CHAPTER 3.1.13.

NEW WORLD SCREWWORM (*COCHLIOMYIA HOMINIVORAX*) AND OLD WORLD SCREWWORM (*CHRYSOMYA BEZZIANA*)

SUMMARY

The New World screwworm[1] *(NWS),* Cochliomyia hominivorax *(Coquerel), and the Old World screwworm*[1] *(OWS),* Chrysomya bezziana *Villeneuve, are both obligate parasites of mammals, including humans, during their larval stages. Both species are in the subfamily Chrysomyinae of the family Calliphoridae of the order Diptera (true flies). Larvae feeding on the skin and underlying tissues of the host cause a condition known as wound or traumatic myiasis, which can be fatal. Infestations are generally acquired at sites of previous wounding, due to natural causes or to animal husbandry practices, but they may also occur in the mucous membranes of body orifices.*

Female flies are attracted to wounds, at the edges of which each female lays an average of 175 (OWS) to 343 (NWS) eggs. The larvae emerge within 12–24 hours and immediately begin to feed, burrowing head-downwards into the wound. After developing through three larval stages (instars) involving two molts, the larvae leave the wound and drop to the ground, into which they burrow to pupate. The duration of the life-cycle off the host is temperature dependent, being shorter at higher temperatures, and the whole cycle may be completed in less than 3 weeks in the tropics.

Treatment is generally effected by application of organophosphorus insecticides into infested wounds, both to kill larvae and to provide a residual protection against reinfestation. Preventive measures include the spraying or dipping of susceptible livestock with organophosphorus compounds and, more recently, use of avermectins (especially doramectin) as subcutaneous injections to animals 'at risk'. Strict control of the movement of animals out of affected areas also acts as a preventive measure.

Identification of the agent*: The larvae of NWS and OWS can be easily confused with each other and with the larvae of other agents of myiasis. Accurate diagnosis involves the identification of larvae extracted from the deepest part of an infested wound. The mature, third instars are most reliable for this purpose, and those of NWS can be identified by their darkly pigmented dorsal tracheal trunks extending from the twelfth segment of the body forward to the tenth or ninth. This pigmentation is unique to the larvae of NWS among the species encountered in wound myiasis. Confirmation of OWS relies on the recognition of a characteristic combination of spinulation, the number of lobes on the anterior spiracles (4–6), and pigmentation of secondary tracheae.*

In the adult stage, species in the genus Cochliomyia *can be separated from other genera involved in wound myiasis by confirmation of a metallic body colour, ranging from light blue to green, with three dark longitudinal stripes always present on the thorax. The separation of NWS from the very similar* C. macellaria *and the identification of adult OWS are discussed in this chapter.*

Serological tests*: At present there are no applicable serological tests, nor are they indicated in the identification of this disease. However, serology may have a future role in studies of the prevalence of myiasis.*

1 In this chapter, the term 'New World' refers to the Americas and the term 'Old World' refers to Europe, Africa and Asia.

***Requirements for vaccines and biological control**: There are no vaccines or biological products available, except for the use of sterilised male flies in the sterile insect technique* (*SIT*). *In this technique, vast numbers of sterilised male flies are sequentially released into the environment, where their matings with wild females produce infertile eggs, leading to an initial population reduction and, progressively, eradication.*

A. INTRODUCTION

The New World screwworm fly (NWS), *Cochliomyia hominivorax* (Coquerel), and the Old World screwworm fly (OWS), *Chrysomya bezziana* Villeneuve, are species of two genera of the subfamily *Chrysomyinae* of the dipteran family *Calliphoridae* (blowflies). Both species are obligate parasites of mammals and, rarely, birds. The zoonotic implications are considerable because humans, especially the young, elderly or infirm, can be infested, with severe and sometimes fatal consequences (Spradbery, 1994). More recent reports of human cases of screwworm myiasis by *C. hominivorax* and *C. bezziana* include Olea *et al.* (2014) and Aggarwal *et al.* (2014), respectively. Despite being in different genera and geographically separated, the two species have evolved in remarkable parallel. They have almost identical life histories because they fill identical parasitic niches in their respective geographical zones. The following discussion will relate to both species, except where indicated.

Unlike most other species of blowflies, adult female screwworms do not lay their eggs on carrion. Instead, they lay them at the edges of wounds on living, injured mammals or at their body orifices. Virtually any wound is attractive, whether natural (from fighting, predators, thorns, disease, and/or tick and insect bites) or man-made (from shearing, branding, castrating, de-horning, docking, and/or ear-tagging). Commonly infested natural wounds are the navels of newborn animals, and the vulval and perineal regions of their mothers, especially if traumatised. If eggs are deposited on mucous membranes, the larvae can invade undamaged natural body openings such as the nostrils and associated sinuses, the eye orbits, mouth, ears, and genitalia.

Within 12–24 hours of the eggs being laid, larvae emerge and immediately begin to feed on the wound fluids and underlying tissues, burrowing gregariously head-downwards into the wound in a characteristic screwworm fashion. As they feed, tearing the tissue with their hook-like mouthparts, the wound is enlarged and deepened, resulting in extensive tissue destruction. Infested wounds often emit a characteristic odour, which can be the first indication that at least one animal in a group is infested. Although the odour is not always apparent to humans, it is obviously highly attractive to gravid females (Hall, 1995), which lay further batches of eggs, so increasing the extent of the infestation. A severe infestation that is left untreated may result in the death of the host.

Screwworm larvae pass through three stages (or instars), separated by cuticular molts that facilitate rapid growth, and they reach maturity about 5–7 days after egg hatch. They then stop feeding and leave the wound, falling to the ground into which they burrow and pupate. The pupa develops within the puparium, a barrel-shaped protective structure formed by hardening and darkening of the cuticle of the mature larva. On completion of development, adult flies usually emerge from the puparium in the morning and work their way up to the soil surface, where they extend their wings for hardening prior to flight. Males become sexually mature and able to mate within 24 hours, but the ovaries of females need to mature over 6–7 days, and females only become responsive towards males, mating when about 3 days old. About 4 days after mating, female flies are ready to oviposit. They seek a suitable host and lay their eggs, all oriented in the same direction, like a tiled roof, firmly attached to each other and to the oviposition substrate. The numbers of eggs laid per batch vary depending on many factors (e.g. fly strain, disturbance during oviposition), but the average first batch has in the order of 175 eggs for OWS and 343 for NWS (Spradbery, 1994). Following the first egg batch, further batches are laid at intervals of 3–4 days (Thomas & Mangan, 1989). Adult flies live on average for 2–3 weeks in the field during which time they feed at flowers, and the females also take in protein, e.g. from serous fluids at animal wounds and decomposing animals.

The rate of development of the immature stages is influenced by environmental and wound temperatures, being slower at low temperatures, although true diapause does not occur. This effect is most pronounced in the off-host pupal stage, which can vary from 1 week to 2 months' duration depending on the season (Laake *et al*., 1936). Thus, the complete life cycle of NWS may take 2–3 months in cold weather, whereas in temperate conditions with an average air temperature of 22°C, it is completed in about 24 days (James, 1947), and in tropical conditions averaging 29°C it is completed in about 18 days (Thomas & Mangan, 1989).

The degree to which NWS and OWS can tolerate cold has had a major influence on their distributions, best documented for NWS. Historically, the range of NWS extended from the southern states of the United States of America (USA), through Mexico, Central America, the Caribbean islands and northern countries of South America to Uruguay, northern Chile and northern Argentina (James, 1947). This distribution contracted during the winter months but expanded during the summer months, producing a seasonality at its edges and year round populations in the central areas – the New World tropics. Use of the sterile insect technique (SIT) in major programmes has resulted in eradication of NWS from the USA, Mexico, Curacao, Puerto Rico, and the Virgin Islands and, in Central

America, from Guatemala, Belize, El Salvador, Honduras, Nicaragua Costa Rica (Wyss, 2001) and Panama. Panama was recognised as free from NWS in 2006 and a permanent barrier zone was established primarily in the Darien province of eastern Panama. This serves as the northern limit of NWS in the Americas. A NWS eradication programme was also officially launched in Jamaica in July 1998, as part of a plan to eradicate the species from the entire Caribbean. This programme encountered severe setbacks due to a complex combination of management and technical difficulties (Vreysen *et al*., 2007), which eventually led to the failure of the programme on the island. Although NWS is a New World species, in 1988, it was detected in Libya in North Africa where it threatened to become firmly established. However, it was eradicated in 1991 by an intensive SIT campaign (Lindquist *et al*., 1992). The threat of spread of screwworms aided by modern rapid transport systems is ever present, necessitating constant vigilance from quarantine and other front-line animal health and medical officers in unaffected areas. Imported cases of NWS have been reported in Mexico, USA, and even in the United Kingdom.

An outbreak of NWS occurred in Florida, USA, in 2016–17 and was eliminated by use of the SIT from ground release chambers. Cases were found predominantly in wildlife (particularly Florida Key deer, *Odocoileus virginianus clavium*) with only a few in domesticated animals (dogs, cats and pet pigs) (USDA, 2017).

The distribution of OWS is confined to the Old World, as the name suggests, throughout much of Africa (from Ethiopia and sub-Saharan countries to northern South Africa), the Middle East Gulf region, the Indian subcontinent, and south-east Asia (from southern China [People's Rep. of] through the Malay Peninsula and the Indonesian and Philippine islands to Papua New Guinea) (James, 1947; Sutherst *et al*., 1989; Zumpt, 1965). OWS was reported from Hong Kong for the first time in 2000, infesting dogs, and a first human case was reported in 2003 (Ng *et al*., 2003). OWS myiasis has also been reported from Algeria (Abed-Benamara *et al*., 1997), in a local shepherd, and in Mexico (Romero-Cabello *et al*., 2010). However, in the absence of other reported cases, particularly animal cases, a continuing presence in either region seems unlikely and the original cases could have been misidentified, emphasising the need for correct identification of samples. The situation in the Gulf area and surrounding regions is dynamic, with reports confirmed from Iran, Iraq and, most recently, Yemen (Robinson *et al*., 2009). Epizootics of traumatic myiasis can follow introductions into such areas, especially where the livestock owners and veterinarians are unfamiliar with OWS (Siddig *et al*., 2005). The climatic requirements of the two screwworm species are very similar and their potential distributions, if unrestrained, would overlap considerably (Sutherst *et al*., 1989).

Treatment of infested wounds usually relies on the application of organophosphorus insecticides such as coumaphos (also dichlofenthion or fenchlorphos), taking due note of the manufacturer's safety instructions (Graham, 1979; Spradbery *et al.*, 1994). The insecticide should be applied at 2- to 3-day intervals until the wound has healed.

Prevention of screwworm infestation can be achieved by spraying or dipping of livestock, for example if member of the group was found to be infested, if animals were traversing or leaving an infested area, or following wound-inducing animal husbandry practices, e.g. shearing and castration.

Indirect prevention of screwworm infestation includes the avoidance of wounding procedures at the times of year when flies are numerous, the careful handling of livestock to minimise wounding, the removal of sharp objects (e.g. wire strands) from livestock pens, and the use of measures to reduce other wound-causing parasites, in particular ticks, e.g. by dipping and by insecticide impregnated ear-tags.

B. DIAGNOSTIC TECHNIQUES

Table 1. *Test methods available and their purpose*

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Agent identification						
Morphology	+++	+++	+++	+++	+++	n/a
Hydrocarbon analysis	n/a	n/a	n/a	+	n/a	n/a

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Mitochondrial DNA analysis	n/a	n/a	n/a	+	n/a	n/a
Detection of immune response						
Serology	n/a	n/a	n/a	n/a	+	n/a

Key: +++ = recommended method, validated for the purpose shown; ++ = suitable method but may need further validation; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; – = not appropriate for this purpose; n/a = purpose not applicable.

1. Identification of the agent

Identification of the eggs and first instars of the agents of myiasis based on morphology can be difficult. First instar larvae submitted to a laboratory can be identified following the descriptions and identification key provided by Szpila *et al.* (2014).

Larvae collected for diagnosis should be removed from the deepest part of the wound to reduce the possibility of collecting non-screwworm species, which may infest the shallower parts of the wound. Living specimens should first be examined for pigmentation of the dorsal tracheal trunks (Figures 1 and 4) and then be preserved in 80% ethanol and returned to the laboratory for examination under a dissecting microscope at up to ×50 magnification (for further techniques see: Hall & Smith, 1993; Spradbery, 1991; Zumpt, 1965). If larvae are placed directly into most preservative solutions they contract and darken. However, optimal preservation of larvae, in their natural extended state, can be made by killing them in boiling water (15–30 seconds immersion) before storage in 80% ethanol. This killing method had no negative effect on subsequent extraction of mitochondrial DNA, amplified by polymerase chain reaction (PCR) (Wardhana *et al*., 2012), but it might impact other molecular techniques and this should be borne in mind.

Second instars have only two spiracular slits in each of the posterior spiracular plates compared with the three slits of third instars (Figures 2 and 3). Second instars of NWS can be diagnosed by the presence of dark pigmentation of the dorsal tracheal trunks, for over half their length in the terminal segment. Other species have less extensive pigmentation of the dorsal tracheal trunks, for example, these trunks are pigmented for no more than one-third of their length in the twelfth segment of OWS. The anterior spiracles of second instar NWS have from seven to nine branches compared with about four branches in OWS (Kitching, 1974). More positive identification may be gained by rearing living, immature larvae to third instars. This can be done on the standard meat medium used for large-scale rearing of NWS before the introduction of gel diets, i.e. in the proportion of 1 litre water, 1.3 kg ground horse or beef meat, 50 g dried bovine blood, and 1.5 ml formalin (Taylor & Mangan, 1987), mixed and maintained at 35–38°C and 70% relative humidity. For simply rearing up larvae for identification, the exact meat and blood types are not essential, and more readily available fresh blood could be used instead of dried blood.

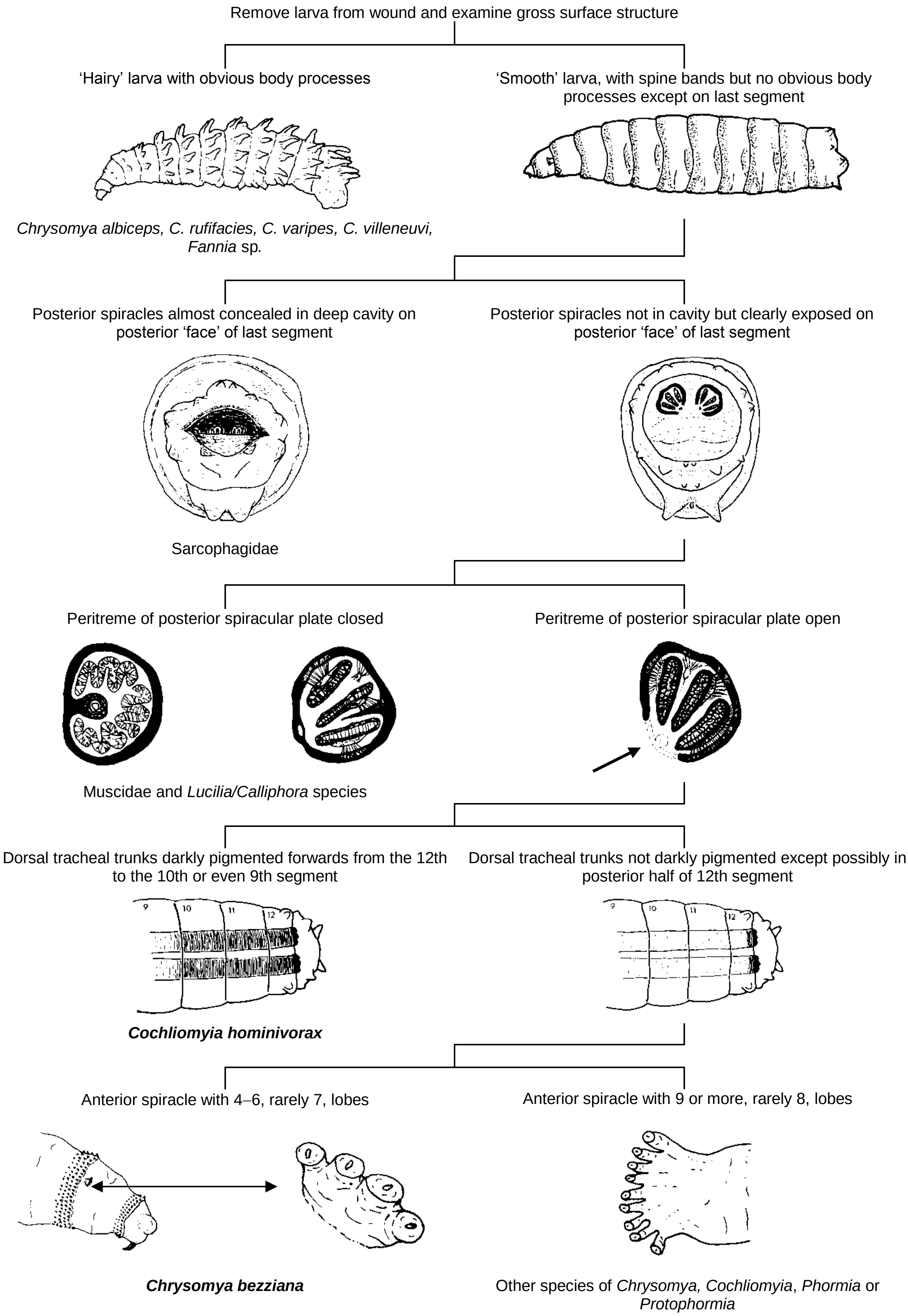

Fig. 1*. Identification key for the diagnosis of third instars of* Cochliomyia hominivorax *and* Chrysomya bezziana *from cases of wound myiasis. To avoid misidentifications, it is essential that the key is worked through from the first step for each specimen.*

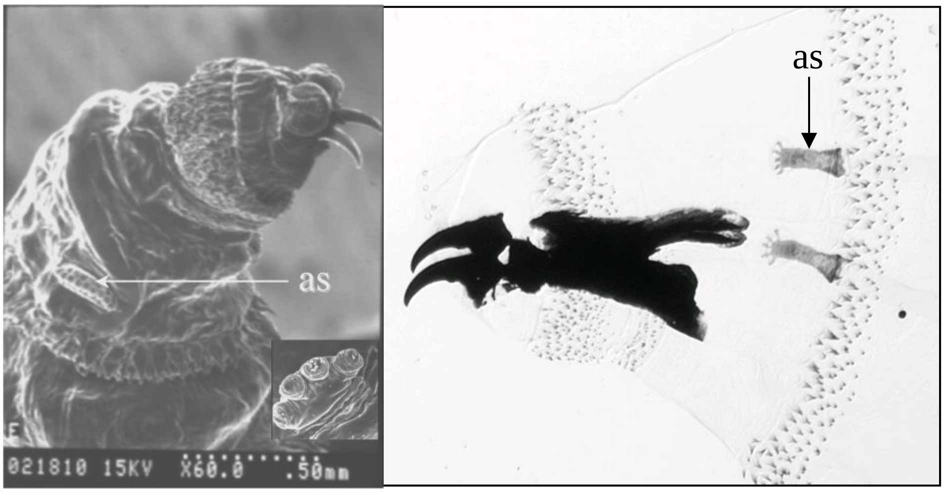

Fig. 2*. Head and next two segments of third instar of* Cochliomyia hominivorax (*left, viewed by scanning electron microscopy, inset is the anterior spiracle of* Chrysomya bezziana) *and of* Chrysomya bezziana (*right, viewed by compound light microscopy, note the thorn-like spines and that this slide preparation has been cleared using 10% KOH so that the anterior spiracles on both sides of the first thoracic segment are visible*)*;*
as = anterior spiracle.

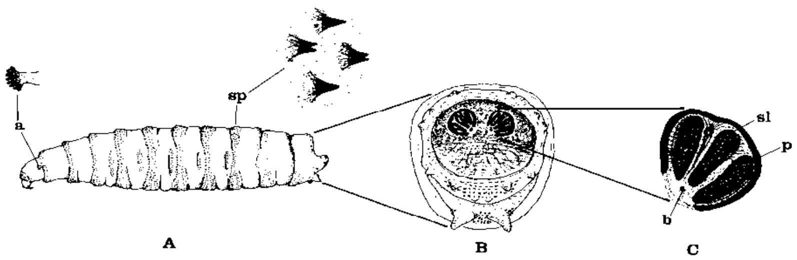

Fig. 3*. Characteristics of third instar of* Cochliomyia hominivorax*:* (*A*) *whole larva, lateral aspect;* (*B*) *posterior face of terminal segment;* (*C*) *posterior spiracular plate; a = anterior spiracle; b = button adjacent to opening in peritreme; p = peritreme; sl = spiracular slit; sp = spines.* (*After Laake* et al. [*1936*].)

Third instars of both NWS and OWS have a robust, typical maggot shape, with a cylindrical body from 6 to17 mm long and from 1.1 to 3.6 mm in diameter, pointed at the anterior end (Laake *et al*., 1936; Spradbery, 1991). Fully mature larvae of both NWS and OWS develop a reddish-pink tinge over the creamy white colour of younger larvae. Both screwworm species have prominent rings of spines around the body and these spines appear large and conspicuous under a microscope, when compared with most non-screwworm species, the longest averaging 130 µm. In NWS the spines can be either single or double pointed, but in OWS they are always single pointed and thorn-like (Figure 2). The anterior spiracles of NWS each have from six to eleven well separated branches, but usually from seven to nine (Figure 2). In OWS, the anterior spiracles each have from three to seven branches, but usually from four to six (Figure 2). The latter character should not be used on its own to identify OWS, because third instars of the obligate myiasis-causing species *Wohlfahrtia magnifica* (Diptera: Sarcophagidae), whose distribution overlaps that of OWS in the Middle East, have similarly branched anterior spiracles. Hence, in using any identification key, such as that in Figure 1, it is essential that each specimen be taken through the whole key to avoid misidentifications. On the posterior face of the terminal segment of both NWS and OWS, the posterior spiracular plates all have a darkly pigmented, incomplete peritreme partially enclosing three straight, slightly oval-shaped slits, which point towards the break in the peritreme. These diagnostic features are illustrated in Figure 3. Of greatest diagnostic value are the dorsal tracheal trunks, which extend forwards from the posterior spiracular plates and are darkly pigmented up to the tenth or ninth segment in NWS (Figure 1; see also: Hall & Smith, 1993; James, 1947; Spradbery, 1991; Zumpt, 1965 for identification keys). This feature is seen most easily in living larvae. Those in preservative may need dissection to remove opaque tissues covering the trunks. The dorsal tracheal trunks of OWS are darkly pigmented only in the twelfth segment. However, in OWS the secondary tracheae branching off the dorsal tracheal trunks are pigmented from the twelfth segment forwards to at least the tenth segment (confirmed in specimens throughout the range, from Malaysia, Bahrain and Zimbabwe; M.J.R. Hall, unpublished). Conversely, in NWS these secondary tracheae are not pigmented, only the dorsal tracheae are. Hence, the tracheal pigmentation appears almost reversed between the two screwworm species (Figure 4).

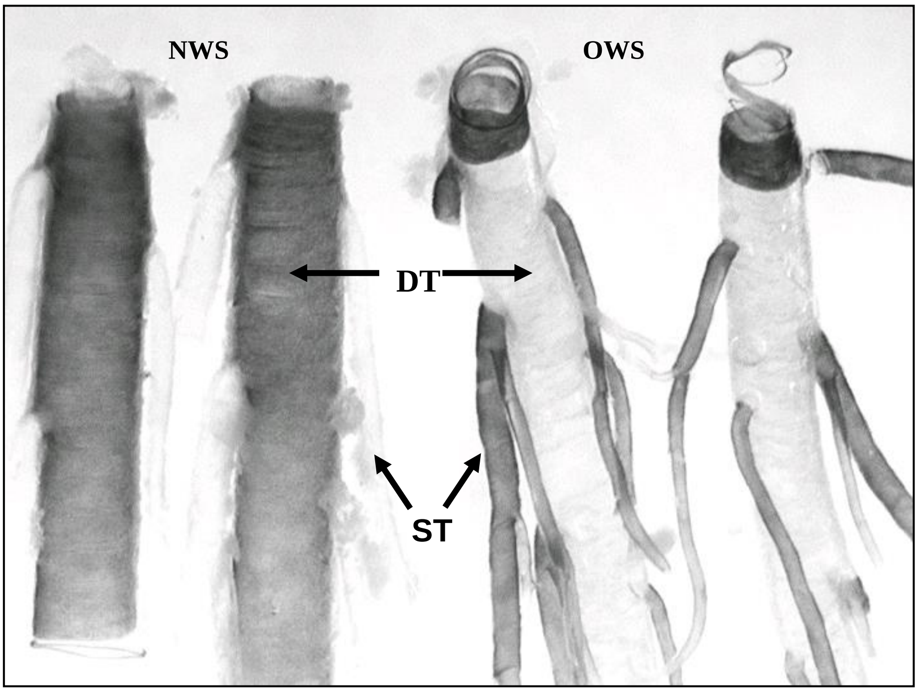

***Fig. 4.** Dorsal tracheal trunks of third instar of* Cochliomyia hominivorax *(left) and* Chrysomya bezziana *(right) dissected forwards from the posterior spiracles (top) to ninth abdominal segment (bottom). Note that the pigmentation of the main dorsal trunks (DT) and the smaller secondary tracheae (ST) is almost reversed between the species.*

Adult: Adult flies needed for identification purposes are often collected using wind-oriented traps (Broce *et al*., 1977) and sticky traps (Spradbery, 1991) baited with a synthetic odour (Mackley & Brown, 1984). A modified bucket-trap combined with a newly developed attractant caught an average of 3.1 times as many OWS as a sticky trap baited with the earlier lure and was more selective for OWS (Urech *et al.,* 2012). Real-time PCR methods can detect OWS in such bulk fly traps even when the prevalence is as low as one OWS in 1,000 other flies (Jarrett *et al*., 2010). Alternative sampling systems, using electrocuting grids or sticky surfaces at odour-baited visual targets, have been used for research purposes (Hall, 1995). Identification of adult flies is seldom required for the diagnosis of myiasis, because the larval stages are those most apparent to livestock owners and veterinary personnel. However, a brief description follows.

i) **NWS**: The body length is usually 8–10 mm, with three dark longitudinal stripes on the dorsal surface of the thorax. Although this fly may generally be a deep blue to blue green metallic colour, colour is variable and can range from light blue to green. This combination of colour and pattern is not shared by any other species commonly involved in wound myiasis except the secondary screwworm of the New World, *Cochliomyia macellaria* (Fabricius). These two *Cochliomyia* species can be separated by the presence of black setulae on the fronto-orbital plates of the head of NWS compared with only light yellow hairs on the fronto-orbital plates of *C. macellaria*. The fifth (=fourth visible) abdominal tergite of NWS has only a very slight lateral pollinose dusting, whereas that of *C. macellaria* has a dense dusting, producing a pair of distinct, lateral, silvery-white spots. In addition, females of NWS have a dark brown-black basicosta, whereas those of *C. macellaria* have a yellow basicosta (Figure 5; see also: Dear, 1985; Laake *et al*., 1936; Spradbery, 1991).

ii) **OWS**: The body is up to 10 mm long and has a metallic blue, bluish-purple or blue-green colour, i.e. it is very similar to NWS, but without the thoracic stripes. The lower squama (s in Figure 5) also differs from NWS, being distinctly covered with fine hairs over its entire upper surface in OWS and other *Chrysomya* species, whereas in NWS it is hairless above, except near the base. Adults of OWS can be distinguished from other *Chrysomya* found in cases of myiasis by the combination of black-brown to dark-orange-coloured anterior thoracic spiracles (rather than pale yellow, creamy, or white), with waxy-white, lower squamae (rather than blackish-brown to dirty-grey) (Spradbery, 1991; Zumpt, 1965).

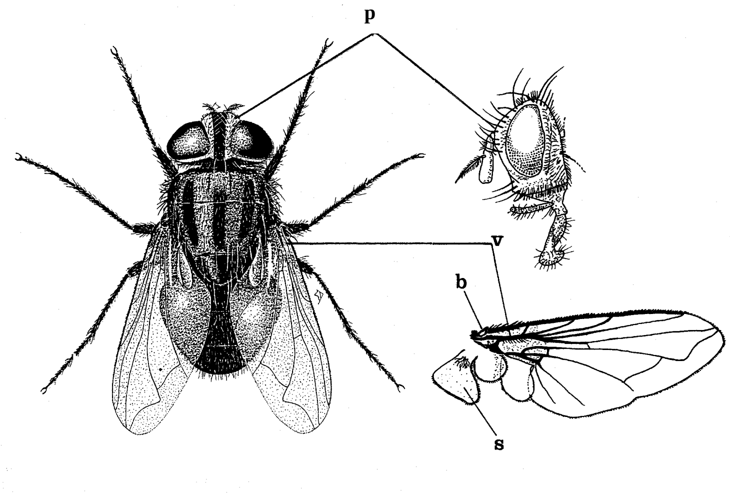

Fig. 5*. Characteristics of adult* Cochliomyia hominivorax*; note longitudinal thoracic stripes; b = basicosta; p = fronto-orbital plate, indicated from above on whole* Cochliomyia hominivorax *and laterally on head of typical calliphorid fly; s = lower squama, surface hairless except at base; v = stem vein with hairs on dorsal posterior surface.*

In addition to the standard morphological techniques discussed previously, more recent techniques for identification of screwworms and their geographical origins include cuticular hydrocarbon analysis (see in Spradbery, 1991) and analysis of mitochondrial DNA (Fresia *et al*., 2011; Wardhana *et al*., 2012). Problems with identification of larvae or adults from cases of myiasis can be referred to the OIE Reference Laboratory for New World screwworm or the FAO Collaborating Centre on Myiasis-Causing Insects and Their Identification[2].

2. Serological tests

No standardised serological tests are presently available, nor are they indicated for diagnosis of this disease. However, experimental studies have shown that serological techniques have potential value in future investigations of the prevalence of screwworm infestations in animal populations to detect antibodies to screwworm post-infestation (Thomas & Pruett, 1992).

C. REQUIREMENTS FOR VACCINES AND BIOLOGICAL CONTROL

There are no biological products such as vaccines, available currently. However, research towards development of potential vaccines is being conducted (Sukarsih Partoutomo *et al*., 2000). The only proven method of eradication of NWS relies on a biological technique, the sterile insect technique, SIT (Lindquist *et al*., 1992), which has also been applied experimentally to OWS (Spradbery, 1994). In this technique, male flies sterilised in their late pupal stage by gamma or x-ray irradiation are sequentially released into the wild in vast numbers. All of their matings with wild females result in infertile eggs only, leading to a progressive population reduction and, eventually, eradication. In operational situations, SIT is supported by the insecticide treatment of screwworm-infested wounds in livestock, by strict control of livestock movement, by the quarantining of infested animals and by an active publicity campaign. SIT is very expensive because of the cost of continuous production and aerial dispersion of sterile flies. Historically, it has been considered cost effective only when used as an eradication strategy in situations where the geography would favour such a programme (e.g. Lindquist *et al*., 1992). Presently, there is only one production facility for sterile adults of New World screwworm, located in Pacora, Panama[3].

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NB: At the time of publication (2019) there were no OIE Reference Laboratories for screwworm (see Table in Part 4 of this *Terrestrial Manual* or consult the OIE web site for the most up-to-date list http://www.oie.int/en/scientific-expertise/reference-laboratories/list-of-laboratories/

NB: FIRST ADOPTED IN 1991 AS NEW WORLD SCREWWORM; FIRST ADOPTED WITH CURRENT TITLE IN 2000. MOST RECENT UPDATES ADOPTED IN 2019.