rimae.....unresolved for *Lucilia sericata* and *Calliphora vicina*
Inner spiracular hair cluster attached more off-centre on rimae.....unresolved for *Chrysomya chloropyga, Chrysomya marginalis* and *Chrysomya albiceps*

Second Instar Larvae; Key 5: Utilising the spines as a diagnostic marker. The analysis was based on the lateral view of the larva. This aspect was examined by means of light microscopy. All the species were identified uniquely with this characteristic.

1. Single-pointed spines.....2
   Single and multi-pointed spines.....6

2. Tips of spines rounded.....*Chrysomya marginalis*
   Tips of spines sharp.....3

3. Predominantly anterior spine bands.....4
   Posterior spine bands also present.....5

4. Very few spines on lateral aspect of posterior segments.....*Lucilia sericata*
   Good coverage of spines on lateral aspects of all segments.....*Lucilia cuprina*

5. Anterior spine band completes up to segment 8/9.....*Calliphora vicina*
   Anterior spine band completes up to segment 6.....*Sarcophaga cruentata*

6. Anterior spine band complete up to segment 10.....*Chrysomya chloropyga*
   Spine band only visible for first few segments due to excessive folding between segments.....*Chrysomya albiceps*

This analysis is presented with some trepidation, due to the variances noted when reviewing the extent of the complete spine bands as found during the current study with that noted in other publications.

When using the spines as a diagnostic marker, the best results will be achieved when the lateral aspect of the larva is evaluated. The dorsal aspect only reveals the completeness of the spine bands and the type of spines present. Only a few species will be uniquely identified when spine areas are accessed from this view. The ventral view also will not yield optimum results since the longitudinal split in the spine band of *L. cuprina* and the even fainter split noticed in the spine band of *L. sericata* were not distinct features to base identification on. Even if an attempt was made to use this feature, fewer species will be uniquely identified.

Second Instar Larvae; Key 6: Utilising the spiracular field as a diagnostic marker. This aspect was examined by means of scanning electron microscopy. Only some of the species were identified uniquely with this characteristic.

1. Deep-set spiracular atrium.....*Sarcophaga cruentata*
   Spiracular field an open structure.....2

2. Perispiracular tubercles large with crown of spinules.....*Chrysomya albiceps*
   Perispiracular tubercles small or moderately sized without crown of spinules.....3

3. Sparse covering of hair on rim of spiracular field.....unresolved for *Lucilia cuprina*, *Lucilia sericata* and *Chrysomya chloropyga*
   Significant covering of hair on rim of spiracular field.....unresolved for *Chrysomya marginalis* and *Calliphora vicina*

Second Instar Larvae; Key 7: Utilising the anal area as a diagnostic marker. This aspect was examined by means of scanning electron microscopy. Only some of the species were identified uniquely with this characteristic.

1. Anal horns triangular.....2
   Anal horns rounded.....4

2. Only single-pointed spines in area around anal area.....*Sarcophaga cruentata*
   Multi-pointed spines also in area around anal area.....3

3. Single-pointed spines with rounded tips.....*Chrysomya marginalis*
   Multi-pointed spines with sharp tips.....*Chrysomya albiceps*

4. Multi-pointed spines in area around anal area.....*Chrysomya chloropyga*
   Single-pointed spines in area around anal area.....unresolved for *Lucilia cuprina*, *Lucilia sericata* and *Calliphora vicina*

Second Instar Larvae; Key 8: A combined key utilising those features visible from the dorsal aspect of the larva. Features of the spine bands (completeness on segments) and the integument will be considered. These aspects were examined by means of scanning electron microscopy. Only some of the species were identified uniquely when viewed from this angle.

1. Hair on dorsum of posterior segments.....*Chrysomya chloropyga*
   No hair on integument.....2

2. Pronounced processes and papillae of integument.....*Chrysomya albiceps*
   No processes or papillae of integument.....3

3. Rounded spine tips.....*Chrysomya marginalis*
   Sharp-tipped spines.....4

4. Predominantly anterior spine bands.....unresolved for *Lucilia cuprina* and *Lucilia sericata*
   Anterior and posterior spine bands.....5

5. Complete anterior spine bands from segments 2 to 6.....*Sarcophaga cruentata*
   Complete anterior spine bands from segments 2 to 8/9.....*Calliphora vicina*

Many larvae were curved in such a way that the spiracular plates were revealed when the larvae where placed on its ventral aspect. In these instances the features of the spiracular plate can be utilised in this analysis. *Lucilia* species could not be distinguished from each other in the above key. However, should the elements of the spiracular plate and in particular the posterior spiracles be visible, these two species could be uniquely identified. The above key is started at the point unresolved for *Lucilia* species, i.e. from step 4

4. Predominantly anterior spine bands.....5
   Anterior and posterior spine bands.....6

5. Middle-inner spiracular hair cluster compacted.....*Lucilia cuprina*
   Middle-inner spiracular hair cluster branched.....*Lucilia sericata*

6. Complete anterior spine bands from segments 2 to 6.....*Sarcophaga cruentata*
   Complete anterior spine bands from segments 2 to 8/9.....*Calliphora vicina*

Second Instar Larvae; Key 9: A combined key utilising those features visible from the lateral aspect of the larva. Features of the spine bands (completeness on segments) and the integument will be considered. These aspects were examined by means of scanning electron microscopy. All the species were identified uniquely when viewed from this angle.

1. Hair on dorsum of posterior segments.....*Chrysomya chloropyga*
   No hair on integument.....2

2. Pronounced processes and papillae of integument.....*Chrysomya albiceps*
   No processes and papillae of integument.....3

3. Rounded bulges of integument.....*Chrysomya marginalis*
   No rounded bulges of integument.....4

4. Predominantly anterior spine bands.....5
   Anterior and posterior spine bands.....6

5. Few spines in spine bands of posterior segments on lateral aspects.....*Lucilia sericata*
   Good coverage of spines in spine bands on lateral aspect of larva.....*Lucilia cuprina*

6. Complete anterior spine bands up to segments 2 to 6.....*Sarcophaga cruentata*
   Complete anterior spine bands up to segments 2 to 8/9.....*Calliphora vicina*

Second Instar Larvae; Key 10: A combined key utilising those features visible from the ventral aspect of the larva. Features of the integument, spines, the labrum and the anal area were considered. These aspects were examined by means of scanning electron microscopy. Only some of the species were identified uniquely when viewed from this angle.

1. Pronounced processes and papillae of integument.....*Chrysomya albiceps*
   No processes or papillae of integument.....2

2. Margins of labrum not distinctly differentiated from margins of pre-oral cavity.....3
   Labrum distinctly structured, two curved lobes.....*Sarcophaga cruentata*

3. Anal horns triangular.....*Chrysomya marginalis*
   Anal horns rounded.....4

4. Multi-pointed spines around anal area.....*Chrysomya albiceps*
Single-pointed spines around anal area.....unresolved for *Lucilia cuprina*, *Lucilia sericata* and *Calliphora vicina*

Second Instar Larvae; Key 11: A combined key utilising those features visible from the posterior aspect of the larva. Features of the spiracular field and the posterior spiracles were considered. These aspects were examined by means of scanning electron microscopy. Not all the species were identified uniquely when viewed from this angle.

1. Deep-set spiracular atrium.....*Sarcophaga cruentata*
Open spiracular plate.....2

2. Large perispiracular tubercles with spinules at tips.....*Chrysomya albiceps*
Moderately sized or small perispiracular tubercles without spinules at tips.....3

3. Sparse covering of hair on rim of spiracular plate.....4
Moderate covering of hair on rim of spiracular plate.....unresolved for *Chrysomya chloropyga* and *Chrysomya marginalis*

4. Middle-inner spiracular hair cluster compacted.....*Lucilia cuprina*
Middle-inner spiracular hair cluster branched.....5

5. Inner spiracular hair cluster attached to middle of rimae.....*Lucilia sericata*
Inner spiracular hair cluster attached of-centre on rimae.....*Chrysomya chloropyga*

Second Instar Larvae; Key 12: An abridged descriptive key utilising all characteristics, irrespective of the angle the larvae was viewed from. Aspects were examined by means of light and scanning electron microscopy. All species were uniquely described with these characteristic.

1a. Integument with prominent processes. (Four lobed labrum, broad distal margins. Solid dorsal cornu; gradually curving mouth hooks; robust hypopharyngeal skeleton; upward turned parastomal bar; protuberances at base of hooks. Spiracular hair clusters branched; inner spiracular hair cluster attached to upper-third of rimae. Predominantly multi-pointed spines on anterior margins of segments. Spiracular field an open structure; large perispiracular tubercles; distal tip crowned with spinules; sparse covering of hair on rim of spiracular field. Triangular anal horns; multi-pointed spines around anal area.).....*Chrysomya albiceps*

1b. Relatively smooth integument.....2

2a. Hair on dorsum of posterior segments of most specimens. (Simplified labrum with margins undistinguished from margins of pre-oral cavity. Solid dorsal cornu; gradually curving mouth hooks; height of anterior margin of hypopharyngeal skeleton >50% of height of posterior margin of base of mouth hooks. Spiracular hair clusters branched; inner spiracular hair cluster attached on upper-third of margin of rimae. Predominantly multi-pointed spines on anterior margins of segments 2 to 10. Spiracular field open structure; small perispiracular tubercles; sparse covering of hair on rim of spiracular field. Rounded anal horns; multi-pointed spines around anal area.).....*Chrysomya chloropyga*

2b. No hair on dorsum of posterior segments.....3

3a. Rounded protrusions of integument. (Simplified labrum with margins undistinguished from margins of pre-oral cavity. Solid dorsal cornu; sickle-shaped mouth hooks. Spiracular hair clusters branched; inner spiracular hair cluster attached to upper-third of rimae. Single-pointed spines with rounded tips. Spiracular field open structure; small perispiracular tubercles; significant coverage of hair on rim of spiracular field. Triangular anal horns; single-pointed spines around anal area.).....*Chrysomya marginalis*

3b. No rounded protrusions of integument.....4

4a. Deep set spiracular atrium. (Smooth integument. Two-lobed labrum; rounded distal margins. Split dorsal cornu. Posterior spiracles hidden from view in spiracular cavity. Sharp-tipped single-pointed spines; anterior spine band to segment 6; also posterior spine bands. Small perispiracular tubercles; moderate coverage of hair on rim of spiracular field. Triangular anal horns; single-pointed spines around anal area.).....*Sarcophaga cruentata*

4b. Spiracular field an open structure.....5

5a. Significant coverage of hair on rim of spiracular field. (Smooth integument. Simplified labrum with margins undistinguished from margins of pre-oral cavity. Solid dorsal cornu; acutely angled mouth hooks. Spiracular hair clusters branched; inner spiracular hair cluster attached to middle of margin rimae. Sharp-tipped, single-pointed spines; anterior spine band to segment 8/9; also posterior spine bands. Spiracular field an open structure; small perispiracular tubercles. Anal horns rounded; single-pointed spines around anal area.).....*Calliphora vicina*

5b. Sparse covering of hair on rim of spiracular field.....6

6a. Middle-inner spiracular hair cluster compacted. (Smooth integument. Simplified labrum with margins undistinguished from margins of pre-oral cavity. Solid dorsal cornu; gradually curving mouth hooks; height of anterior margin of hypopharyngeal skeleton narrow in relation to height of posterior margin of base of mouth hooks; clearly visible, robust labial sclerite. Sharp-tipped, single-pointed spines; predominantly anterior spine bands; good coverage of spines on lateral aspect of segments. Spiracular field an open structure; small perispiracular tubercles; sparse coverage of hair on rim of spiracular field. Anal horns rounded; single-pointed spines around anal area.).....*Lucilia cuprina*

6b. Middle-inner spiracular hair cluster branched. (Smooth integument. Simplified labrum with margins undistinguished from margins of pre-oral cavity. Solid dorsal cornu; gradually curving mouth hooks; height of anterior margin of hypopharyngeal skeleton narrow in relation to height of posterior margin of base of mouth hooks; labial sclerite not distinct. Inner spiracular hair cluster attached to middle of rimae. Sharp-tipped, single-pointed spines; predominantly anterior spine bands; very few spines on lateral aspect of segments. Spiracular field an open structure; small perispiracular tubercles; sparse coverage of hair on rim of spiracular field. Anal horns rounded; single-pointed spines around anal area.).....*Lucilia sericata*

4.4. KEYS FOR THIRD INSTAR

As in second and first instar larvae twelve characteristics were evaluated for their diagnostic value. However, one of these characteristics was evaluated in terms of two methodologies, bringing to the table 13 aspects third instar larvae can be evaluated in terms of. Of the 13 aspects evaluated, four were of no diagnostic value. As in second instar larvae some of these characteristics (ventral organ, oral ridges and labium) were discarded due to the lack of variation noted in these characteristics in the different species and due to the overlap noted in the ranges of the number of buttons on the anterior spiracles of the different species. The antennomaxillary complex was as in second instar larvae, a distinguishing characteristic between the sarcophagid and the calliphorids. A partial separation could be achieved through two of the characteristics, namely the integument and the spines. A larger range of characteristics than that found for second instar larvae were robust enough to be used on a stand-alone basis.

These six characteristics on which a full separation can be achieved were; (i) the labrum, (ii) the cephalopharyngeal skeleton, (iii and iv) the posterior spiracles by means of light and scanning electron microscopy, (v) the spiracular plate and (vi) the anal area. Due to the labrum being vastly different in the species examined and not being based on a common ground plan, no key will be presented for it.

Third Instar Larvae; Key 1: Utilising the integument as a diagnostic marker. This aspect was examined by means of scanning electron microscopy. Only some of the species were identified uniquely with this characteristic. The benefit of this characteristic is that the difference between smooth and hairy (*Chrysomya albiceps*) and also for that species with its prolapsed integument (*Chrysomya marginalis*) a diagnostic can be done with the aid of a stereo microscope. This allows for rapid identification, without any preparation for these species needed.

1. Integument with prominent processes or papillae .....*Chrysomya albiceps*
   Integument with no prominent processes or papillae.....2

2. Sac-like protrusion of integument.....*Chrysomya marginalis*
   No sac-like protrusions of integument.....3

3. Small, pointed bulges on lateral and ventral aspects of segments.....*Sarcophaga cruentata*
   No pointed bulges on lateral and ventral aspects of segments.....unresolved for *Lucilia cuprina*, *Lucilia sericata*, *Chrysomya chloropyga* and *Calliphora vicina*

Evaluating this characteristic is highly dependent on the correct procedures being followed during the killing and preservation of specimens. Following the correct procedures prevents larvae that do not have prolapsed integuments from exhibiting this type of features as artefacts of distortion due to incorrect killing and preservation methodologies.

Third Instar Larvae; Key 2: Utilising the cephalopharyngeal skeleton as a diagnostic marker. This aspect was examined by means of scanning electron microscopy. All of the species were identified uniquely with this characteristic.

1. Split dorsal cornu.....*Sarcophaga cruentata*
   Solid dorsal cornu.....2

2. Parastomal bar not distinctly visible.....*Chrysomya albiceps*
   Parastomal bar distinctly visible.....3

3. Accessory oral sclerite present.....4
Accessory oral sclerite absent.....5

4. Robust mouth hooks.....*Chrysomya marginalis*
Slender mouth hooks.....*Calliphora vicina*

5. Termination pattern of dorsal cornu rounded.....*Chrysomya chloropyga*
Termination pattern of dorsal cornu pointed.....6

6. Slender mouth hooks.....*Lucilia sericata*
Robust mouth hooks.....*Lucilia cuprina*

The absence of a prominent posterio-dorsal notch at the base portion of the mouth hooks in *C. marginalis*, opposed to it being present in *C. vicina* can also be used as a distinguishing characteristic at step 4.

Third Instar Larvae; Key 3: Utilising the posterior spiracle as a diagnostic marker. This aspect was examined by means of light microscopy. All of the species were identified uniquely with this characteristic.

1. Peritreme complete.....2
Peritreme incomplete.....4

2. Plunging projection between middle and outer spiracular openings.....*Lucilia sericata*
No plunging projection between middle and outer spiracular openings.....3

3. Buttons of posterior spiracles orientated in ventro-medially position.....*Lucilia cuprina*
Buttons of posterior spiracles orientated to medial position.....*Calliphora vicina*

4. Thin spiracular openings.....*Sarcophaga cruentata*
Substantial spiracular openings.....5

5. Thin peritreme.....*Chrysomya marginalis*
Substantial peritreme.....6

6. Peritreme form cup-like projection at position of button.....*Chrysomya albiceps*
Peritreme form no cup-like projections at position of button.....*Chrysomya chloropyga*

At step 4 the ear-shaped posterior spiracles and the spiracular openings not pointing to the position of the button in *S. cruentata* opposed to the O-shaped posterior spiracles and the spiracular opening orientated towards the position of the button in the other species could also be utilised as distinguishing characteristics.

At step 6, the projections the peritreme forms between the spiracular openings can be used to distinguish these species from each other. In *C. albiceps* the peritreme was slightly arched in between the spiracular openings. In *C. chloropyga* a knob-like projection was formed between the inner and middle spiracular openings and a sharp-pointed projection was formed between the middle and outer spiracular openings.

Third Instar Larvae; Key 4: Utilising the posterior spiracle as a diagnostic marker. This aspect was examined by means of scanning electron microscopy. All of the species were identified uniquely with this characteristic.

1. Buttons of posterior spiracles orientated to midline of spiracular plate.....*Calliphora vicina*
   Buttons of posterior spiracles orientated in ventro-medial position.....2

2. Posterior spiracles ear-shaped.....*Sarcophaga cruentata*
   Posterior spiracles O-shaped.....3

3. Middle-inner spiracular hair cluster compacted.....4
   Middle-inner spiracular hair cluster branched.....5

4. Dorsal spiracular plate area infringed upon.....*Lucilia cuprina*
   Dorsal spiracular plate area not infringed upon.....*Lucilia sericata*

5. Outer spiracular hair cluster attachment area similar to other spiracular hair clusters, i.e. attached to small area.....*Chrysomya albiceps*
   Outer spiracular hair cluster attachment area larger than other spiracular hair clusters.....6

6. Dorsal spiracular plate area infringed upon.....*Chrysomya chloropyga*
   Dorsal spiracular plate area not infringed upon.....*Chrysomya marginalis*

Sarcophaga cruentata could also be distinguished from the rest of the species by the thin spiracular openings opposed to the more substantial spiracular openings noted for the other species.

Third Instar Larvae; Key 5a: Utilising the spines as seen from ventrally as a diagnostic marker. This aspect was examined by means of scanning electron microscopy. Not all species could be identified uniquely with this characteristic.

1. Predominantly single-pointed spines.....2
   Single and multi-pointed spines.....3

2. Round-tipped spines.....*Chrysomya marginalis*
   Sharp-tipped spines.....unresolved for *Calliphora vicina* and *Sarcophaga cruentata*

3. Multi-pointed spines have notched tips.....4
Multi-pointed spines almost split to base.....5

4. Creeping welt as ridge.....*Chrysomya albiceps*
Creeping welt flat with surface.....*Chrysomya chloropyga*

5. Distinct longitudinal split in creeping welt.....*Lucilia cuprina*
Generally no split or very indistinct split in creeping welt.....*Lucilia sericata*

At step 4 the degree to which the tips of the spines split could be utilised to distinguish amongst these two species. The tips were split slightly in *C. albiceps*, whereas a more pronounced split was noted in the tips of *C. chloropyga* spines.

Third Instar Larvae; Key 5b: Utilising the spines as seen laterally as a diagnostic marker. This aspect was examined by means of scanning electron microscopy. Not all species could be identified uniquely with this characteristic. Unfortunately due to the fusiform area not being observed optimally in *C. vicina* specimens examined, this species could not be analysed further where the fusiform area was used in the key. However, this key illustrates that a lateral view was also insufficient to uniquely identify all species even at the point in the key (step 3) where the characteristics of the fusiform area were not used.

1. Predominantly single-pointed spines.....2
Single and multi-pointed spines.....3

2. Round-tipped spines.....*Chrysomya marginalis*
Sharp-tipped spines.....unresolved due to fusiform area problem discussed previously *Calliphora vicina* and *Sarcophaga cruentata*

3. Multi-pointed spines have notched tips.....4
Multi-pointed spines split to almost base.....unresolved for *Lucilia cuprina* and *Lucilia sericata*

4. Notch in tip of spines slight.....*Chrysomya albiceps*
Notch in tip of spines distinct.....*Chrysomya chloropyga*

Third Instar Larvae; Key 6: Utilising the spiracular field as a diagnostic marker. This aspect was examined by means of scanning electron microscopy. All of the species were identified uniquely with this characteristic.

1. Deep-set spiracular atrium.....*Sarcophaga cruentata*
   Open, flat spiracular plate.....2

2. Small, indistinct perispiracular tubercles.....*Lucilia sericata*
   Clearly visible perispiracular tubercles.....3

3. Perispiracular tubercles with crown of spinules.....*Chrysomya albiceps*
   Perispiracular tubercles without crown of spinules.....4

4. Middle dorsal tubercle closer to outer dorsal tubercles than to inner dorsal tubercle.....*Chrysomya chloropyga*
   Middle dorsal tubercle equidistant from inner and outer dorsal tubercles.....5

5. Slightly sunken-in spiracular plate.....*Chrysomya marginalis*
   Spiracular plate not sunken-in.....6

6. Slightly angled / hinged spiracular plate.....*Lucilia cuprina*
   Spiracular plate not angled / hinged.....*Calliphora vicina*

Third Instar Larvae; Key 7: Utilising the anal area as a diagnostic marker. This aspect was examined by means of scanning electron microscopy. All of the species were identified uniquely with this characteristic.

1. Triangular anal horns.....2
   Anal horns not triangular.....6

2. Anal horn form equilateral triangle.....3
   Anal horn form isosceles triangle.....4

3. Anal pads smaller than anal horns.....*Lucilia cuprina*
   Anal pads and horns similar in size.....*Calliphora vicina*

4. Anal pads smaller than anal horns.....*Chrysomya marginalis*
   Anal pads and horns similar in size.....5

5. Fine, small spines cover anal pads.....*Chrysomya chloropyga*
   Robust spines cover anal pads.....*Chrysomya albiceps*

6. No spines on anal pads.....*Lucilia sericata*
Spines on anal pads.....*Sarcophaga cruentata*

Step 5 could also be resolved by considering the spines around the anal area. Multiple rows of robust spines surrounded the anal area in *C. albiceps*, whereas approximately two rows of smaller spines surrounded the anal area in *C. chloropyga*.
Step 6 could be resolved through the shape of the anal horns. The anal horns were elongated structures in *S. cruentata*, whereas being more compact, rounded structures in *L. sericata*.

Third Instar Larvae; Key 8: A combined key utilising those features visible from the dorsal aspect of the larva. Features of the spines and the integument will be considered. These aspects were examined by means of scanning electron microscopy. Only some of the species were identified uniquely when viewed from this angle.

1. Integument with processes and papillae.....*Chrysomya albiceps*
Integument with no processes or papillae.....2

2. Predominantly single-pointed spines.....3
Single and multi-pointed spines.....4

3. Round-tipped spines.....*Chrysomya marginalis*
Sharp-tipped spines.....unresolved for *Calliphora vicina* and *Sarcophaga cruentata*

4. Slight notch in tips of multi-pointed spines.....*Chrysomya chloropyga*
Comb-like structure, multi-pointed spines split to almost base.....unresolved for *Lucilia cuprina* and *Lucilia sericata*

The same key can be used for the lateral view since the fusiform area; a feature of the spines available for analysis on the lateral aspect of larvae, was not sufficiently varied in the species examined.

Third Instar Larvae; Key 9: A combined key utilising those features visible from the ventral aspect of the larva. Features of the integument, spines and anal area will be considered. These aspects were examined by means of scanning electron microscopy. All species were identified uniquely when viewed from this angle.

1. Integument with processes and papillae.....*Chrysomya albiceps*
   Integument with no processes or papillae.....2

2. Distinct longitudinal split in creeping welt.....*Lucilia cuprina*
   No split or indistinct split in creeping welt.....3

3. Triangular anal horns.....4
   Anal horns not triangular.....6

4. Anal horn form equilateral triangle.....*Calliphora vicina*
   Anal horn form isosceles triangle.....5

5. Anal pads smaller than anal horns.....*Chrysomya marginalis*
   Anal pads and horns similar in size.....*Chrysomya chloropyga*

6. No spines on anal pads.....*Lucilia sericata*
   Spines on anal pads.....*Sarcophaga cruentata*

Since the labrum is also available from this view, the identification can be used to confirm the identification.

Third Instar Larvae; Key 10: A combined key utilising those features visible from the posterior aspect of the larva. Features of the spiracular plate and the posterior spiracles were considered. These aspects were examined by means of scanning electron microscopy. All species were identified uniquely when viewed from this angle.

1. Deep-set spiracular atrium.....*Sarcophaga cruentata*
   Open spiracular plate.....2

2. Perispiracular tubercles with crown of spinules.....*Chrysomya albiceps*
   Perispiracular tubercles without crown of spinules.....3

3. Buttons of posterior spiracles orientated medially.....*Calliphora vicina*
   Buttons of posterior spiracles orientated ventro-medially.....4

4. Slightly sunken-in spiracular plate.....*Chrysomya marginalis*
Spiracular plate not sunken-in.....5

5. Middle-inner spiracular hair cluster compacted.....6
Middle-inner spiracular hair cluster branched.....*Chrysomya chloropyga*

6. Indistinct perispiracular tubercles.....*Lucilia sericata*
Distinct perispiracular tubercles.....*Lucilia cuprina*

Third Instar Larvae; Key 11: An abridged descriptive key utilising all characteristics, irrespective of the angle the first instar larvae was viewed from. Aspects were examined by means of light and scanning electron microscopy. All species were uniquely described with these characteristic.

1a. Deep-set spiracular atrium. (Integument with small, pointed bulges on lateral and ventral aspects of segments 5 to 11. Labrum M-shaped. Split dorsal cornu. Buttons of posterior spiracles face ventro-medially; posterior spiracles ear-shaped; thin spiracular slits; spiracular slits not orientated to button; incomplete, thin-walled peritreme; dorsal spiracular plate area not infringed on. Single-pointed and sharp-tipped spines. Small perispiracular tubercles. Anal horns elongated structures; spines around anal area and on anal pads.).....,.....*Sarcophaga cruentata*
1b. Open spiracular plate.....2

2a. Integument with processes. (Labrum in form of moustache on a lip. Solid dorsal cornu; parastomal bar not distinct structure due to large hypopharyngeal sclerite; three bumps at base of mouth hooks. Buttons of posterior spiracles face ventro-medially; O-shaped posterior spiracles; moderately size spiracular slits orientated to button; incomplete peritreme of substantial thickness; peritreme form cup-like projections around button, ach slightly between spiracular slits; spiracular hair branched; small area of attachment of outer spiracular hair cluster; dorsal spiracular plate area infringed on. Single and multi-pointed spines; distinct notched tips. Open spiracular plate; distinct perispiracular tubercles; middle dorsal tubercle equidistant from inner and outer dorsal tubercles. Anal horns in form of isosceles triangle; anal pads and horns similarly sized; robust spines on anal pads and form multiple rows around anal area.).....*Chrysomya albiceps*
2b. Integument with no processes.....3

3a. Integument with rounded protrusions. (Labrum proximal part box-shaped, distal portion with slight split in tip attached to main body at perpendicular angle, distal margin serrated. Solid dorsal cornu, accessory oral sclerite; robust mouth hooks, no prominent posterio-dorsal notch to base of mouth hooks. Buttons of posterior spiracles orientated ventro-medially; O-shaped posterior spiracles; moderately size spiracular slits orientated to button; incomplete thin peritreme; spiracular hair clusters branched; large area of attachment of outer spiracular hair cluster; dorsal spiracular plate area was not infringed on. Round-tipped, single-pointed spines. Relatively open spiracular plate somewhat sunken-in; noticeable perispiracular tubercles; middle dorsal tubercle equidistant from inner and outer dorsal tubercles. Anal horns in form of isosceles triangle; anal pads smaller than anal horns.).....*Chrysomya marginalis*

3b. No rounded protrusions of integument.....4

4a. Buttons of posterior spiracles orientated medially. (Smooth integument. Half-moon shaped proximal portion of labrum; slight split to narrow, sharp-tipped distal portion. Solid dorsal cornu; accessory oral sclerite; small, slender mouth hooks; prominent posterio-dorsal projection to base. Buttons of posterior spiracle orientated medially; O-shaped posterior spiracles; moderately sized spiracular slits orientated to button; complete peritreme; no inner projection of peritreme; spiracular hair clusters branched; small area of attachment of outer spiracular hair cluster; dorsal spiracular plate area was not infringed on. Single-pointed, sharp-tipped spines. Open spiracular plate; noticeable perispiracular tubercles; middle dorsal tubercle equidistant from inner and outer dorsal tubercles. Anal horns form equilateral triangle; anal pads and horns similarly sized.).....*Calliphora vicina*

4b. Buttons of posterior spiracles orientated ventro-medially.....5

5a. Middle-inner spiracular hair cluster branched. (Smooth integument. Labrum roughly box-shaped; proximal margin convex; distal margin with serrated edges; tip split somewhat. Solid dorsal cornu; dorsal cornu terminate rounded. Buttons of posterior spiracle orientated ventro- medially; O-shaped posterior spiracles; moderately sized spiracular slits orientated to button; incomplete peritreme of substantial thickness; knob-like and sharp-tipped inner projections; spiracular hair clusters branched; large area of attachment of outer spiracular hair cluster; dorsal spiracular plate area was infringed on. Single and multi-pointed spines, pronounced split in tips of multi-pointed spines. Open spiracular plate, clearly visible perispiracular tubercles; middle dorsal tubercle closer to outer dorsal tubercles than to inner dorsal tubercle. Anal horns in form of isosceles triangle; anal horns and pads similarly sized, small spines on anal pads and approximately two rows of spines around anal area.).....*Chrysomya chloropyga*

5b. Middle-inner spiracular hair cluster compacted.....6

6a. Distinct perispiracular tubercles. (Smooth integument. Overall shape of labrum triangular, distal tip pointed. Solid dorsal cornu; pointed termination pattern; robust mouth hooks. Buttons of posterior spiracles orientated ventro-medially; O-shaped posterior spiracles; moderately sized spiracular slits orientated to button; complete peritreme of substantial thickness; slight inner projections; middle-inner spiracular hair clusters compacted; small area of attachment of outer spiracular hair cluster; dorsal spiracular plate area was infringed on. Single and multi-pointed spines; multi-pointed spines had comb-like form with split to almost base of spines. Open spiracular plate; middle dorsal tubercle equidistant from inner and outer dorsal tubercles. Anal horns form equilateral triangle; anal pads smaller than anal horns; no spines on anal pads, only around anal area.).....*Lucilia cuprina*

6b. Indistinct perispiracular tubercles. (Smooth integument. Overall shape of labrum triangular, distal tip broad. Solid dorsal cornu; pointed termination pattern; long, slender mouth hooks. Buttons of posterior spiracle orientated ventro-medially; O-shaped posterior spiracles; moderately size spiracular slits orientated to button; complete, thin-walled peritreme with deep plunging projection between outer and middle spiracular slit; middle-inner spiracular hair clusters compacted; small area of attachment of outer spiracular hair cluster; dorsal spiracular plate area was not infringed on. Single and multi-pointed spines; multi-pointed spines comb-like form with split to almost base of spines. Open spiracular plate; middle dorsal tubercle closer to outer dorsal tubercle than to inner dorsal tubercle. Anal horns rounded; anal pads smaller than anal horns; no spines on anal pads, only around anal area.).....*Lucilia sericata*

4.5. KEYS FOR PUPARIA

Seven characteristic of the puparium were evaluated for their diagnostic value. Two of these characteristics i.e. (i) the frontal field and (ii) the bubble membrane could be used to uniquely describe all the species. A partial separation could be achieved utilising five of these characteristics i.e. (i) the shape of the puparium, (ii) the texture of the puparium, (iii) the lateral ridge, (iv) the spiracular plate and (v) the anal area. The analysis of the combined keys used characteristics viewed by light microscopy as well as scanning electron microscopy. This is possible for puparia, since “fresh” material can be examined by means of light microscopy and then be prepared for scanning electron microscopy without suffering structural damage.

Puparia; Key 1: Utilising the shape of the puparium as a diagnostic marker. This aspect was examined by means of light microscopy. Only some of the species were identified uniquely with this characteristic.

1. Pointed, narrow anterior end.....2
   Rounded, broad anterior end.....5

2. Constrictions between segments.....*Chrysomya albiceps*
   Smooth transition between segments.....3

3. Rounded posterior end.....*Calliphora vicina*
   Truncated posterior end.....4

4. Narrow posterior end.....*Chrysomya chloropyga*
   Broad posterior end.....*Chrysomya marginalis*

5. Narrow, truncated posterior end.....*Sarcophaga cruentata*
   Broad, rounded posterior end.....unresolved for *Lucilia cuprina* and *Lucilia sericata*

Puparia; Key 2: Utilising the surface texture of the puparia as a diagnostic marker. This aspect was examined by means of light microscopy. Only some of the species were identified uniquely with this characteristic.

1. Rough surface with course transverse striations.....2
   Smooth surface with fine, superficial transverse striations.....4

2. Processes and papillae on puparium surface.....*Chrysomya albiceps*
   No processes and papillae on puparium surface.....3

3. Surface texture uniform across dorsal surface.....*Chrysomya chloropyga*
   Difference in surface texture between anterior and posterior halves of puparium....*Chrysomya marginalis*

4. Slight indentations on lateral surface.....*Sarcophaga cruentata*
   No indentations on lateral surface.....5

5. Narrow, wavy ridges on lateral surface.....*Calliphora vicina*
   Barely visible ridges on lateral surface.....unresolved for *Lucilia cuprina* and *Lucilia sericata*

Puparia; Key 3: Utilising the lateral ridge as a diagnostic marker. This aspect was examined by means of scanning electron microscopy. Only some of the species were identified uniquely with this characteristic.

1. Narrow lateral ridge.....2
   Broad lateral ridge area.....unresolved for *Chrysomya chloropyga, Chrysomya marginalis* and *Chrysomya albiceps*

2. Lateral ridge perched on a narrow raised area.....*Calliphora vicina*
   No raised area noted.....unresolved for *Lucilia cuprina*, *Lucilia sericata* and *Sarcophaga cruentata*

Puparia; Key 4: Utilising the frontal field as a diagnostic marker. This aspect was examined by means of scanning electron microscopy. All the species were identified uniquely with this characteristic.

1. Distinct lip-like fold or ridges and loops.....2
   Wrinkled folds.....3

2. Lip-like fold on ventro-medial aspect of frontal field.....*Sarcophaga cruentata*
   Distinct ridges and loops of frontal field.....*Chrysomya albiceps*

3. Large folds.....4
   Small folds.....5

4. Large folds contained between margins of anterior spiracles.....*Chrysomya chloropyga*
   Large folds not contained between margins of anterior spiracles.....*Chrysomya marginalis*

5. Superficial folds.....*Lucilia sericata*
   Deeper-set folds.....6

6. Larger folds around mouth scar arranged radially symmetrical.....*Lucilia cuprina*
   Larger folds around mouth scar not radially symmetrically arranged.....*Calliphora vicina*

Puparia; Key 5: Utilising the bubble membrane as a diagnostic marker. This aspect was examined by means of scanning electron microscopy. All the species were identified uniquely with this characteristic.

1. No bubble membrane.....*Sarcophaga cruentata*
   Bubble membrane.....2

2. Bubble membrane in slight depression.....3
   Bubble membrane level with puparium surface or slightly raised.....4

3. Widely spaced bubbles, arranged as two disrupted rings around central ones.....*Chrysomya marginalis*
   Closely grouped bubbles, smaller bubbles surrounding larger ones in centre.....*Calliphora vicina*

4. Two rings of bubbles surrounding central bubbles.....*Chrysomya albiceps*
   Not like above.....5

5. Similar sized bubbles.....6
   Combination of large and small bubbles.....*Chrysomya chloropyga*

6. Structured arrangement, ring of bubbles surrounding centralised ones.....*Lucilia sericata*
   Unstructured arrangements, grouped together with no specific pattern.....*Lucilia cuprina*

Puparia; Key 6: Utilising the spiracular field as a diagnostic marker. This aspect can be examined by means of light and scanning electron microscopy. Not all of the species were identified uniquely with this characteristic.

1. Deep-set spiracular atrium.....*Sarcophaga cruentata*
   Open spiracular plate.....2

2. Large perispiracular tubercles.....*Chrysomya albiceps*
   Small perispiracular tubercles.....3

3. Slightly bulging spiracular plate.....unresolved for *Lucilia cuprina. Lucilia sericata* and *Calliphora vicina*
   Spiracular plate not bulging.....4

4. Posterior spiracles visible when viewed dorsally or laterally.....*Chrysomya marginalis*
   Posterior spiracles not visible when viewed dorsally or laterally.....*Chrysomya chloropyga*

It should be noted that the key was worked out for the posterior view up to step 2. Analysing the specimens in terms of the bulging of the spiracular plate (step 3) could only be performed when viewing the puparium dorsally or laterally.

Puparia; Key 7: Utilising the anal area as a diagnostic marker. This aspect was examined by means of scanning electron microscopy. Not all of the species were identified uniquely with this characteristic.

1. Elements of the anal area clearly distinguished from puparium surface.....*Chrysomya chloropyga*
   Elements of the anal area not clearly distinguished from puparium surface.....2

2. Anterior margin of anal area bounded by horse-shoe ridge.....*Chrysomya albiceps*
   No horse-shoe ridge present.....unresolved for *Lucilia cuprina, Lucilia sericata, Chrysomya marginalis, Calliphora vicina* and *Sarcophaga cruentata*

Puparia; Key 8: A combined key utilising those features visible from the dorsal aspect of the puparium. The features considered were: puparium surface texture, shape of the puparium and certain aspects of the spiracular plate that could be determined from the dorsal view. These aspects were examined by means of light microscopy. Only some of the species were identified uniquely when viewed from this angle.

1. Broad, rounded anterior end.....2
   Narrow, pointed anterior end.....3

2. Narrow, truncated posterior end.....*Sarcophaga cruentata*
   Broad, rounded posterior end.....unresolved for *Lucilia cuprina* and *Lucilia sericata*

3. Distinct processes and papillae on puparium surface.....*Chrysomya albiceps*
   No distinct processes and papillae on puparium surface.....4

4. Difference in texture of anterior and posterior halves of puparium.....*Chrysomya marginalis*
   Puparium surface uniform with regard to texture.....5

5. Posterior spiracles visible on spiracular plate when viewed dorsally.....*Calliphora vicina*
   Posterior spiracles not visible on spiracular plate when viewed dorsally.....*Chrysomya chloropyga*

Puparia; Key 9: A combined key utilising those features visible from the lateral aspect of the puparium. The features considered were: puparium surface texture, shape of the puparium, the respiratory horn (where it is still present), the lateral ridge and certain aspects of the spiracular plate that could be determined from the lateral view. Most of these aspects were examined by means of light microscopy; the bubble membrane and the lateral ridge were examined by means of scanning electron microscopy. All species can be uniquely identified should the puparia still have its bubble membrane intact. However, in older puparia where the structure of the bubble membrane was destroyed by the emerging respiratory horn, only some of the species were identified uniquely when viewed from this angle.

1. Smooth puparium surface.....2
   Rough puparium surface.....5

2. Recessed spiracular plate.....*Sarcophaga cruentata*
   Bulging spiracular plate.....3

3. Visible ridges on midline, dorso-medially and ventro-medially.....*Calliphora vicina*
   Barely noticeable ridges.....4

4. Large and small bubbles, unstructured arrangement of bubbles.....*Lucilia cuprina*
   Bubbles uniform in size, ring of bubble surrounded centralised ones.....*Lucilia sericata*

5. Processes and papillae on puparium surface.....*Chrysomya albiceps*
   No processes and papillae on puparium surface.....6

6. Posterior spiracles visible on spiracular plate.....*Chrysomya marginalis*
   Posterior spiracles hidden from view on spiracular plate.....*Chrysomya chloropyga*

The features of the lateral ridge could be used to make a distinction between the species at step 1.

Puparia; Key 10: A combined key utilising those features visible from the ventral aspect of the puparium. The features considered were: puparium surface texture, shape of the puparium and anal area. These aspects were examined by means of light and scanning electron microscopy. Not all species were uniquely identified when viewed from this angle.

1. Smooth puparium surface.....2
   Rough puparium surface.....4

2. Broad, rounded posterior end.....3
   Narrow, truncated posterior end.....*Sarcophaga cruentata*

3. Narrow, pointed anterior end.....*Calliphora vicina*
   Broad, rounded anterior end.....unresolved for *Lucilia cuprina* and *Lucilia sericata*

4. Processes and papillae on puparium surface.....*Chrysomya albiceps*
   No processes and papillae on puparium surface.....5

5. Anal area distinctly visible on puparium surface.....*Chrysomya chloropyga*
   Anal area indistinct on puparium surface.....*Chrysomya marginalis*

Puparia; Key 11: A combined key utilising those features visible from the posterior aspect of the puparium. The features considered were: spiracular plate and the posterior spiracles. Although the posterior spiracles are not puparium specific structures, this third instar characteristic was still available in the puparium for usage. These aspects were examined by means of scanning electron microscopy. All species can be uniquely identified when viewed from this angle.

1. Deep-set spiracular atrium.....*Sarcophaga cruentata*
   Open spiracular plate.....2

2. Large perispiracular tubercles.....*Chrysomya albiceps*
   Small perispiracular tubercles.....3

3. Middle-inner spiracular cluster compacted.....4
   Middle-inner spiracular cluster branched.....5

4. Dorsal plate area infringed upon.....*Lucilia cuprina*
   Dorsal plate area not infringed upon.....*Lucilia sericata*

5. Small attachment area of outer spiracular cluster to rimae.....*Calliphora vicina*
Large attachment area of outer spiracular cluster to rimae.....6

6. Dorsal plate area infringed upon.....*Chrysomya chloropyga*
Dorsal plate area not infringed upon.....*Chrysomya marginalis*

Puparia; Key 12: An abridged descriptive key utilising all characteristics, irrespective of the angle the puparium was viewed from. Aspects were examined by means of light and scanning electron microscopy. All species were uniquely described with these characteristic.

1a. Deep-set spiracular atrium. (Broad, rounded anterior end; narrow truncated posterior end. Smooth puparium surface; indentations laterad. Narrow lateral ridge. Frontal field with ventro-medial lip-like flap. No bubble membrane. Anal area indistinct.).....*Sarcophaga cruentata*
1b. Open spiracular plate.....2

2a. Rounded posterior end.....3
2b. Truncated posterior end.....5

3a. Anterior end broad and rounded.....4
3b. Anterior end narrow and pointed. (Rounded, broad posterior end. Smooth puparium surface; small, clearly visible ridges lateral on segments. Narrow lateral ridge, perched on top of narrow raised area. Frontal field with small, etched-in folds, arranged radially symmetrical around mouth scar. Bubble membrane in depression; bubble of dissimilar sizes, closely grouped; larger bubbles centrally located. Open spiracular plate, bulging outwards somewhat; indistinct perispiracular tubercles. Anal area indistinct.).....*Calliphora vicina*

4a. Folds on frontal field, superficial. (Anterior end rounded and broad; broad, rounded posterior end. Smooth puparium surface with barely visible ridges laterad on segments. Narrow lateral ridge. Bubble membrane level with puparium surface; bubbles similarly sized and closely grouped; ring of bubbles surrounded central ones. Open spiracular plate, slightly bulging outwards; indistinct perispiracular tubercles. Anal area indistinct.).....*Lucilia sericata*
4b. Folds on frontal field, more deep-set. (Anterior end rounded and broad; broad, rounded posterior end. Smooth puparium surface with barely visible ridges laterad on segments. Narrow lateral ridge. Bubble membrane level with puparium surface; different sized bubbles closely grouped; unstructured arrangement of bubbles. Open spiracular plate, slightly bulging outwards; indistinct perispiracular tubercles. Anal area indistinct.).....*Lucilia cuprina*

5a. Processes and papillae on puparium surface. (Pointed, narrow anterior end; broad, truncated posterior end. Broad lateral ridge area. Distinct loops and ridges in frontal field. Bubble membrane slightly raised from puparium surface; bubbles similarly sized, two concentric rings of bubbles surround central ones. Open spiracular plate angled/ hinged with distinct perispiracular tubercles. A distinct semi-circular ridge around anal area.).....*Chrysomya albiceps*

5b No processes and papillae on puparium surface.....6

6a. Difference in texture of anterior and posterior halves of puparium. (Pointed, narrow anterior end; broad, truncated posterior end. Generally rough puparium surface. Broad lateral ridge area. Frontal field with large and small folds; larger folds not contained within margins of anterior spiracles. Bubble membrane in slight depression, bubble similarly sized, widely spaced. Open spiracular plate with indistinct perispiracular tubercles, posterior spiracles visible when spiracular plate viewed dorsally or laterally. Anal area indistinct.).....*Chrysomya marginalis*

6b Puparium surface uniform with regard to texture. (Pointed, narrow anterior end; broad, truncated posterior end. Generally rough puparium surface. Broad lateral ridge area. Frontal field with large and small folds; larger folds contained within margins of anterior spiracles. Bubble membrane level with puparium surface, bubbles not of similar sizes; closely grouped bubbles; ring of smaller bubbles surrounded larger centrally located ones. Open spiracular plate with indistinct perispiracular tubercles; posterior spiracles not visible when spiracular plate viewed dorsally or laterally. Elements of the anal area distinct structures.).....*Chrysomya chloropyga*

4.6. CLOSING STATEMENT

The unique identification of the immature stages of calliphorid and sarcophagid flies found at the scene of a homicide is crucial in the calculation of the post mortem interval. More often identification was based on the more recognisable third instar stages. However, it is not always possible to rear the earlier immature stages through to the third instar or to the adult stage. It is therefore important to be able to identify any of the immature stages. The current study looked at establishing descriptions and compiling keys for the immature stages of forensically important calliphorids and sarcophagids occurring in the central Free State region. Although information was available for some of the immature stages of some of the species, it was important to evaluate known characteristics for intraspecific variation from a regional perspective. This study also filled the gap where no or little information was available regarding the morphological features of the immature stages. Characteristics never used before for identification purposes were identified, the most notable being the labrum of third instar larvae and the frontal field of puparia. A suite of characteristics was evaluated

and almost all eggs could be uniquely identified. On the basis of individual features or through the use of a combination of characteristics all larval instars as well as puparia could be identified uniquely. The keys presented should make the identification of the immature stages of forensically important calliphorids and the sarcophagid occurring in the central Free State region an easy exercise.

CHAPTER 5

REFERENCES

5. REFERENCES

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Cognisance is taken of the correct spelling of the author's surname in the case of Erzinçlioglu. The differential spelling employed in the text and the reference list is not an oversight, but a reflection of the spelling as given in the various journal articles.