World Health Organization

Entomological manual for onchocerciasis elimination programmes

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Contents

Preface

Medical entomology is essential for the control and elimination of neglected tropical diseases, particularly onchocerciasis. However, there is a shortage of medical entomologists worldwide, especially in countries that are most affected by these diseases, where resources are scarce and promising job opportunities are not offered by the medical entomology sector. Training more health and field workers in entomology therefore remains a critical gap, as highlighted in the road map for neglected tropical diseases 2021–2030.

This manual is a new resource for strengthening the capacity of scientists combatting onchocerciasis. It will be a fundamental tool in the last mile as we approach elimination of onchocerciasis, where entomological evaluations and surveillance will be required in all endemic countries in order to achieve verification of elimination.

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Abbreviations and acronyms

ABR	annual biting rate
APOC	African Programme for Onchocerciasis Control
ATP	annual transmission potential
CDD	community-directed distributor
CI	confidence interval
DALY	disability-adjusted life year
EWT	Esperanza window trap
HLC	human landing collection
L3	third-stage *Onchocerca volvulus* larva
MBR	monthly biting rate
MDA	mass drug administration
NTD	neglected tropical disease
OCP	Onchocerciasis Control Programme
OEPA	Onchocerciasis Elimination Programme for the Americas
PCR	polymerase chain reaction
VVC	village vector collector

Glossary and useful terms

The definitions given below apply to the terms as used in this manual. They may have different meanings in other contexts. Some terms/definitions are reproduced from reference *1*.

Adenitis: inflammation of a gland; often used as lymphadenitis, inflammation of a lymph gland

Anterior: front, front end

Antigen: a foreign substance that induces an immune response

Basitarsus: fifth segment from the end of the leg

Breeding site: area in a river that is favourable for development of larvae and pupae of black fly species

Capillary: the smallest blood vessel; many are located just below the skin's surface

Carapace: the chitinous dorsal shield of Crustaceans

Chromosome: a linear structure within the nucleus of a cell composed of protein and DNA which holds genetic information

Cocoon: a woven silk structure covering a pupa

Community-directed treatment with ivermectin: regular planned ivermectin treatment of village members by local volunteers, thereby empowering communities to take responsibility for ivermectin delivery

Community mobilization: creating partnerships with sectors of a community to address pressing issues

Cytospecies: morphologically similar species that are differentiated by cytological characteristics such as the banding patterns of chromosomes

Cytotaxonomy: study of species according to cytological (cellular) characteristics

Dichoptic: having separated eyes at the top of the head

Diurnal: active during the day

Dorsal: upper surface or back

Entomological evaluation: collection and tracking of data on black fly vectors in time and space to assess progress in onchocerciasis control or elimination

Filament: thin thread

First-line village: one with no other settlements between it and the nearest vector breeding site (< 10 km)

Frons: the space between the eyes at the top of the head

Gonotrophic cycle: interval between successive egg development and oviposition, completed after an adult female *Simulium* spp. has taken a blood meal, produced an egg-batch, found an oviposition site in a stream or river and laid its eggs. It then seeks another blood meal, thereby commencing another gonotrophic cycle. Thus, the length of a gonotrophic cycle is the interval between successive egg-laying.

Gravid: swollen and full of mature or nearly mature eggs

Histoblast: a cell or group of cells that can form tissues

Holoptic: having eyes that meet at the top of the head

Human landing collection: a method for collecting anthropophilic black flies in which humans are used as bait

Hyperendemic: heavily endemic, implying a prevalence of infection ≥ 60%

Hypostomium: a chitinized, cone-shaped, median tooth plate of the larval head capsule, located at the ventral base of the mouthparts

Identification key: a dichotomous key in which the sequence and structure of identification steps are fixed so that, at each stage of a decision, two alternatives (a couplet) are offered, each leading to a result or a further choice

Ivermectin: an anti-parasitic drug originally used in veterinary medicine, which has a broad spectrum of activity

Labrum: the "upper lip" of an insect that helps hold the mouthparts in place

Larvicide: a type of insecticide designed to kill insects at the larval stage

Malpighian tubule: tube in an insect's alimentary canal involved in excretion and osmoregulation

Mectizan®: brand name of ivermectin registered for use in humans in Africa to combat onchocerciasis

Membrane: a thin sheet of tissue

Mermithid: a nematode parasite belonging to the family Mermithidae that often infects the aquatic stages of *Simulium* spp. The genus *Isomermis*, the most common parasite of the *S. damnosum* complex, has an aquatic adult. *Simulium* spp. are infected in the aquatic stages, and the larvae are often carried over into the adult fly. Thus, their presence in an adult female *S. damnosum* usually, but not invariably, indicates that the fly is nulliparous.

Microfilaria: the first larval stage of *O. volvulus* shed by female worms, found in the skin of the human host and taken up by biting female *Simulium* vectors

Morphospecies: a species identified by its external structural features

Naphthalene, tert-hexadecyl mercaptan: chemical compounds that are attractive to *S. damnosum sensu stricto*; used in Esperanza window traps

Nulliparous: not having taken a blood meal and developed eggs and therefore not infected with *O. volvulus*

Onchodermatitis: skin irritation or inflammation due to onchocerciasis

Parous: having taken at least one blood meal and developed and deposited eggs; likely to be infected with *O. volvulus*

Phoretic: living organism attached to another animal but not parasitic on it

Plastic aroma bead: commercially available bead that can be impregnated with chemical compounds to lure black flies

Postgenal cleft: a non-sclerotized ventral aperture of the larval head capsule

Prospection: in *Simulium* ecology, the search for aquatic black fly stages (eggs, larvae, pupae) at potential breeding sites

Respiratory filament: the filament-shaped appendix of a pupal gill that enlarges the surface of the tracheal system to extract dissolved oxygen from water

s.s./s.l.: *sensu stricto* (in the narrow sense)/*sensu lato* (in the broad sense)

Scutum (also Mesonotum): the dorsal mesothorax

Spermatophore: a gelatinous structure containing sperm that is transferred to the female during copulation

Tanglefoot: glue or adhesive used to coat the two surfaces of the Esperanza window trap

Taxon: the basic taxonomic unit, usually referring to genus and species

Tergite: a hard, chitinous plate on the dorsal surface

Topographical map: a detailed map of a land area, with geographical positions and elevations for both natural (rivers, lakes, mountains, swamps) and man-made features

Transmission zone: geographical area in which transmission of *O. volvulus* occurs by locally breeding vectors; can be regarded as a natural ecological and epidemiological unit for intervention

Tubercle: a small projection

Turbid: opaque or thick with suspended matter

Vasodilator: a substance that causes blood vessels to widen in diameter

Wadi: a valley or channel that is dry except during the rainy season

Wing tuft: a prominent group of setae of the wing located on the stem vein of the radial vein

Zoophilic: attracted specifically to animal hosts; animal-biting

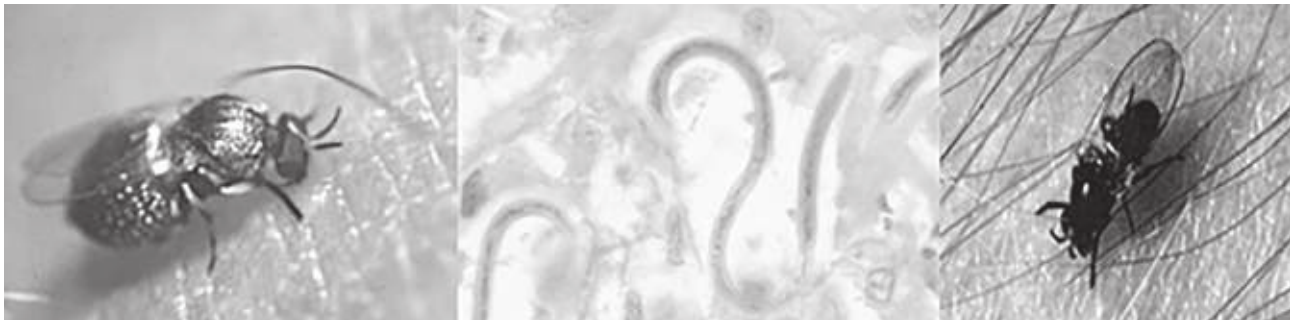
Chapter 1.

Introduction

Learning outcomes for Chapter 1

By the end of this chapter, the reader should be able to:

- understand what a black fly is;
- appreciate the public health importance of black flies;
- describe human onchocerciasis ("river blindness");
- appreciate the socioeconomic and public health impacts of onchocerciasis;
- understand why onchocerciasis should be eliminated;
- describe the process of moving from a control strategy to elimination of onchocerciasis; and
- explain why an entomological manual for onchocerciasis elimination is necessary.



1.1 Black flies

A black fly is any member of the family Simuliidae. Common names of members of the Simuliidae include blackfly or black fly (two words). The latter is used throughout this publication according to standard entomological notation, indicating members of the order Diptera (true flies), and to the Oxford Concise Medical Dictionary (10th edition). The family consists of over 2300 species, the genus *Simulium* being the largest (more than 1800 species). These insects are usually small, black or grey, with short legs and antennae (Fig. 1.1).

Fig. 1.1. Female black fly (*Simulium damnosum*) taking a blood meal.

Source: United States Joint Pathology Center

Adult males feed on nectar, while females of most species feed on nectar and take blood before laying eggs. Black flies have short mouthparts, and females are "pool feeders", i.e. they use their mouthparts to lacerate superficial capillaries in the skin to create a pool of blood and then withdraw it (Fig. 1.1). Their saliva, which contains hyaluronidase (an enzyme that breaks down hyaluronic acid and hyaluronan to facilitate the spread of salivary secretions during blood-feeding) and a mixture of powerful anticoagulants (which inhibit or retard coagulation of blood), vasodilators and immunomodulatory components (specific compound or groups of compounds that modify the immune response), is secreted into the bite wound, preventing platelet aggregation and clotting during blood feeding and ingestion. These bites may cause localized tissue damage and, if the number of feeding flies is sufficient, may cause anaemia. Host selection varies by species, i.e. different species prefer different vertebrate host sources for their blood meals. Black flies feed in the daytime, preferably when wind speeds are low.

Species of public health importance belong to four genera, *Simulium, Prosimulium, Austrosimulium* and *Cnephia*, which may bite humans, while other species prefer to feed on other mammals or birds. The genus *Simulium* is the most widespread globally and includes vectors of several pathogens, including *Onchocerca volvulus,* a filariid nematode that causes river blindness in Africa, Yemen and the Americas. Examples of important vectors are the *S. damnosum* species complex and *S. neavei* species group in Africa and Yemen *(1)* and *S. ochraceum, S. metallicum, S. exiguum* and *S. guianense* in the Americas *(2)*.

1.2 Health importance of *Simulium* spp.

1.2.1 Nuisance and injurious effects not related to *O. volvulus*

Different species of black flies in different parts of the world affect human and animal health in several ways. In addition to transmitting viral, protozoan and nematode pathogens, the bites of some species cause blood loss or render humans and animals vulnerable to various infections, while the bites of other species are an intolerable nuisance.

Bites of black flies sometimes result in severe systemic reactions characterized by headache, fever and nausea as well as adenitis. This condition, called "black fly fever", usually does not last longer than 48 h and occurs after a large number of bites. Numerous bites around the eyes of sensitized individuals may cause the surrounding tissues to become swollen and completely obscure vision. When black flies are hyper-abundant in an area, swarms of female flies landing, crawling on the hair, under clothing, into nostrils and ears and being inhaled can be psychologically distressing. This situation may discourage tourists from entering black fly-infested areas. In addition, extreme numbers of black fly bites and annoyance may significantly reduce the weight gain of livestock, milk production of dairy cattle and egg production of hens.

Many species are zoophilic and transmit pathogens to animals. Some *Simulium* species have been identified as vectors of non-human *Onchocerca* species. For instance, in England, *S. ornatum* is a vector of *O. gutturosa* in cattle, while *O. ochengi* is a common bovine parasite in Africa and is transmitted by members of the *S. damnosum* species complex.

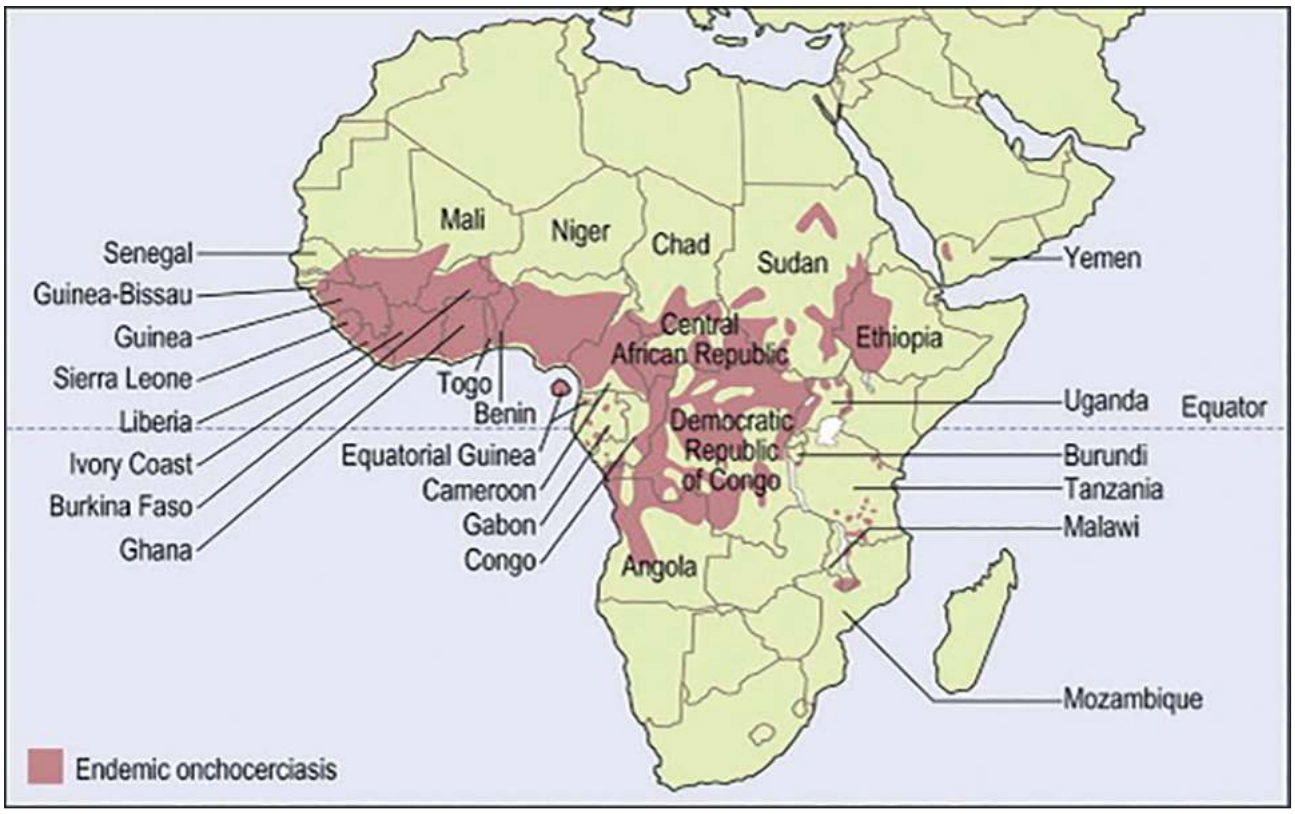
Other black fly species transmit protozoan parasites to a variety of avian hosts. These parasites, which include species of *Leucocytozoon* and *Trypanosoma avium,* may affect large numbers of wild and domestic fowl populations. Several species of *Simulium* are also competent vectors of vesicular stomatitis virus, an RNA virus that infects horses, cattle and swine in the Americas. Infection with this virus creates lesions that mimic foot-and-mouth disease and must be rapidly differentiated to prevent epizootics.

1.2.2 Transmission of *Onchocerca volvulus*

The most important aspect of the relation between humans and black flies is their role as vectors of the nematode parasite, *O. volvulus*. Human onchocerciasis is a chronic parasitic disease that affects millions of people on four continents (Figs 1.2, 1.3).

Africa: Human onchocerciasis was endemic primarily in areas between 15° N and 14° S. More than 99% of people currently threatened by *O. volvulus* live in 31 countries in sub-Saharan Africa (Fig. 1.2).

Fig. 1.2. Onchocerciasis-endemic countries in Africa and the Arabian Peninsula (Yemen).

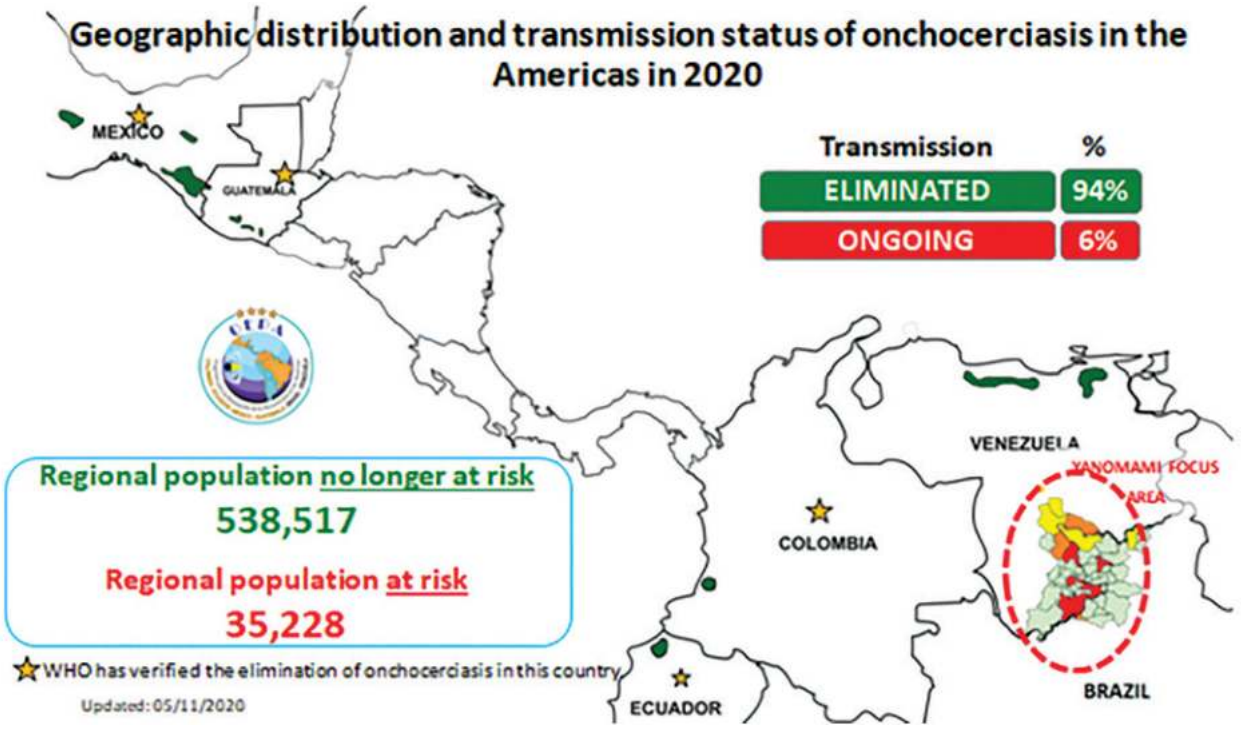


Note that Ivory Coast is now Côte d'Ivoire, Sudan is divided into Sudan and South Sudan, and Tanzania is the United Republic of Tanzania, with Zanzibar.

Arabian Peninsula: Onchocerciasis is endemic in the Arabian Peninsula (Yemen), especially in the area of Taiz, where the vector is *S. rasyani,* a species in the *S. damnosum* species complex. Onchocerciasis in Yemen is confined to areas near permanent wadis ranging in height from 300 m to 1200 m. This focus has been known for many years as endemic, with unique clinical characteristics, notably the occurrence of an atypical and severe form of onchodermatitis, known as *sowda* or reactive onchodermatitis. National control of the disease began in 1992 as an individual case treatment programme by administering ivermectin to people presenting with reactive onchodermatitis. Mass drug administration (MDA) was begun as a pilot project in 2016 and scaled up in 2018 as the Yemen programme moved to the goal of elimination.

The Americas: Human onchocerciasis was originally found in 13 foci in six countries in the Americas (Fig. 1.3). The principal foci were located in coffee plantations at high altitude (150–500 m) or in lowland jungles. Before initiation of the regional control and elimination programme – the Onchocerciasis Elimination Programme for the Americas (OEPA) – these foci were distributed from Mexico in the north to the border of Brazil and the Bolivarian Republic of Venezuela in the south. *S. ochraceum* and *S. metallicum* were the most important primary vectors in the region. Currently, onchocerciasis is endemic in only one focus (the Yanomami focus, which extends across the borders of both countries.

Fig. 1.3. Former geographical distribution of onchocerciasis in the Americas and current status, 2020. For details regarding active foci, see Wkly Epidemiol Rec. 2020;95:484–7.



Source: The Carter Center, Atlanta (GA), USA

1.3 Prevalence of human onchocerciasis

Before control and elimination programmes were begun, 20–40 million people were estimated to be infected, of whom 350 000 were blind and another 500 000 were visually impaired; over 6 million had various onchocercal skin diseases *(3)*, and about 1.6% had lymphatic complications *(2)*. In 2017, WHO estimated that 203 million people were at risk and 20.9 million were infected with *O. volvulus*, of whom 14.6 million had skin disease and 1.15 million had visual impairment *(4)*.

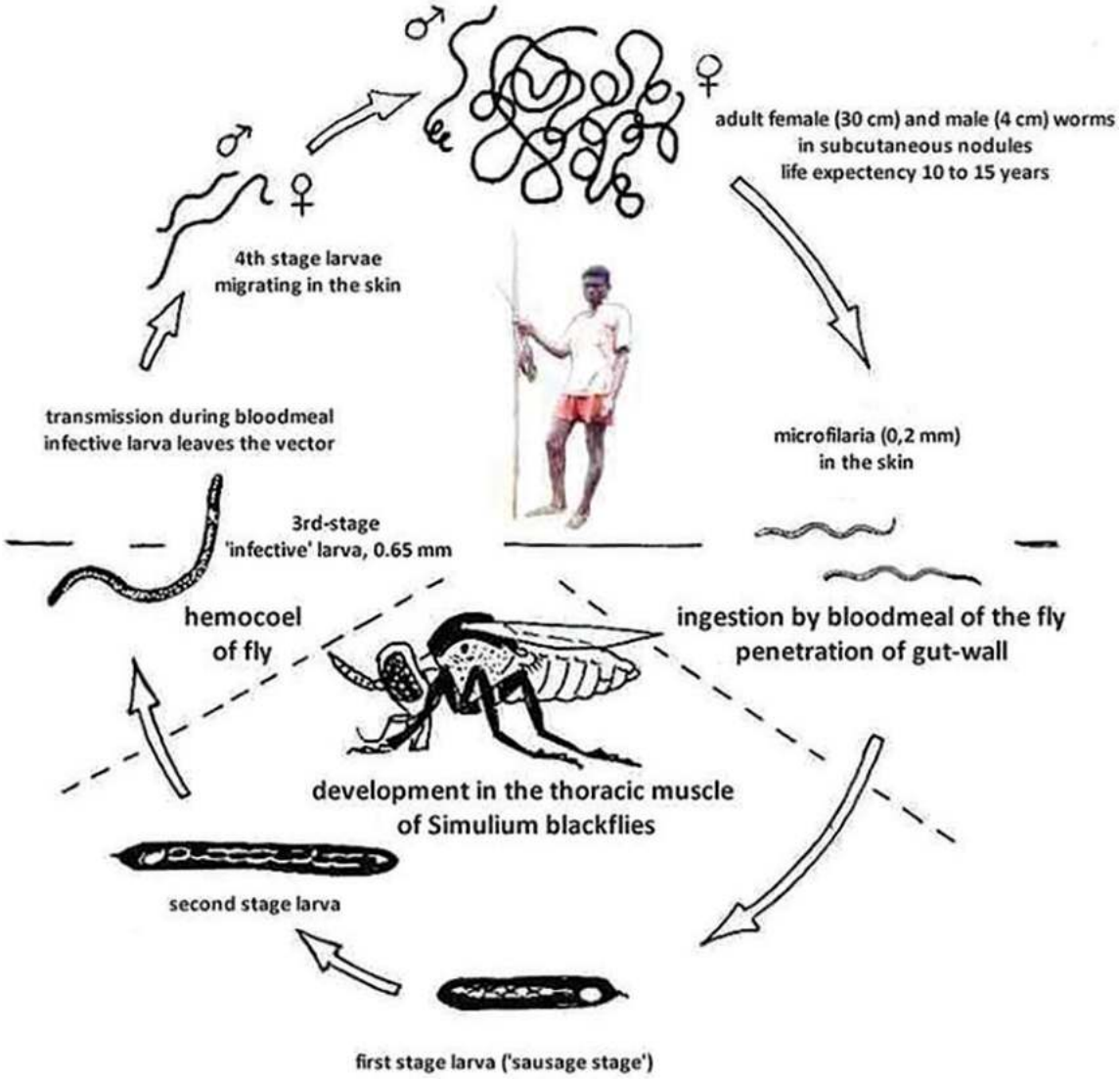
1.4 Distribution of onchocerciasis

The local distribution of onchocerciasis is determined by the ecology and behaviour of the black fly vector. A key aspect is the effective flight range of females searching for a blood meal. As this is usually unlikely to exceed 20 km, the highest rate of parasite transmission is within a 20-km radius of larval breeding sites. The most severely affected communities are almost invariably located within this radius. Because onchocerciasis occurs close to a river, it is believed in many rural endemic communities to be caused by the gods of the rivers. While the origin of the term "river blindness" to describe the disease is not known precisely, observations by Dr Jean Hissette in 1930 in foci along the Sankuru and Uele rivers in the Belgian Congo confirmed the spatial relation between *O. volvulus* ocular pathology and rivers. In the Americas, human onchocerciasis is known as Robles disease, named after the Guatemalan physician who associated the presence of the parasite with human blindness.

1.5 Life cycle of *Onchocerca volvulus* and pathology of human onchocerciasis

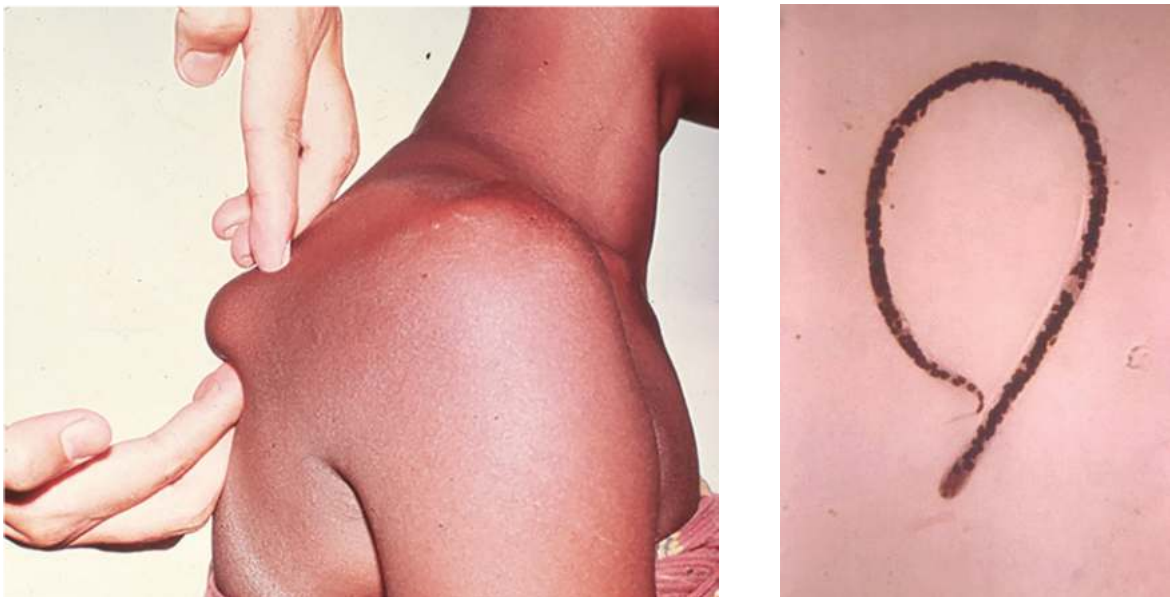
The adult worms (males and females) live mainly in subcutaneous nodules (Figs. 1.4, 1.5A), where females, during the 9–14 years of fecundity, produce millions of microscopic, motile embryos called microfilariae (Fig. 1.5B). The average onchocercal nodule measures 0.5–2 cm and often can be palpated (Fig. 1.5A); however, some nodules may reach a diameter exceeding 6 cm and others may be deeper in the body and not palpable. In most African countries, *O. volvulus* microfilariae are distributed in the lymphatic channels of the skin around the pelvic region to the upper arm, whereas, in the Americas, microfilariae are found more often in the thorax and upper body, including the head.

Fig. 1.4. Life cycle of *Onchocerca volvulus*.



Source: reference *5*

Fig. 1.5. A. Palpated onchocercal nodule on the upper back. B. *O. volvulus* microfilaria. Note the lack of complex internal development. This stage is a motile embryo that requires a black fly as an intermediate host.



A B

Sources: A, United States Joint Pathology Center; B, United States Centers for Disease Control and Prevention

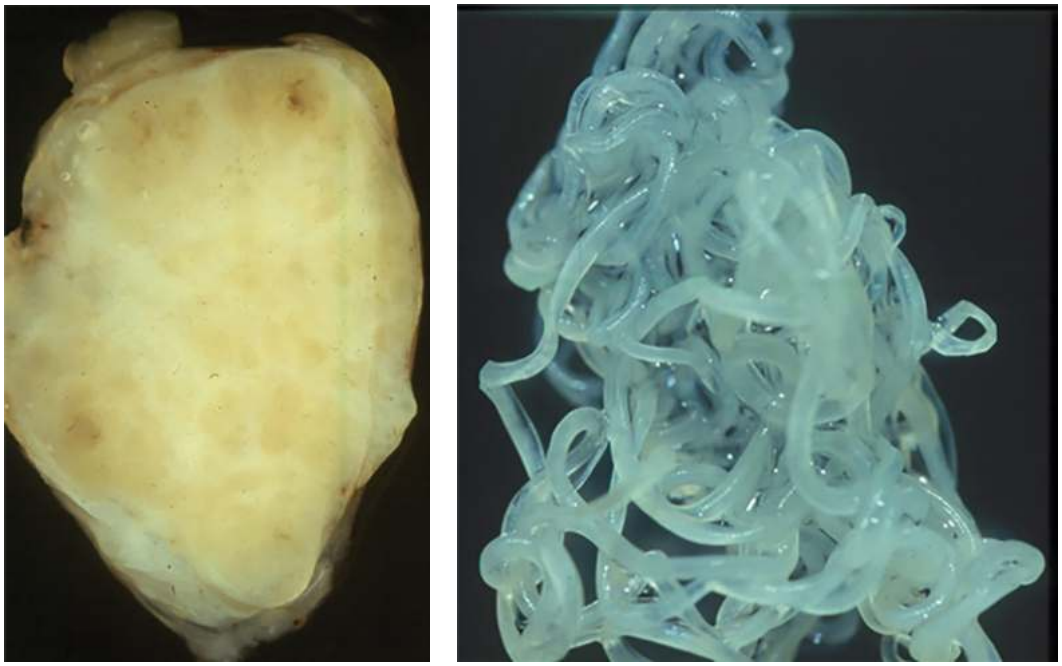
Humans are the sole vertebrate host of *O. volvulus*, i.e. there is no animal reservoir, and the parasite undergoes several developmental stages in both humans and black flies. Female flies feed on blood intermittently, which they require to develop each batch of eggs. When female flies bite infected individuals, they ingest microfilariae from the skin. Once inside the fly, the microfilariae pass down the alimentary tract, reach the midgut, where they then enter the hemocoele (body cavity), infect the fly's thoracic musculature, and continue development. In the Americas, several vector species have a cibarial armature, a series of tooth-like or spicule-shaped chitinous structures in the foregut, which sheds microfilariae, whereas other species secrete a thickened peritrophic membrane to prevent or limit infection. Once in the flight muscles, the surviving microfilariae moult twice (L1 to L2, L2 to L3) and become infective for humans. Some of the L3s find their way from the thoracic musculature to the mouthparts (proboscis, an elongated tube-like structure designed to suck liquids), from which they are transmitted to the human host during a subsequent blood meal. The vector part of the life cycle takes 7–12 days to complete, depending on the air temperature. Following infection, the parasite moults twice in the human host (L3 to L4, L4 to juvenile stage) and then matures to an adult worm, growing over a relatively long period (12–16 months) before reaching patency (maturation of a parasite in which infection is detectable).

Signs of infection with *O. volvulus* are the presence of nodules containing adult worms and the presence of microfilariae in the skin.

1.5.1 Onchocercal nodules

Most of the tissue changes in onchocerciasis that contribute to the clinical manifestations appear to be associated with the death of microfilariae rather than directly to the presence of adult worms; however, adult worms become surrounded in subcutaneous tissues to form characteristic onchocercal nodules (Fig. 1.6A). Each nodule contains a number of long adult female worms (Fig. 1.6B) and much smaller male worms. Microfilarie are produced by the female worm and escape into the skin.

Fig. 1.6. A. Nodule with adult worms embedded in connective tissue. B. Mass of female worms in the nodule revealed after collagenase digestion.



A B

Sources: A, B, United States Joint Pathology Center

1.5.2 Microfilariae

The microfilarial stage of the parasite migrates through the body by a writhing mechanical motion while secreting elastase (an enzyme that breaks down elastin and other proteins) and other enzymes to digest the skin and create pathways. An overwhelming majority of microfilariae (those not ingested by a black fly) eventually die and create a complex array of pathological manifestations, which may include onchocercal skin disease (onchodermatitis), lymphadenitis (sometimes resulting in hanging groin), ocular lesions (punctate and sclerosing keratitis) and systemic manifestations.

1.5.3 Onchodermatitis

Onchodermatitis begins when microfilariae degenerate in the dermis. This is accompanied by inflammation, with degranulation of eosinophilic granules on the cuticle of the microfilaria. Some individuals with onchocerciasis may have clinically normal skin, while others have intense pruritis (itching), which may progress to an inflammatory response over time. Onchodermatitis may present in a variety of forms. Severe skin disease and lymphatic lesions are more prevalent in rainforest zones than in the savannah, while ocular lesions and blindness are more severe in the savannah *(6)*.

1.5.4 Lymphatic complications of human onchocerciasis

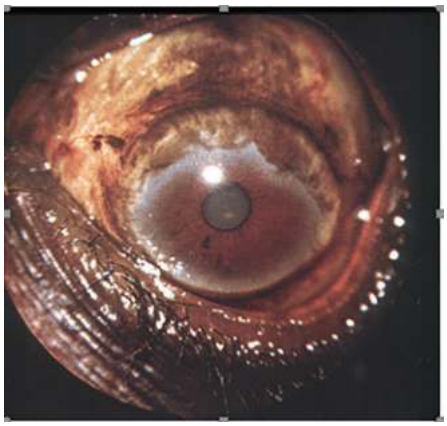
In patients with lymphatic involvement, the changes in their lymph nodes may progress. Antigens released from microfilariae lead to the deposition of immune complexes in the tissue. Over time, this may progress to hanging groin and other genital deformations. Such clinical features are often seen in highly endemic areas in Africa.

1.5.5 Ocular complications of onchocerciasis

The release of large amounts of parasitic antigens after destruction of microfilariae has more serious pathological consequences in the eye than in other organs with more efficient antigen clearance *(7)*. As microfilariae die, they stimulate a limited inflammatory response in the eye. Over time, this results in accumulation of local inflammatory lesions (punctuate keratitis) and scarring (sclerosing keratitis). Because the eye depends on optical clarity for its normal function, even minor scarring can cause significant visual impairment.

Onchocercal blindness may result from disease in different parts of the eye: anterior chamber disease consisting of corneal keratitis (Fig. 1.7), iris disease (iritis and possibly secondary glaucoma and cataract) and anterior chamber disease in the form of chorioretinal and optic nerve disease.

Fig. 1.7. Sclerosing keratitis due to chronic anterior chamber infection with *O. volvulus* microfilariae.



Source: World Health Organization

1.5.6 Systemic manifestations

Several features not clearly associated with the etiology or pathogenesis of onchocerciasis have been described, including the presence of microfilariae in diverse parts of the body, low body weight, general debility, musculo-skeletal pain, epilepsy, dwarfism (Nakalanga syndrome) and nodding syndrome. The community prevalence rates and intensity of these clinical manifestations may depend on the distribution, abundance and biting intensity of the vector flies in endemic areas. These associations are being investigated.

1.6 The burden of onchocerciasis

Because the immature stages of vector flies occur in streams and rivers, the location of larval breeding sites determines the distribution and intensity of onchocerciasis. It is a "disease at the end of the road", where poor rural farmers and villagers who usually dress scantly while working in the field are exposed to the bites of infective flies. Onchocerciasis is chronic and cryptic in nature, and older members of endemic communities have significantly more clinical manifestations than younger people.

Onchodermatitis not only creates psychosocial problems but also has direct and indirect economic impacts. A report by WHO in 1997 *(8)* showed that an individual with severe manifestation of onchodermatitis spent about US$ 20 more per annum on health than individuals who were unaffected. At the low-income levels in poor endemic communities, this extra cost represents 15% of an infected individual's earnings.

One measure of the impact of a disease is disability-adjusted life years (DALYs) lost, which is a measure of early mortality and years lived with disability due to a disease. In 2017, it was estimated that onchocerciasis results in 1.3 million DALYS *(9)*, and a previous analysis of the impact suggested that more than half the burden is due to skin-related morbidity *(10, 11)*.

1.6.1 The burden on social life

Although the bite of a female *Simulium* vector is not initially painful, it may be followed by an irritated, intensely raised ulcerative lesion at the bite site, due to irritation produced by the fly's salivary secretions, a complex mixture of biologically active low-molecular weight proteins. Susceptible people may suffer long-term pruritus and intense scratching severe enough to cause insomnia *(2)*, resulting in lost productivity.

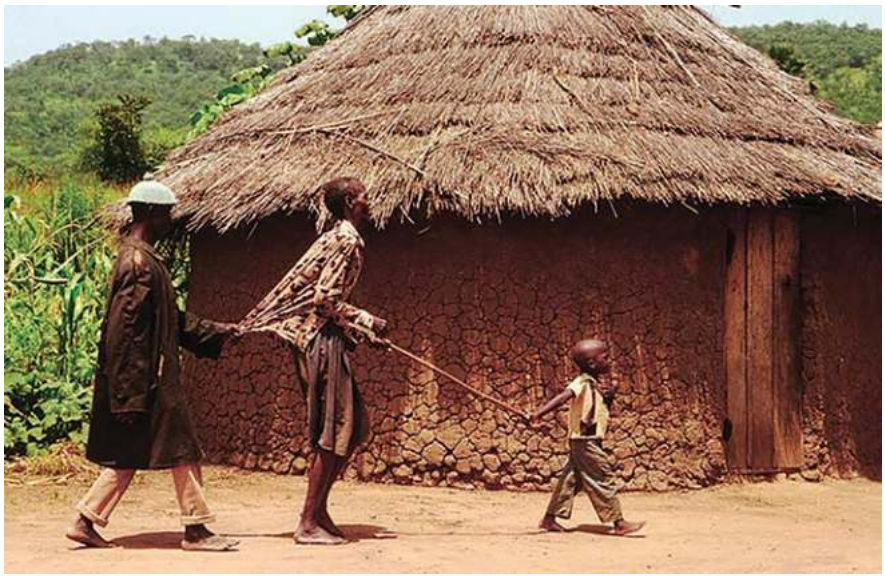
Many changes due to onchodermatitis are visible, which may have distressing negative effects on the lifestyle of affected individuals, including psychosocial stigmatization and social rejection, fear of discrimination *(12)*, low self-esteem *(13)*, psychological distress and limited occupational opportunities *(12, 14)*. The negative impact is especially important for women because of the societal value attached to their skin and beauty *(15, 16)*.

1.6.2 Effect on the labour supply

The effects of onchocerciasis on the labour supply have been a major concern for decades, especially in countries where the disease is highly endemic and infection levels are intense. Published evidence indicates that the disease may impact the labour pool in three ways:

- **as a cause of death**. Prost & Vauglade *(17)* observed that the mortality rate among people over 30 years of age blinded by *O. volvulus* was three to four times higher than that of sighted people of the same age group. The consequence is a decrease in mean life expectancy of about 13 years in 30–40% of adults living in endemic communities *(18)*.
- **as a cause of permanent disability**. Blindness and serious visual impairment due to onchocerciasis reduce the number of labour years in common activities requiring vision *(19)* (Fig. 1.8).

Fig. 1.8. Blindness and/or serious visual impairment due to onchocerciasis reduces the availability of affected individuals for labour that requires vision.



Source: reference *20*

- **partial visual impairment**. Partial loss of sight and/or other non-disabling manifestations may also reduce the efficiency of work. Rolland & Ballay *(21)* in Burkina Faso and Nwoke *(2)* in Nigeria observed that blindness is heavily concentrated in groups of working age and reaches very high levels in hyper-endemic areas. WHO *(22)* reported that the average total number of potential working days lost annually was 170 per person. This implied that nearly 9 million days were lost per year in the 11 areas of West Africa covered by the Onchocerciasis Control Programme (OCP) at that time. While the accuracy of this figure may be questionable because of the often high unemployment in rural areas, the effect of blindness on productivity in onchocerciasis-endemic areas is significant.

1.6.3 The effect on supply of land

Onchocerciasis also has adverse effects on the effective supply of land in Africa. Waddy *(23)* and Hunter *(24)* reported that low population densities due to desertion of many fertile river valleys in the savannah zone of West Africa were due mainly to river blindness (Fig. 1.9).

Fig. 1.9. Abandonment of villages in fertile river valleys in the savannah zone of West Africa.

The combined evidence suggested that depopulation of many riverine areas occurred where onchocerciasis was hyper-endemic. This impact was reversible, as evidenced by a long-term appraisal of OCP activities, which revealed that, where transmission was virtually interrupted by vector control, progression of eye lesions was halted and the incidence of new infections among about 30 million children born during the programme period was almost zero; 25 million ha of land were also reclaimed for agricultural use, enough to provide food for 18 million people *(25)*. All the areas previously abandoned were spontaneously resettled, accompanied by increased economic development.

1.7 From control to elimination of onchocerciasis

Like other neglected tropical diseases (NTDs), onchocerciasis is associated with poverty and is concentrated almost exclusively among impoverished populations, often living in remote, marginalized areas. The disease is one of 10 NTDs targeted for elimination of transmission in the WHO 2030 road map for NTDs *(26)*. Achieving elimination will require access to MDA for every eligible person in areas of transmission. It has been estimated that this will require sustained annual treatment coverage rates ≥ 80% of communities for 15–20 years in Africa *(27)*, and some epidemiological models predict that it will take longer, depending on such conditions as initial infection intensity and treatment regimen *(28)*. Elimination of *O. volvulus* transmission in defined foci has nevertheless been reported in Ethiopia, Nigeria, Sudan and Uganda. Elimination of transmission and eventually the parasite has been verified by standardized criteria in 11 of the 13 foci in the Americas, where twice yearly MDA was the main strategy.

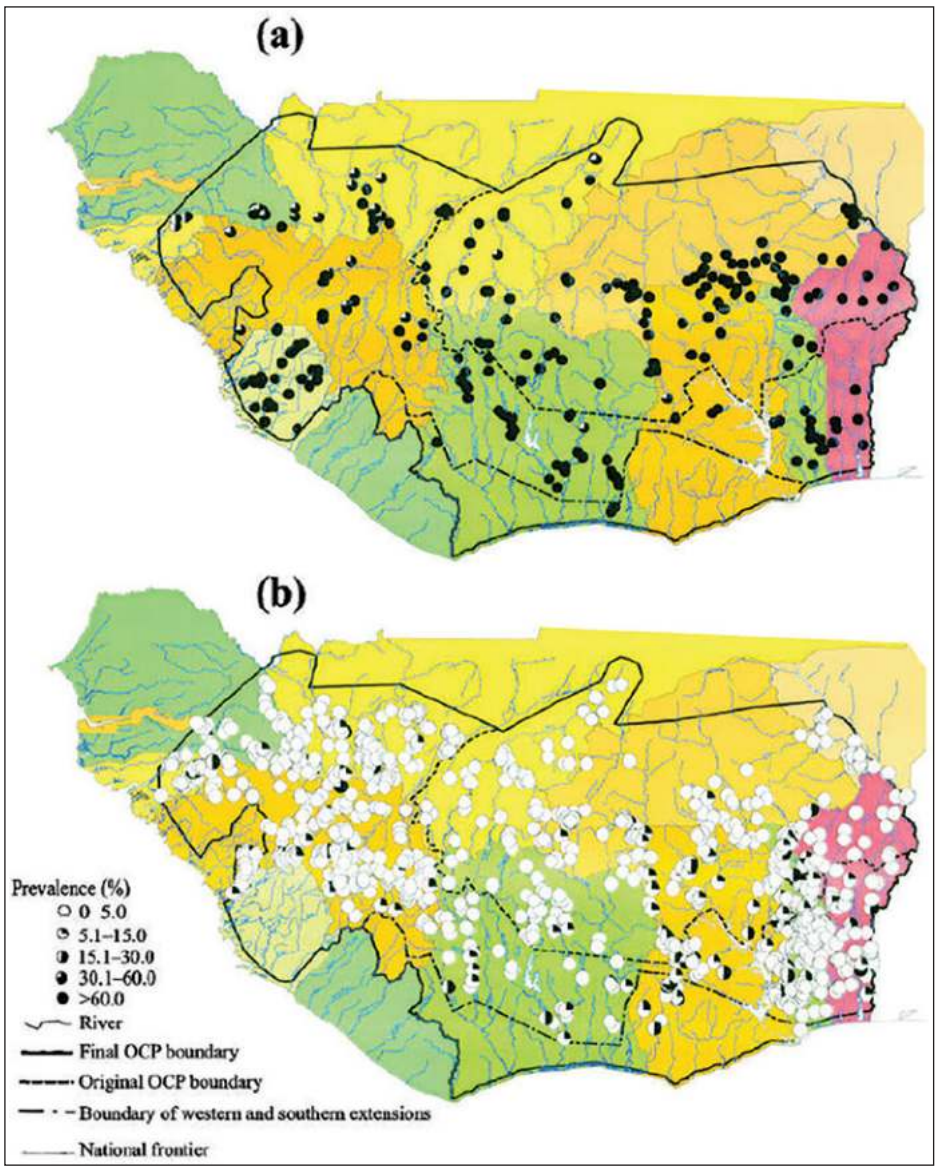
1.8 Justification for an entomological manual for onchocerciasis elimination programmes

1.8.1 Vector control

The role of *Simulium* species as vectors of *O. volvulus* has been known for almost 100 years. Initial black fly control consisted of reducing the numbers of these insects as a nuisance. As black flies became incriminated not only as a serious nuisance but also as vectors of *O. volvulus*, more attention was paid to the parasite spread by these insects. The recognition that onchocerciasis causes severe skin disease and blindness and has a strongly negative socioeconomic impact on endemic communities and areas led to establishment of a supranational programme. WHO, the World Bank and the United Nations Development Programme, with WHO as the executing agency, organized the regional OCP in West Africa in 1974 and began operations to control river blindness 1 year later in 11 countries (Fig. 1.10).

From its inception until 1987, the OCP relied exclusively on vector control to achieve its aims. For a decade (1974–1984), the OCP conducted prolonged, regular larviciding of rivers with rapidly biodegradable insecticides. The impact of the programme was measured epidemiologically and entomologically *(29)*. Epidemiological evaluation was based on detection by microscopy of microfilariae in skin snips to determine the prevalence and intensity of infection in the community and ophthalmological evaluations to detect microfilariae in the eye. In entomological evaluations, humans were used as attractants to catch flies, which were then dissected to determine the presence of infective-stage larvae of *O. volvulus*. Transmission indices, particularly the annual transmission potential (ATP), were calculated and used as standard measurements (see Chapter 11). OCP prepared entomological and epidemiological manuals for training to assist programme personnel in performing the necessary evaluations.

Fig. 1.10. Prevalence of infection and disease intensity in areas covered by the Onchocerciasis Control Programme in West Africa in 1974 (a) and 10 years later (b).



1.8.2 The advent of Mectizan®

With recognition of Mectizan® (ivermectin) as the drug of choice for treatment of onchocerciasis and the unprecedented decision by Merck & Co. Inc. to donate the drug for as long as necessary, a new chapter in onchocerciasis control began. Access to the drug provided organizations like the OCP and OEPA with a public health tool to control disease and possibly eliminate *O. volvulus*, although there were formidable logistical challenges to ensuring access to ivermectin by the people who needed it. Nevertheless, the availability of ivermectin gave regional control programmes the opportunity to use mass chemotherapy as the main tool in place of or as an adjunct to larviciding.

The OCP began to distribute ivermectin in priority populations in 1987. The epidemiological picture of the disease in many other endemic African countries was, however, unknown, and it became urgent to identify endemic zones rapidly so that ivermectin could be distributed to those most affected. This led to development of rapid epidemiological mapping of onchocerciasis. Rather than examining microfilarial-positive skin snips, the new approach involved clinical examination of a random sample of 30–50 adults (aged ≥ 20 years) in a selected village for onchocercal nodules to determine the epidemiological pattern of the disease in a transmission focus. The procedure was field-tested in a nationwide epidemiological survey in Cameroon by Ngoumou & Walsh *(30)*. After the success of this exercise, field manuals and guidelines were prepared and rapid epidemiological mapping of onchocerciasis became the primary tool for identifying transmission zones.

About 3 years after initial use of ivermectin in the OCP, additional countries adopted MDA in collaboration with United Nations agencies and nongovernmental development organizations, which created a coordinating structure in 1992 to promote the control efforts. The OCP continued to operate until 2002, with many accomplishments. Skin disease was significantly reduced throughout the Region (40 million people freed from infection), 600 000 cases of blindness were prevented, the size and distribution of the *O. volvulus* population in the region was substantially decreased, 18 million infants born after inception of the programme faced no risk of developing onchocerciasis, and ≥ 25 million ha of fertile, riverine land were re-opened.

1.8.3 Onchocerciasis Elimination Programme for the Americas

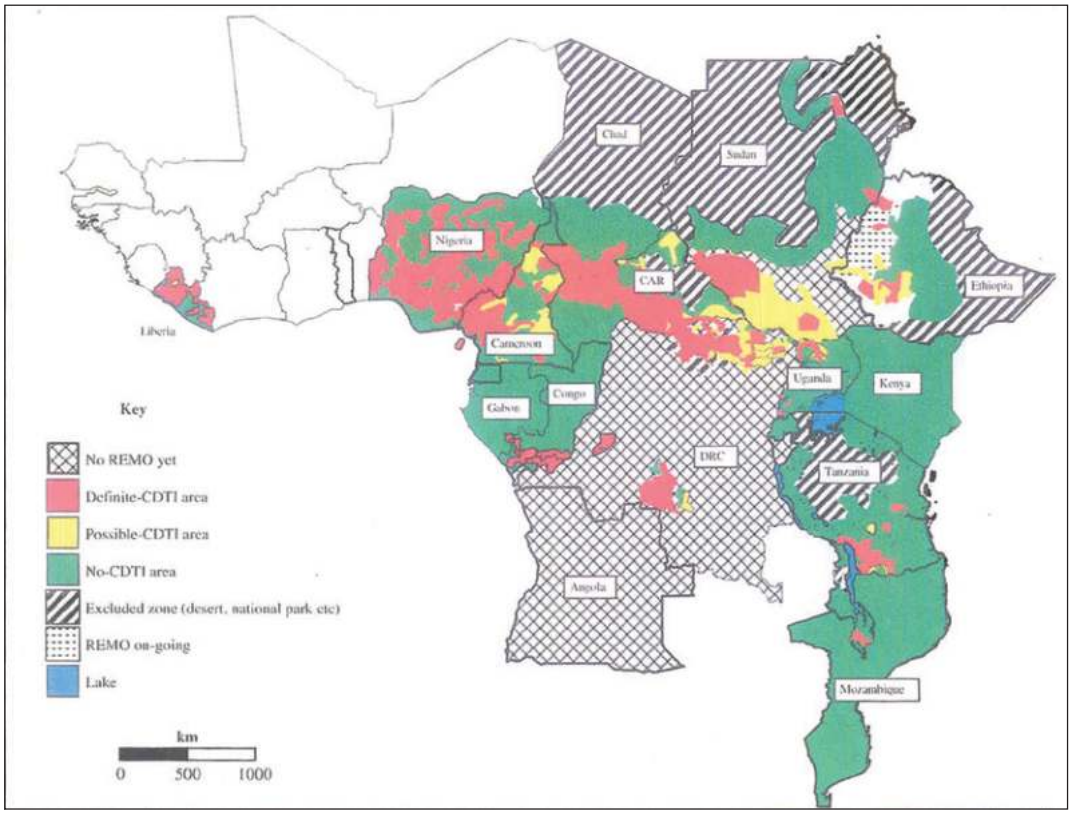
A regional programme for elimination of *O. volvulus* from all affected countries in the Americas was established in 1992. At that time, there were 13 active foci in six countries, from Mexico in the north to Brazil in the south (Fig. 1.3). The strategy was based on earlier studies in Liberia and a 3-year pilot study in Guatemala, which showed more rapid results of twice-yearly administration at the community level, covering ≥ 85% of the eligible population, than one annual treatment. At the beginning of the programme, more than 536,000 people were at risk of infection. Treatment coverage of > 85% of the eligible population was reached by 2006, and no new cases of onchocercal blindness have been reported in the Region since 2009. The original transmission zone has been reduced by 94%, the remainder being in a trans-border jungle focus between Brazil and the Bolivarian Republic of Venezuela.

1.8.4 African Programme for Onchocerciasis Control

The launch of the African Programme for Onchocerciasis Control (APOC) in 1995 opened a new chapter in control of the disease on that continent (Fig. 1.11). The original objective of APOC was to control the disease by establishing effective, self-sustaining, community-directed treatment with ivermectin through collaborative partnerships in the framework of primary health care in the remaining endemic areas of Africa. Vector elimination by environmentally safe methods in selected foci was also considered, but was not a major control tool as it had been in the OCP *(20)*.

With improvements in technology, the strategies for epidemiological and entomological evaluations changed. APOC originally used a combination of data from dissection and pool screening of flies (for parasite infectivity rate) analysed in the polymerase chain reaction (PCR) (Chapters 9 and 10). Flies were caught by human collectors to determine monthly biting rates (MBRs) and to calculate annual transmission statistics to be integrated with evaluations based on rapid epidemiological mapping of onchocerciasis and skin snips.

Fig. 1.11. Countries in the African Programme for Onchocerciasis Control.



Source: Onchocerciasis Control Programme in West Africa

REMO, rapid epidemiological mapping of onchocerciasis; CDTI, community-directed treatment with ivermectin.

CAR, Central African Republic; DRC, Democratic Republic of the Congo. Note that Sudan is now divided into Sudan and South Sudan, and Tanzania is the United Republic of Tanzania, with Zanzibar.

1.8.5 The era of onchocerciasis elimination

The first WHO guidelines were prepared for interruption of *O. volvulus* transmission in the Americas, which were first operationalized in a focus in Guatemala *(31)*. Subsequently, APOC supported work in Mali and Senegal to demonstrate the feasibility of onchocerciasis elimination in Africa *(32)*, which led WHO to consider moving from control to elimination. An informal meeting of experts on the elimination of onchocerciasis transmission in Africa with current tools was organized by APOC at Ouagadougou, Burkina Faso, in 2009, which provided a definition of onchocerciasis elimination and a conceptual framework that was subsequently refined by the Technical Consultative Committee of APOC *(33)*.

Establishment of the new goal of elimination required a major change in strategy. Data from Africa and the Americas were used to refine the WHO guidelines for stopping MDA and verification of the elimination of onchocerciasis *(27)*. The new criteria required use of the immunoglobulin G4 response to the Ov16 recombinant antigen as determined by enzyme-linked immunosorbent assay in children, rather than detection of microfilariae in skin snips. Entomological evaluations were directed to assessment of the prevalence of infective larvae in the heads of black flies by O-150 PCR. Fly dissection was no longer required but could be used to determine parity rates. Currently, as areas of low prevalence of onchocerciasis may now require MDA, rapid epidemiological mapping of onchocerciasis is no longer important and has been replaced by serology of the immunoglobulin G4 response to Ov16. In some locations, host-seeking flies are now collected in Esperanza window traps (EWTs) rather than by human landing capture. Vector control is now recommended as a complementary strategy to MDA in areas that are not progressing towards elimination. With changes in how evaluations are performed, tools and the goals of the programmes, a new entomological manual was necessary so that each affected country is prepared to assess its progress towards this goal.

Chapter 2.

General characteristics and life cycle of black flies

Learning outcomes for Chapter 2

By the end of this chapter, the reader should be able to:

- classify black fly (*Simulium*) species;
- appreciate the biological characteristics of *Simulium* spp.; and
- describe and explain the life history of *Simulium* spp.

2.1 Classification of the genus *Simulium*

Phylum: Arthropoda (insects and their allies)

Class: Insecta

Order: Diptera

The order Diptera is composed of two-winged or "true flies". Insects in this order are characterized by:

- conspicuous compound eyes, most species also having three simple eyes, the ocelli, set in a triangle at the top of the head;
- only one pair of membranous functional wings; and
- the hind wings reduced to halters (the second pair of wings in Diptera that are reduced and serve as balancing organs).

Family Simuliidae (black flies)

- Most members are black, hence the common name; however, some species are brown, golden or even yellow.
- Immature stages are aquatic, and adult female flies of many species are aggressive blood-feeders.

Genus *Simulium* and species

The type genus of the family Simuliidae, *Simulium*, comprises more than 1800 species. The type species, *S. colombaschense*, was a notorious pest in the middle region of the Danube Basin *(34)*, with annual emergence of enormous populations that attacked humans and cattle. Several species in the genus are important vectors of a number of pathogens, including the parasite *O. volvulus*. Vector species that can transmit *O. volvulus* are found in 28 sub-Saharan African countries, Yemen and six countries in Central and South America (Brazil, Colombia, Ecuador, Guatemala, Mexico and the Bolivarian Republic of Venezuela). Different vector species of *Simulium* are found in different regions. For example:

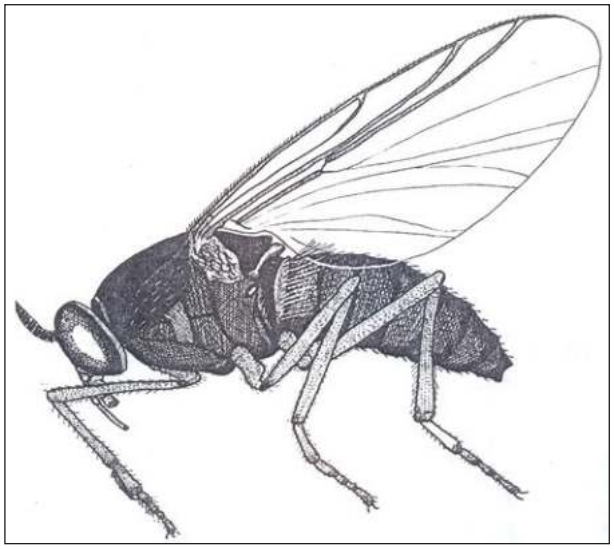
- *S. sirbanum* and *S. damnosum* in the African savannah
- *S. neavei*, primarily in East African forest regions
- *S. yahense* and *S. squamosum* in African rainforest regions
- *S. leonense* in the lowlands of Sierra Leone
- *S. sanctipauli* in large coastal rivers of West Africa
- *S. ochraceum* in upland areas of Central America and Mexico
- *S. metallicum* and *S. exiguum* in hilly, sparsely wooded areas of Central and South America
- *S. guianense* in the rainforest of Brazil and the Bolivarian Republic of Venezuela

Many black fly species are very similar in appearance, and detailed chromosomal or molecular analysis may be required to confirm the correct identification. For this reason, they are placed in a species complex (e.g. *S. damnosum* complex). For example, see Post et al. *(35)*. Related species that are similar in appearance but can be separated with traditional taxonomic tools are classified into a group category (e.g. *S. neavei* group). More detailed descriptions of morphological characters used for identification are presented in Chapter 4.

2.2 General characteristics of an adult black fly

Adult female black flies (Fig. 2.1) are usually small (2–4 mm long), robust and dark, with a stout body and a characteristic humped thorax. The head has conspicuous compound eyes, which exhibit sexual dimorphism. A typical male simuliid has large eyes that touch above the antennae (holoptic) (Fig. 2.2A), whereas the eyes of the female are well separated above the antennae (dichoptic) (Fig. 2.2B).

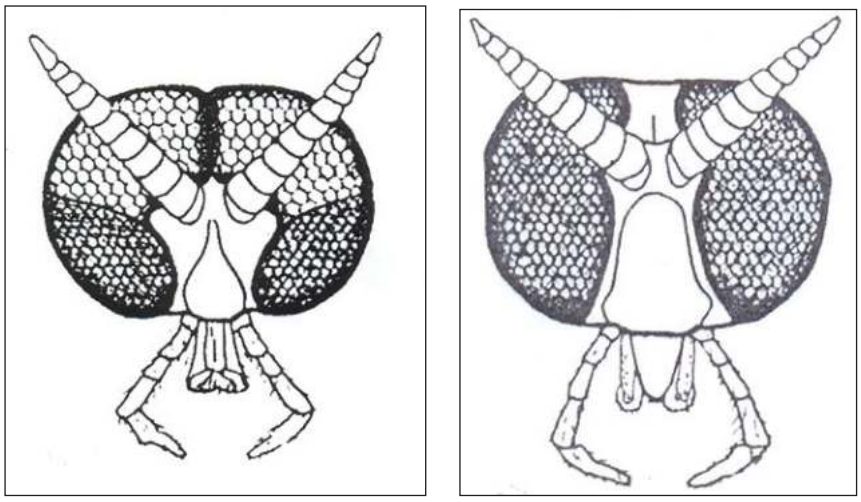
Fig. 2.1 Lateral view of an adult female black fly showing characteristic features.



Source: reference *36*

Note the typical shape of the thorax and the thickened anterior veins of the wings.

Fig. 2.2. Front view of the head of a black fly showing A, holoptic eyes of males and B, dichoptic eyes of females.



A B

Source: reference *36*

The antennae are shorter than the thorax and are distinctively segmented into 9–11 segments. The wings are short, broad, transparent and without scales, ornamentation or prominent hairs. The wing venation is characteristic: only the veins near the anterior margins are well developed and stout, while the remaining veins are membranous or weak (Fig. 2.1). The abdomen is short and covered with inconspicuous, closely oppressed fine hairs. The genitalia of both sexes are very conspicuous. In practical terms, the distinctive male genitalia of simuliids are characteristic of each species and can often be used for taxonomic purposes.

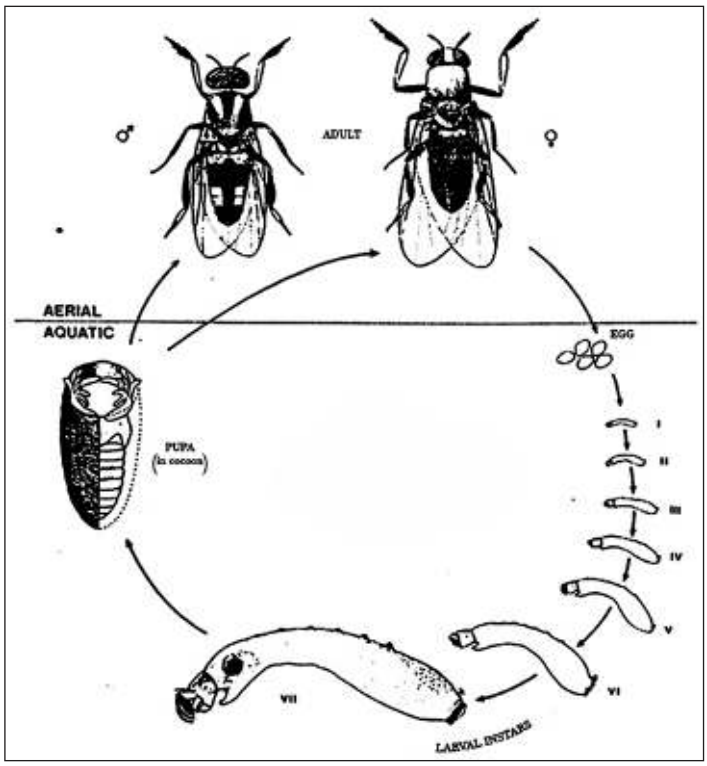
Adult black flies are diurnally active, and females of some species can travel long distances in search of a blood meal. Others use seasonal winds due to large weather fronts to migrate over relatively vast areas.

2.3 Life cycle of *Simulium*

Only female *Simulium* spp. feed on blood, by means of a syntrophium, a structure consisting of several mouthparts working in unison. The skin is penetrated by attachment of the labrum to the host's skin, followed by the cutting action of the mandibles. The maxillary laciniae and hypopharynx are then inserted into the cut to enlarge the wound. Saliva is injected through the hypopharynx into the lesion, which prevents blood from clotting and allows it to move into the wound and form a pool. Other components of the saliva act as a local immune suppressant and vasodilator. Female black flies exhibit gonotrophic concordance, i.e. a single blood meal provides sufficient nutrients to develop one batch of eggs. Black flies draw other fluids into their alimentary tract as well mainly by means of a powerful cibarial pump, a muscular structure located in the head. Both sexes take nectar meals from plants for energy.

Black flies undergo complete metamorphosis, with four distinct stages of development (Fig. 2.3): egg, larva, pupa and adult (imago).

Fig. 2.3. Life cycle of *Simulium damnosum*.



Source: modified from references *1 and 37*

2.3.1 Egg stage

The pattern of oviposition differs by species. For instance, some female flies alight on protruding substrates such as rocks, stones and vegetation to lay their eggs. *Simulium ochraceum*, an important vector in the Americas, scatters its eggs over the surface of flowing water while in flight. In other species, females crawl under the water and become completely submerged during oviposition. The oviposition site for *S. neavei*, a vector in East Africa, has yet to be determined.

The types of water preferred by female black flies for oviposition differ by species. Some *Simulium* spp. prefer trickles of water, slow-flowing streams or lake outlets, while others, such as members of the *S. damnosum* species complex, important vectors in most African countries, prefer fast-flowing, well-oxygenated streams, rivers and rapids containing a high nutrient load.

Simulium eggs are 0.1–0.4 mm long and triangular and are usually laid in large batches (≥ 150). The eggs are pale or whitish when first deposited and darken as they mature. Black fly eggs lack an internal wax layer and therefore must remain wet to moist to embryonate. To avoid desiccation, egg batches are laid in sticky masses or strings on a level with or just below the water line on submerged objects.

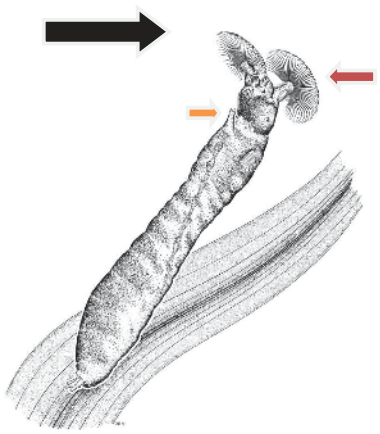
The duration of development and hatching of the eggs to larvae depends on the water temperature. This takes an average of 30–36 h at 26 °C and 4 days at 21 °C. The eggs of *S. sirbanum*, however, may fully develop within 1 day in extremely warm water (32 °C) *(38)*.

2.3.2 Larval stage

Larvae hatch after a few days to a few weeks, depending on the water temperature and region. Newly hatched larvae maintain their hold on the egg case by means of a silken thread secreted by the salivary glands until they can attach to more substantial submerged objects using a circlet of small hooklets on the last body segment. There may be six to eight larval instars, each separated by a moult. *S. damnosum* typically has seven larval instars (Fig. 2.3).

Simulium larvae have characteristic features that distinguish them from other aquatic insects (Fig. 2.4). The head is usually black and has a pair of prominent feeding brushes, commonly called a cephalic fan (Fig. 2.4, red arrow). Ventrally, just below the head, is a small pseudopod, the proleg (Fig. 2.4, orange arrow), which is armed with a small circle of hooklets. A *Simulium* larva has a weak, segmented, cylindrical body, which is usually white, although the body may be dark or sometimes greenish in some species.

Fig. 2.4. The last larval instar of *Simlium vittaum*, showing two characteristic morphological features: the cephalic fan (red arrow) and the proleg (orange arrow). The black arrow (top) indicates the direction of movement of the water column.



Source: drawing by Dr David Maddison, Oregon State University (OR), USA

Larval development of *S. damnosum* usually comprises seven instars separated by moults. Thus, instars 1 and 2 are very small larvae (< 1.25 mm) and have an antenna with one (instar 1) or two articles (instar 2). Instar 3 does not have scales on the body, and the antenna has three articles. Instar 4 has scales only on the posterior third of the abdomen, while instar 5 has scales on the entire abdomen; in addition, the histoblasts of the wing and the mesothoracic leg are not fused.

Instars 6 and 7 have scales on the abdomen and thorax; the histoblasts of the wing and the mesothoracic leg are apparent. The histoblasts of the respiratory organs are pale at instar 6 and become dark at instar 7 before pupation.

Mature last instar larvae of *S. damnosum* are recognized by the presence of a dark "gill spot" or respiratory histoblast, through which the developing gills of the pupa can be seen on the lateral side of the thorax. For cytotaxonomic studies, larval instars with a darkened larval respiratory filament or histoblast are best selected for chromosome work (see Chapter 7).

The duration of larval development varies by species and is inversely proportional to water temperature and the quantity and quality of food conveyed by the water column (Fig. 2.4). On average, *S. damnosum* larvae complete development of this stage within 8–10 days. In general, black fly larvae are filter feeders and use their outstretched cephalic "fans" coated with mucous to filter food particles (primarily diatoms, algae, microscopic fungi and bacteria). Some species, however, use their mouthparts to scrape algae and other particles from substrates, and others collect particulate matter from decomposing plant material *(39)*. Many black fly larvae are mobile, but their movements on a substrate are usually reduced. Each larva clings to a support with its posterior crown of hooks and a silken thread for anchors. Larvae may also move within a stream by drifting in the current to new locations.

2.3.3 Pupal stage

At the end of the larval stage, the last larval instar spins silk secreted from the salivary glands into a protective, slipper-shaped, brownish cocoon and moults inside to a pupa. This stage remains anchored to the same substrate as the larva. Mature pupae generally look like adults wrapped in a membrane. They are yellowish when young but become increasingly dark as they develop. At the "old" pupal stage, the eyes have the same sexual dimorphism as the adult fly. Pupae do not feed and are completely embedded inside a cocoon, fixed at the support, with the exception of the respiratory filaments.

The thorax bears a pair of filamentous, cuticular gills at its dorsal end, in the front (Fig. 2.5A, arrow). The number, shape and port of these respiratory filaments are very characteristic and species specific, sometimes allowing identification in the field.

The duration of the pupal stage depends on the ambient temperature but lasts an average of 3–4 days. The duration is slightly longer in the female than in the male. On emerging from the pupal case, the young adult fly floats to the surface of the water enclosed in a bubble of air and takes flight within minutes.

Some African black flies, characterized by members of the *S. neavei* group, have an unusual aquatic existence. The larvae (except for the first instar) and pupae do not occur on submerged vegetation and rocks but are attached to the bodies of aquatic arthropods such as the immature stages of mayflies and various crustaceans, particularly freshwater crabs. This association is termed phoretic.

Fig. 2.5. A. Cutaway diagram of a black fly pupa showing the outer cocoon and developing adult fly inside. Note the long respiratory filaments (gills). B. *Simulium* pupae attached to an aquatic plant.

A B

Source: A, Dr Wolfgang Lechthaler, Eutaxa.com; B, Carlos Pradera, *Desinsectador 08-2017. <https://desinsectador.com/2017/08/06/simulium-erythrocephalum-sobre-potamogeton-nodosus/>*

2.3.4 Adult flies

Adult black flies mate in swarms, usually at aquatic emergence sites *(40)*. Male flies hover over the site, using a marker as an orientation guide to maintain the integrity of the swarm, and are attracted visually to the movement of female flies as they move up into the swarm. As seen in Fig. 2.2A, male flies have dorsal compound eyes, which enable them to detect movement over a wide lateral and vertical field. Once contact is made and coupling between the two sexes has ended, the female retains sperm by means of a spermatophore, a hardened case containing sperm placed on the last abdominal segment. Insemination occurs as the sperm enter the reproductive tract. Female black flies mate once in their lifetime.

Flies of both sexes usually imbibe nectar and plant juices containing carbohydrates shortly after emergence and mating. These juices are stored in a crop and serve as a primary energy source as female flies begin searching for a blood meal. Nulliparous flies may move long distances from their breeding sites, while parous flies are less likely to do so, preferring to search for a blood host closer to the aquatic breeding sites.

Female flies use hierarchical cues to first find and then orient to a host. Long-distance orientation begins after detection of colour and movement of the host. Darker colours (black, blue) are attractive to black flies. Once a potential host is seen, mid-range orientation is stimulated by plumes of carbon dioxide emanating from the host, which bring the fly closer. The final set of host-seeking stimulants consists of a variety of chemicals present in sweat and on the skin of the host.

Chapter 3.

Breeding sites of black fly vectors

Learning outcomes for Chapter 3

By the end of this chapter, the reader should be able to:

- identify the breeding sites of black fly larvae;
- recognize different types of aquatic sites in which black fly larvae occur;
- find and identify substrates used for attachment by black fly larvae;
- describe the types of vegetation associated with black fly breeding sites;
- recognize the different types of man-made aquatic habitats used by black fly larvae;
- understand the importance of hydrological and biological determinants of black fly larval productivity; and
- identify the crabs on which phoretic larvae of *S. neavei* group occur.

3.1 Introduction

Identification of the aquatic locations of *Simulium* species larvae is an important first step in vector control and surveillance, which will determine the success of an onchocerciasis control programme. These aquatic habitats are commonly called "breeding sites" or "breeding places".

In the ecology of fast-flowing rivers, black flies play an important role in the invertebrate community. Larvae filter water through their fan-like mouthparts (Chapter 2) to remove dissolved and particulate inorganic and organic compounds, which serve as food. The content of the filtrate depends on the geology, topography, climate, time of day, depth of water body and biota. Physico-chemical attributes also affect the life of aquatic organisms and influence the distribution of *Simulium* larvae.

For black fly larvae to become established in an aquatic habitat, the essential conditions are:

- the presence of substrates for laying eggs and subsequent attachment of larvae and pupae;
- a satisfactory water column velocity, e.g. 0.4–2.4 m/s for members of the *S. damnosum* spp. complex and minimal flow for members of the *S. neavei* species group, which are phoretic on freshwater crabs; and
- sufficient food particles in plankton.

These conditions must be met simultaneously.

3.2 Each breeding site has a distinct fly population

African vectors of *O. volvulus* belong to two groups of *Simulium* flies: those that breed in fast-flowing rivers (*S. damnosum* species complex and *S. albivirgulatum*) and those living in phoretic association with invertebrates, e.g. crabs. The latter live in or close to small, turbulent rivers in East Africa but can survive during the dry season in holes with stagnant water.

Some typical breeding sites for vector species in Africa and the Americas are described and illustrated below, with the species of *Simulium* commonly encountered at those sites.

S. damnosum sensu lato (s.l.) larvae typically prefer fast-flowing, broken "white-water" in the rapids of rivers, but medium-sized streams may also be populated, especially in upland areas. In the dry season, larvae are found in more slowly-flowing rivers. *S. damnosum s.l.* larvae usually occur in water with relatively high oxygen saturation (75–100%).

This species complex breeds in savannah, lowland forest and highland areas. In most savannah breeding sites, the occurrence of this species complex is seasonal, depending on the geology of the riverbed and changing hydrological conditions (water height and discharge) (Fig. 3.1).

S. damnosum sensu stricto (s.s.) and ***S. sirbanum*** are typical savannah species. Their larvae and pupae are found in large rivers that become dry or dwindle to an insignificant flow seasonally. Populations are re-established after the dry season by immigrating or migrating flies.

Fig. 3.1. Mayo Galke near Tcholliré, North Cameroon, an almost perennial breeding site for savannah species (*S. damnosum sensu stricto* and *S. sirbanum)*.

Source: photograph by A. Renz

This productive site can result in an annual biting rate (ABR) of up to 87 000 bites per person (see Chapter 11).

S. damnosum s.l. breed mainly in rapids and broken water, with two exceptions. First, at the height of the rainy season, a river may become so high and the flow of water so fast that rocky rapids are flooded and larvae breed in a dispersed manner on vegetation, such as partially submerged branches of trees along the river (i.e. not concentrated in rapids) (Fig. 3.2A). This is referred to as "linear breeding". The second exception is that, in some savannah areas at the height of the dry season, a river may not dry up completely but be reduced to little more than a trickle. Under such condition, clusters of larvae may be found in spots where the flow is fastest (Fig. 3.2B).

Fig. 3.2. A. Linear breeding of *S. damnosum* larvae on submerged trees during the rainy season. B. Dry-season breeding site of *S. damnosum s.s.* in the Aruu falls in northern Uganda, where very small numbers of larvae survive in trickles during the dry season.

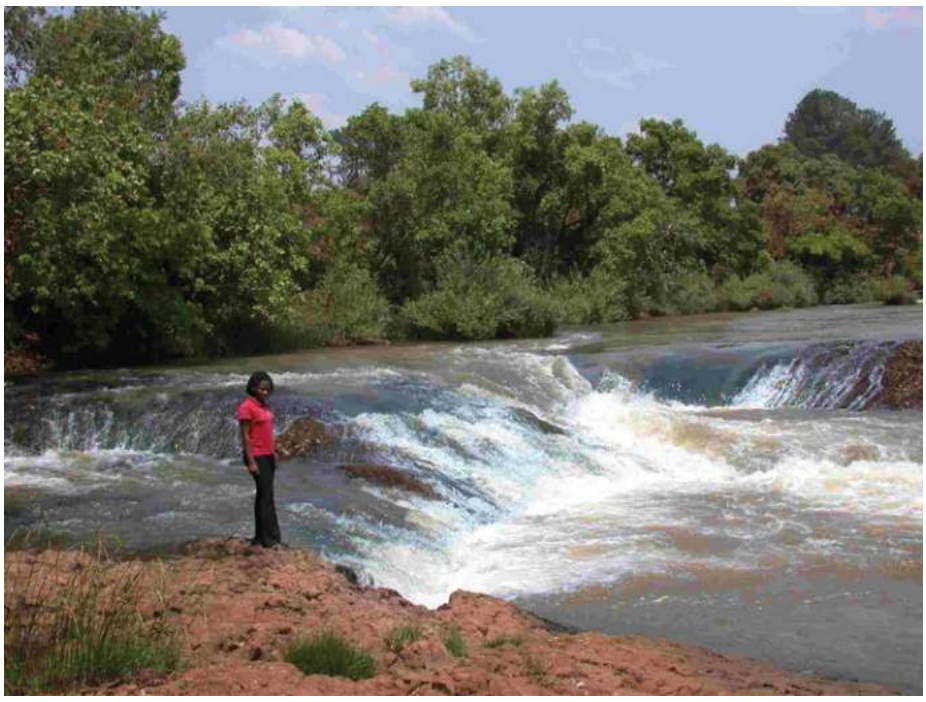
A

B

Sources: photographs by R. Post

S. squamosum occurs in a number of different forms (see identification of adult flies, Chapter 4). In highland savannah areas, this species breeds mainly in perennial rivers, which in mountainous areas are sometimes quite small, or in broad rivers in lowland areas typical of a savannah habitat in Guinea or Sudan (Fig. 3.3).

Fig. 3.3. Vina du Sud near Ngaoundéré, Cameroon, an annual breeding site for *S. squamosum* in the Guinea savannah of Cameroon. The annual biting rate may reach 250 000.



Source: photograph by A. Renz

S. squamosum larvae may also occur in small tributaries in forests (Fig. 3.4), hidden under the tree canopy and difficult to see on satellite maps. The species shares this type of aquatic habitat with two other vector species, ***S. yahense*** and ***S. mengense***.

Fig. 3.4. A rainforest breeding site typical for *S. squamosum* larvae. Note the dense vegetation surrounding the site.

Source: photograph by A. Renz

S. sanctipauli is confined to the rainforests of West Africa, where larvae prefer large, open rivers (Fig. 3.5).

Fig. 3.5. A *S. sanctipauli* breeding site. Note the large number of white-water rapids.

Source: photograph by R. Garms

S. soubrense larvae occur in smaller rivers in forests (Fig. 3.6).

Fig. 3.6. *S. soubrense* larval breeding site in the Bagbe River, Côte d'Ivoire.

Source: photograph by R. Garms

A variety of *S. damnosum* forms occur in East Africa, some of which are active vectors of *O. volvulus*. These include the typical savannah vectors *S. damnosum s.s.* and *S. sirbanum* as well as local forms that may be anthropophilic, such as *S. kilibanum*. A number of other cytoforms may also be found, several of which actively bite humans. For example, in the Mahenge Mountains onchocerciasis focus in the United Republic of Tanzania, the Nkusi J forms of *S. damnosum* and *S. kilibanum* were the predominate species and probably the vectors of *O. volvulus (41)* (Fig. 3.7).

Fig. 3.7. The Mzelezi River in the Mahenge, United Republic of Tanzania, onchocerciasis focus. This perennial river provides excellent sites for *S. damnosum s.l.* larval breeding.

Source: photograph by A. Hendy

S. rasyani breeds in permanently flowing wadis in the west of Yemen, which carry water from the Sarawat mountain range westwards into the Red Sea. The rivers in the wadis are shallow but flow at about 0.5–1 m/s. The bed is often stony or sandy, and white-water rapids are rare. Larvae and pupae are found mainly on half-submerged vegetation along the edges of the rivers (Fig. 3.8).

Fig. 3.8. *S. rasyani* larval breeding site in Yemen.

Source: photograph by R. Garms

S. albivirgulatum is a vector species in the Congo basin, between the Tshuapa and Congo rivers. Larvae breed in slow, smooth-flowing rivers lined by gallery (riparian) forest, where adult female flies transmit the parasite.

Several *Simulium* species, mainly the *S. neavei* spp. group in East Africa, have evolved a phoretic association with crustaceans and large aquatic insects (mayflies and dragonflies). *S. neavei,* an important vector of *O. volvulus* in East and Central Africa, occurs in streams where its larvae and pupae are attached to decapods of the genus

Potamonautes (freshwater crabs). This black fly and other phoretic species are confined to shaded forest areas (Fig. 3.9) and often disappear when deforestation occurs.

S. neavei and *S. ethiopiense* are attached, usually in small numbers of up to 10 individuals, on the back and sides of the crab's cephalothorax (body), the claws (chelipeds) and the basal segments of the legs. Crab density can reach up to one crab per metre in small tributaries and 10 or more in large rivers.

Fig. 3.9. A river in the Mpamba-Nkusi onchocerciasis focus in mid-western Uganda. *Potamonautes* river crabs, with *S. neavei* larvae and pupae attached, live in the turbid falls and retract into holes when the river dries up.

Source: photograph by T. Lakwo

3.3 Other types of breeding site in Africa

Larval habitats suitable for black fly larvae may be created inadvertently by the construction of bridges, river fords, irrigation ditches, road culverts, dams and other devices to control river flow. Examples are shown in Figs 3.10 and 3.11.

Fig. 3.10. Large hydroelectric dams create excellent breeding sites for *S. damnosum s.l.*

Fig. 3.11. A small dam in Nigeria which serves as a man-made breeding site for *S. damnosum s.l.*

Source: both photographs by R. Post

3.4 Examples of breeding sites in the Americas

Onchocerca volvulus was transported to the Americas from Africa several hundred years ago, and parasite populations became established in six countries. Interestingly, *O. volvulus* was able to adapt to a number of indigenous *Simulium* species to complete this part of its life cycle. Overall, seven species have been incriminated as primary or secondary vectors in the region. Before MDA with ivermectin, about 70% of all cases occurred in Guatemala and Mexico, where the main vector was *S. ochraceum*. Larvae of this highly anthropophilic species occur in small, cool mountain streams that flow all year. Water at a temperature of 16–20 °C and a flow rate of 0.1–1.0 L/s is preferred. This species is unusual in that it can colonize artificial streams with these physical qualities, and *S. ochraceum* larval breeding on coffee plantations where irrigation is used is common (Fig. 3.12).

Fig. 3.12. *S. ochraceum* larval breeding site on a coffee plantation in Guatemala.

Source: photograph by E. Cupp

Current MDA with ivermectin has eliminated about 95% of *O. volvulus* populations in the Americas, with only two active, adjoining cross-border foci along the border between Brazil and the Bolivarian Republic of Venezuela.

The main vector there is *S. guianense s.l.*, a cluster of at least three cytoforms *(42)*, the larvae of which occur in large populations in broad, fast-flowing, open-canopy rivers generally similar to *S. sanctipauli* (Fig. 3.13).

Fig. 3.13. A. Open-canopy larval breeding site of *S. guianense*. B. Tributary with aquatic vegetation that serves as a peripheral larval breeding site.

A B

Source: photographs by O. Noya-Alarcon

3.5 Physico-chemical characteristics of larval breeding sites

The environmental characteristics of breeding sites are correlated with the distribution of different species of black flies. Among the most important are temperature, pH and ion content. Other physical and biological factors determine the suitability of a site for larval production, including hydrological and biological parameters that directly affect the productivity of a *Simulium* breeding site. For example, the hydrological conditions for *S. damnosum s.l.* breeding sites are a temperature of 16–24 °C and a pH of 7.7–10.0 for the East African complex and a temperature of 22–33 °C and a pH of 5.7–6.2 for the West African complex *(43, 44)*. Flow rate, food quality, predation and infection by microscopic pathogens regulate larval populations. Other factors, particularly the use of agricultural chemicals (fertilizers, insecticides) and their run-off into streams and rivers, can reduce larval populations.

Breeding sites of the *S. damnosum* complex are almost always in fast-flowing, broken white-water rapids, which are often associated with hard rocks, geological fault lines and rapid changes in altitude. Examination of geological maps is often useful, because pre-Cambrian basement geology consists of rock formations, and rapids are common. Geological maps (including small-scale geological maps) also show fault lines, which can promote rapids on rivers. Topological maps are essential for finding relevant features associated with rapids, preferably at a scale of 1:50000 (or possibly 1:250000) (see Chapters 5 and 6). Such maps may include marks that show the position of significant rapids (or waterfalls) on rivers. Rapids are often associated with points on a map where a river is shown to be wider (shallower because of hard bedrock), with islands, or narrower (flowing faster through a gorge) or makes a sudden, angular change of direction (following a geological fault line).

The hydrological and ecological parameters that determine the suitability of breeding sites are therefore:

- water levels (m), water discharge (m^3/s) and seasonality;
- speed of water flow (m/s);

- support for larvae and pupae (plants, roots, leaves, rocks);
- nutrients (algae, plankton, diatoms, bacteria, plant detritus);
- predators (fish, dragonflies for larvae and birds and large insects for adult flies);
- pathogens (viruses, bacteria, fungi, microsporidia, ciliates and mermithid nematodes); and
- run-off of agricultural chemicals.

3.6 Other important factors

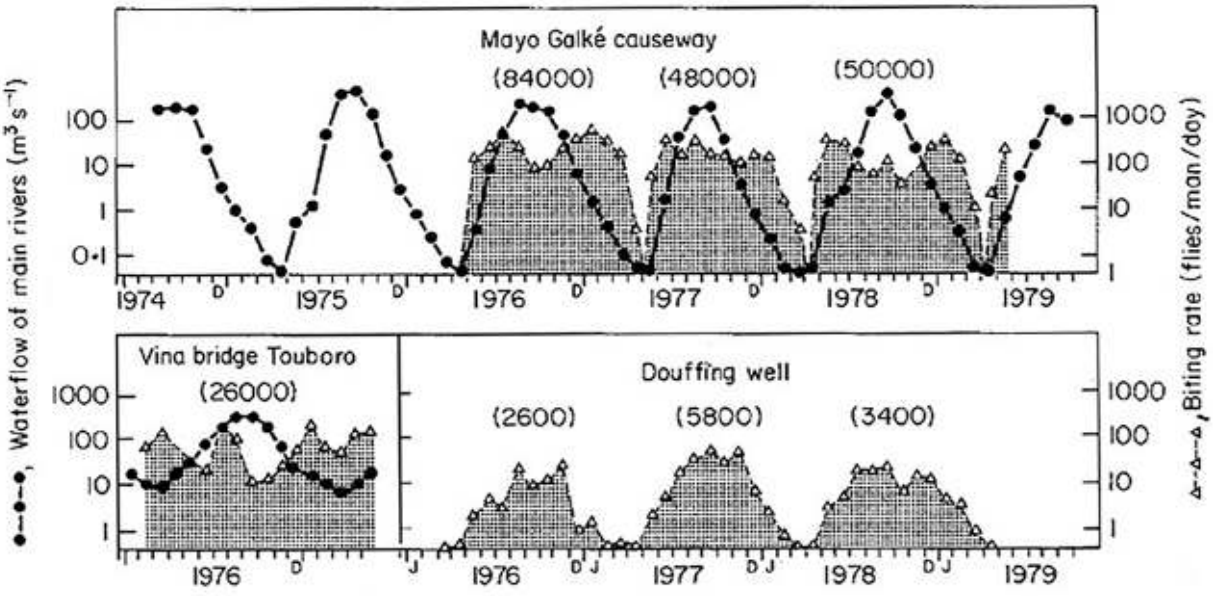
3.6.1 Substrates and plants associated with *S. damnosum* larval breeding sites

Gravid flies search for suitable substrates (leaves and stems of plants, roots or rocks) and land and crawl under the water to oviposit. The principal condition is that the substrate not be covered by algae or diatoms, which can prevent larvae from attaching. Larger larvae may clean the substrate with their mouthparts (Chapter 2) before attaching themselves by the circular ring of hooks on their abdomen. Artificial substrates such as plastic strips can serve as oviposition sites for population surveys.

3.6.2 Hydrological data on river height and water discharge

In many river systems in Africa, hydrological data on water levels and river discharge were recorded daily for many years (see for example reference *45*). As water levels vary dramatically during the year in the savannah, from a few litres per second to hundreds of metres cubed per second during flooding in the rainy season, a logarithmic presentation of water discharge and the daily biting rate can often be correlated (Fig. 3.14).

Fig. 3.14. Hydrological (m^3/s) and entomological data (daily biting rate) for some untreated seasonal and perennial *Simulium* spp. breeding sites in the savannah of northern Cameroon.



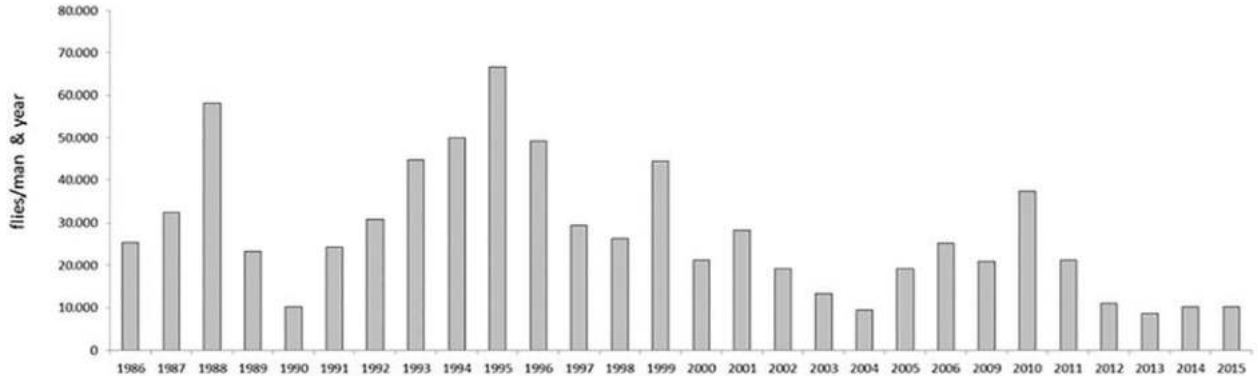
Source: reference *46*

Note the logarithmic scales.

3.6.3 Seasonal and annual variations in the productivity of breeding sites

Although there is some seasonal dynamic in human-biting rates, the monthly productivity rate (emergence of adult flies) of a local breeding site is difficult – or even impossible – to forecast. In large rivers, populations tend to build up at the beginning of the rains and again at the end of the dry season. In seasonally flowing rivers, breeding sites are productive only during the rainy season, when the water flows. The actual size of the fly populations at a given site and time (month) may differ dramatically from one year to the next (Fig. 3.15). This variation is a strong argument for rigorous, regular entomological follow-up with the same methods in order to obtain meaningful data that can be correlated and analysed.

Fig. 3.15. Variations in annual biting rates at the Vina du Nord River at Soramboum, Cameroon, over 28 years.



Source: data from A. Renz

Note the general trend of decreasing fly-populations over time.

3.7 Summary

Generally, black flies breed in swift-flowing, well-aerated or -oxygenated rivers and streams, while others breed in small rivulets. Large black fly populations at a breeding site indicate clean, healthy streams, as most species do not tolerate organic pollution.

Larval breeding sites differ widely among the major vectors of *O. volvulus*. For example, *S. damnosum s.l.* larvae are usually found along major rivers with fast-moving water, whereas *S. ochraceum* in the Americas is an "area" breeder, whereby larvae may occur in multitudes of generally distributed trickles and rivulets (small streams and ditches). *Simulium damnosum s.l.* may breed during the dry season when water levels drop precipitously and larvae inhabit small trickles of water.

Phoretic *Simulium* larvae and pupae occur on *Potamonautes* spp. crabs and dragonfly larvae in Central and East Africa, typically in small rivers, where the crabs live in small holes.

All the potential breeding sites of black fly vectors have aquatic substrates such as grasses, leaves and stems, twigs, plant roots and rocks for the attachment of pre-imaginal or aquatic stages (eggs, larvae and pupae). The most important criteria for all these substrates are that they be immobile and submerged in the water. Broken water rapids occur where hard, resistant surface rocks outcrop in river channels, whereas rivers flow smoothly where the land surface is formed of softer sedimentary rocks.

Fly populations vary from year to year and season to season, requiring standardized longitudinal surveillance. Man-made structures such as dams and culverts often create black fly larval breeding sites and should also be monitored.

Chapter 4.

Identification of black fly vectors in Africa

Learning outcomes for Chapter 4

By the end of this chapter, the reader should be able to:

- recognize the eggs, larvae, pupae and adults of black flies;
- distinguish the larvae, pupae and adults of *S. damnosum* species complex and *S. neavei* group from those of other black flies;
- recognize the diagnostic value and variability of some larval taxonomic characters within the *S. damnosum* species complex;
- use the larval taxonomic key to identify the main African *Simulium* subgenera and confirm the presence of vector species;
- use the principal characters of pupae to confirm the presence of vector species; and
- use adult morphological characteristics to identify anthropophilic African *Simulium* spp.

4.1 Introduction

Black fly vectors are identified in two to three stages. First, the insect specimen collected must be identified as a black fly, requiring familiarity with the various black fly stages. Secondly, if the specimen collected is a black fly, the morphospecies (identified by its external structural features) or at least the vector species should be identified. Thirdly, if the specimen is a member of a species complex, the cytospecies, which cannot be identified from morphological characters and must be differentiated from unique chromosomal features, should be determined (Chapter 7).

The world black fly inventory *(47)* includes three subgenera of the genus *Simulium* that include vector species, namely (i) *Edwardsellum* (55 valid, distinct cytospecies or cytoforms plus several named morphospecies without cytological backgrounds) *(48)*; (ii) *Lewisellum* (15 species, including the *S. neavei* group); and (iii) *Metomphalus*, with one species, *S. albivirgulatum*. Collectively, these comprise the vectors of *O. volvulus* in Africa. Within these subgenera are several morphoforms, cytoforms and molecular forms awaiting clarification of their sibling species status and agreement by systematists specialized in the family Simuliidae. The known distribution of vector taxa in Africa, including habitats, is shown in Table 4.1.

Table 4.1. Black fly vectors of *O. volvulus* and distribution on the African continent.

Subgenus	Taxon	Geographical region, countries	Habitat and epidemiology
Edwardsellum	*S. damnosum* subcomplex	Africa south of the Sahara	Savannah
	S. damnosum sensu stricto	West, Central and East Africa; Angola, Benin, Botswana, Burkina Faso, Burundi, Cameroon, Central African Republic, Chad, Congo, Côte d'Ivoire, Democratic Republic of the Congo, Equatorial Guinea (including Bioko), Ethiopia, Gabon, Ghana, Guinea, Guinea-Bissau, Kenya, Liberia, Malawi, Mali, Mozambique, Namibia, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, Somalia, South Africa, South Sudan, Sudan, Swaziland, Togo, United Republic of Tanzania, Zambia, Zimbabwe	Savannah
	S. sirbanum	Benin, Burkina Faso, Cameroon, Central African Republic, Côte d'Ivoire, Ghana, Guinea-Bissau, Mali, Niger, Nigeria, Sierra Leone, Sudan, Togo, Uganda	Dry savannah
	S. dieguerense	West Africa; Guinea, Mali	Savannah
	S. sanctipauli subcomplex	West Africa	Forest
	S. sanctipauli	Côte d'Ivoire, Ghana, Guinea, Liberia, Mali, Nigeria, Sierra Leone, Togo	Forest
	S. soubrense	Benin, Côte d'Ivoire, Ghana, Guinea, Liberia, Mali, Nigeria, Sierra Leone, Togo	Forest
	S. leonense	Guinea, Liberia, Sierra Leone	Forest
	S. konkourense	Guinea, Guinea-Bissau, Liberia, Sierra Leone	Forest, mountains

(continued)

Table 4.1. (Continued)

Subgenus	Taxon	Geographical region, countries	Habitat and epidemiology
	S. squamosum subcomplex	West, Central and East Africa	Forest, mountains
	S. kaffaense	Ethiopia	Mountains
	S. squamosum sensu stricto	Cameroon, Benin, Central African Republic, Côte d'Ivoire, Democratic Republic of the Congo, Ghana, Guinea, Liberia, Nigeria, Sierra Leone, Togo	Forest, mountains
	S. yahense	Benin, Cameroon, Côte d'Ivoire, Equatorial Guinea (including Bioko), Ghana, Guinea, Liberia, Nigeria, Sierra Leone, Togo	Forest, highlands
	Kibwezi subcomplex	Central and East Africa	Forest, mountains
	S. mengense	Central Africa; Cameroon	Forest
	Sanje subcomplex	Eastern to southern Africa	Mosaic and mountains
	S. kilibanum	Burundi (?), Democratic Republic of the Congo, Uganda, United Republic of Tanzania (?)	Forest, mountains
	S. thyolense	Malawi, United Republic of Tanzania	Forest, mountains
Lewisellum	*S. neavei* group	Central and East Africa	Mostly highland forests
	S. neavei	Angola (?), Democratic Republic of the Congo, Kenya, Uganda	Mostly highland forests
	S. woodi	Malawi, United Republic of Tanzania, Zambia (?)	Mostly highland forests
	S. ethiopense	Ethiopia	Mostly highland forests
Metomphalus	*S. albivirgulatum*	Central and southern Africa; Angola, Botswana, Democratic Republic of the Congo, Congo, Gabon, South Africa, Zambia, Zimbabwe	Lowland forest

There are 2384 extant black fly species worldwide, but the vast majority do not bite humans, i.e. are non-anthropophilic. A small number are autogenous (females can develop eggs without a blood meal), but the rest are zoophilic and bite non-human mammals and/or birds. A few zoophilic species land on humans but do not usually take a blood meal (for example, *S. griseicolle* in Sudan). Other zoophilic species occasionally bite humans, especially when they are in association with the black fly's normal host. For example, *S. vorax* usually bites cattle but will occasionally bite cattle-herders. Some normally zoophilic species regularly (and inexplicably) bite humans in only parts of their geographical range. Examples are *S. bovis* in northern Uganda and *S. dentulosum,* which do not transmit *O. volvulus*, and *S. albivirgulatum* in central Democratic Republic of the Congo, which does transmit the parasite.

Correct identification of *O. volvulus* vectors in Africa is crucial for successful elimination programmes in order to monitor progress, confirm interruption of transmission or implement vector control in addition to MDA. Morphotaxonomy of the adult females of both *S. damnosum sensu lato (s.l.)* and *S. neavei* requires finding unique morphological features for separation and classification into generic and specific groupings. These are described here, with data for other *Simulium* spp.

The objective of this chapter is to expand on the basic information presented in Chapter 2 and make the reader aware of some reliable morphological characters used to identify the species of *S. damnosum s.l.* and other vectors involved in the transmission of *O. volvulus*. Identification keys for the different stages (immature and adults) are illustrated, and the more important diagnostic morphological features are highlighted.

4.2 How to recognize that the specimens collected are eggs, larvae, pupae and adults of simuliids and not of other insects

4.2.1 Eggs of black flies

Black fly eggs are deposited in sticky clusters on submerged objects, such as rocks, leaves and other aquatic vegetation, or sometimes on substrates that emerge from the water's surface but are in a splash zone. Eggs are 0.15–0.20 mm through their long axis and ovoid–triangular (Fig. 4.1). Simuliid eggs lack a thickened chorion and are sensitive to desiccation.

Fig. 4.1. A, a cluster of old eggs on vegetation. B, typical egg batches on a substrate, the lower one with first instars just hatched. The inserted drawing (middle right) illustrates the triangular shape of black fly eggs.

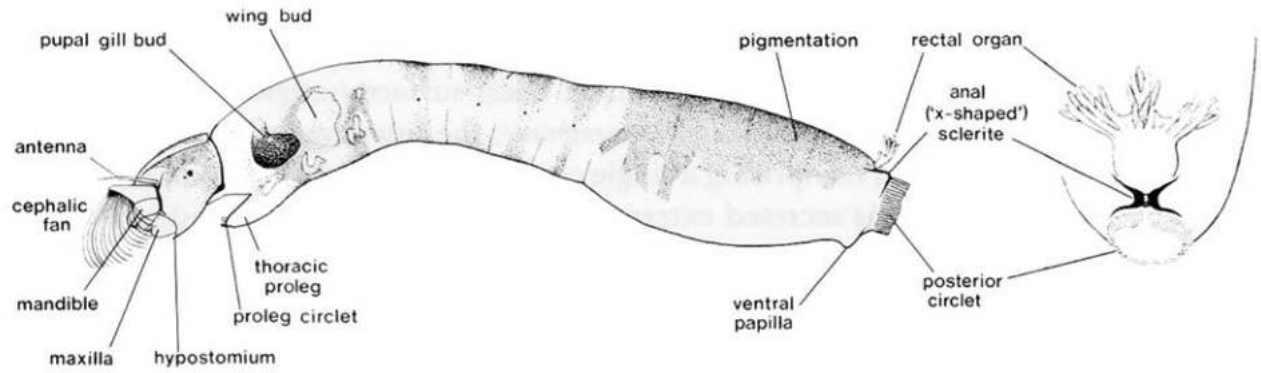
A B

Sources: photographs by A. Krueger; drawing from reference *49* (Fig. 6.1, p. 243)

4.2.2 Larvae of black flies

Simulium larvae have characteristic features (Fig. 4.2), which distinguish them from other aquatic insects. The **head capsule** is more strongly defined than the thorax and abdomen, which are only weakly segmented. The head is usually darkish and has a pair of prominent feeding brushes, commonly called cephalic fans. The thorax and abdomen are separated externally by a slight constriction. Ventrally, the **thorax** bears a small but very characteristic unpaired pseudopod called the thoracic proleg, which is armed with a small circle of minute hooklets. The posterior half of the **abdomen** is club-shaped and is often pigmented dorsally. The abdomen ends with a terminal proleg bearing a **posterior circlet** of small hooks. The anus opens dorsal to the posterior circlet, and the rectal organ may be extruded from it.

Fig. 4.2. A, Morphological features of a simuliid larva; B, photograph of a black fly larva (*S. damnosum s.l.*).



A

B

Sources: drawing from reference *50*; photograph by A. Krueger

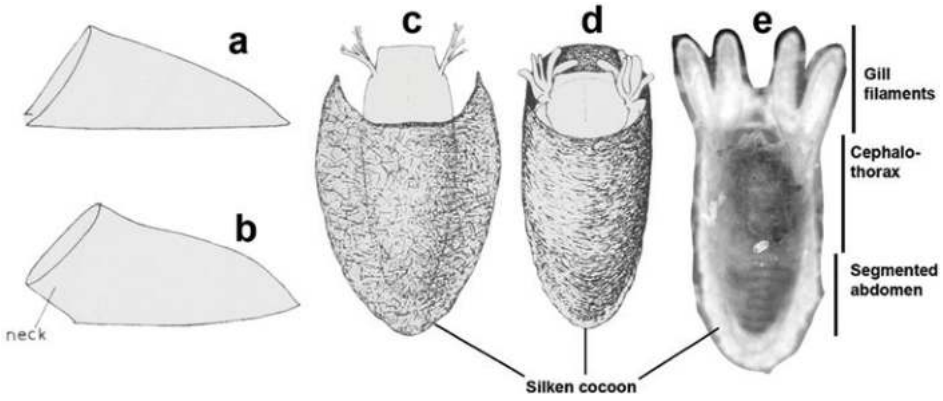
Further structures of diagnostic value include the antennae, mandibles, maxillae, hypostomium, gill spot (bud), anal sclerite and ventral papillae. The size of the last instar larvae varies considerably among species, from 3.4 to 12 mm, most species measuring 5–10 mm.

4.2.3 Pupae of black flies

In most species, the last larval instar spins a **silken cocoon**, in which it pupates. The structure and appearance of the cocoon are very characteristic. They may be slipper- or shoe-shaped (Fig. 4.3 A, B), with an open or closed anterior neck (Fig. 4.3 C, D) but always with the closed caudal end directed upstream and the open end downstream. In general, the colour of the cocoon is pale at first and darkens as the pupa ages. The sizes of black fly pupae vary, also depending on larval size, but they are usually 3–5 mm (without the gills).

The head and thorax of the pupa are combined into a single cephalothorax, and the abdomen is segmented (Fig. 4.3 E). The latter bears tiny spines and hooks, which engage with the threads of the cocoon and retain the pupa in place. The cephalothorax bears a pair of more or less elongated, branched **pupal gills**, which trail downstream of the cocoon. In some species, however, the gills are short and extend barely beyond the lip of the cocoon. Their shapes and branching patterns are very important characters for identification of species (see Table 4.2). Members of the ***S. damnosum*** complex have shoe-shaped, closed cocoons (Fig. 4.3 B, D), whereas species of the ***S. neavei*** group have slipper-shaped, open cocoons (Fig. 4.3 A, C).

Fig. 4.3. Characteristic features of black fly pupae. A, Slipper-shaped cocoon. B, Shoe-shaped cocoon. C, Open cocoon. D, Closed cocoon. E, Original specimen (*S. griseicolle*) with pupal characters.



Sources: A–D modified from reference *50*; E, photograph by A. Krueger

Adults emerge from the pupal case through a slit at the back of the pupal thorax and float to the surface on a bubble of air.

4.2.4 Adult black flies

Adults are small (2–7mm), dark, stout-bodied and "hump-backed" (see Fig. 2.1) and are generally larger than blood-sucking ceratopogonids (biting midges).

Head: The head of an adult black fly has conspicuous **compound eyes**, which exhibit sexual dimorphism (see Fig. 2.2). The eyes of a typical male are contiguous in front (holoptic), whereas the eyes of the female are separated by a broad forehead or "frons" (dichoptic). The **short horn-like antennae** are the same in both sexes and consist of small, disc-like segments, compacted to give a beaded appearance. Unlike other nematoceran flies, the antennae of black flies are covered only with minute, short hairs. Most commonly, there are 11 antennal segments.

Thorax: The typical "hump-backed" appearance of black flies originates from the prominent, elevated dorsal midsection of the thorax, the **mesonotum** (or scutum). In many species, the mesonotum displays characteristic colour patterns or may be covered by metallic silvery or golden scales, but there is pronounced sexual dimorphism. The **wings** are short (1.5–6.0mm), broad, colourless and transparent, with a large anal lobe and characteristic venation (see Figs 4.9 and 4.10 below). The radial veins are well developed along the anterior margin of the wing, with weaker median and cubital veins posteriorly. The radial sector may be unbranched or have two branches. There is a forked submedian fold between the median (M2) and the cubital (Cu1) veins. Despite its weak appearance, the wing is highly efficient, and some simuliid species can fly many kilometers in still air.

Abdomen: The abdomen is made up of eight segments, the last three of which compose the genitalia and are not obvious. The female terminalia (the last two or three visible segments of the abdomen with appendages that serve as mating structures) are compact and relatively inconspicuous. In many black fly species, the dorsal abdominal segments (tergites) bear groups or stripes of silvery or golden scales.

Males differ from females by a larger hump on the thorax, enlarged reddish eyes, different mesonotal patterns and a more slender abdomen, which is tipped by a pair of claspers, which are usually visible.

4.3 How to recognize that the specimens collected are eggs, larvae, pupae and adults of *Simulium damnosum* complex and *Simulium neavei* group and not of other black fly species

4.3.1 Specific identification of *Simulium* larvae

The eggs of one simuliid species cannot be differentiated from those of another.

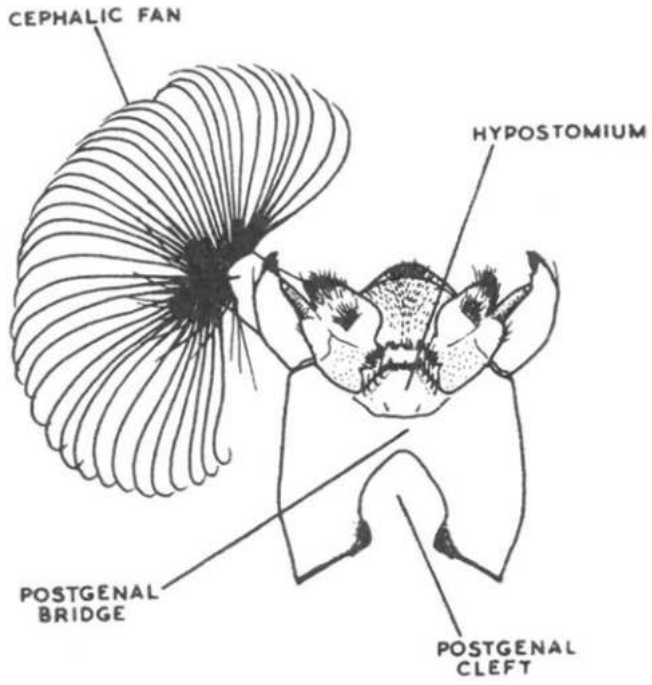
(a) Larval stages (instars)

Although specific identification of simuliid larvae generally applies to "mature" larvae, i.e. 6th–7th instar, it may be necessary to differentiate earlier instars. Fig. 2.3 illustrates the relative size differences of *S. damnosum* larval instars, and Chapter 2 provides a general description of key morphological features for each instar.

(b) Identification of African *Simulium* spp. larvae

Most larval characters vary during development. For example, the number of rays in the cephalic fans (Fig. 4.4), the number of hooklet rows in the proleg circlets (Fig. 4.2A), the number of antennal segments (Fig. 2.3) and the number of secondary lobules in the anal gills all increase as the larva grows. After attainment of the four-segmented condition, the antennal segments continue to change their relative lengths. Two characteristics, however, the form of the **hypostomium** and the shape of the **postgenal cleft**, appear to remain fairly constant during maturation of the larva, although the actual size of these structures naturally increases in successive instars as the head capsule grows.

Fig. 4.4. Characteristics of the larval head used in identification. Ventral view. Only one of the cephalic fans is shown.



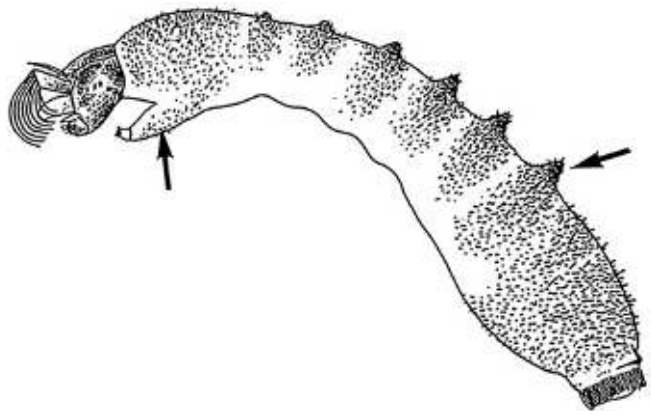
Source: reference *52*

The shape of the postgenal cleft is perhaps the most valuable single character in the taxonomy of African *Simulium* larvae and may be useful in determining the identity of even the smallest larva. With the hypostomium, the postgenal cleft is fundamentally useful in making a correct sub-generic identification if the actual species cannot be determined. The "*neavei*" type of hypostomium (Fig. 4.7) is found only in larvae of the *S. neavei* group species and not in any other African black fly species; thus, it is diagnostic for this group (subgenus *Lewisellum*). If this taxonomic character is seen in very early instar larvae before their attachment to a phoretic host, they can be distinguished from non-*neavei* larvae living in the same habitat.

(c) Unique characteristics of *S. damnosum* larvae

Larvae of *S. damnosum s.l.* are medium-sized and often dark and densely covered with scale-like setae all over the body, in contrast to other African black flies, but the most reliable character for specific identification is the dense vestiture of setae covering its thoracic proleg (Fig. 4.5). Other black fly species may have dense body scales, but no other African species has them on the proleg. *S. damnosum s.l.* also has abdominal dorsal tubercles, which are not found in any other African black fly, but these are sometimes quite small and visible only under a microscope.

Fig. 4.5. Larva of *S. damnosum s.l.* with scales on the thoracic proleg and abdominal dorsal tubercles (arrows).

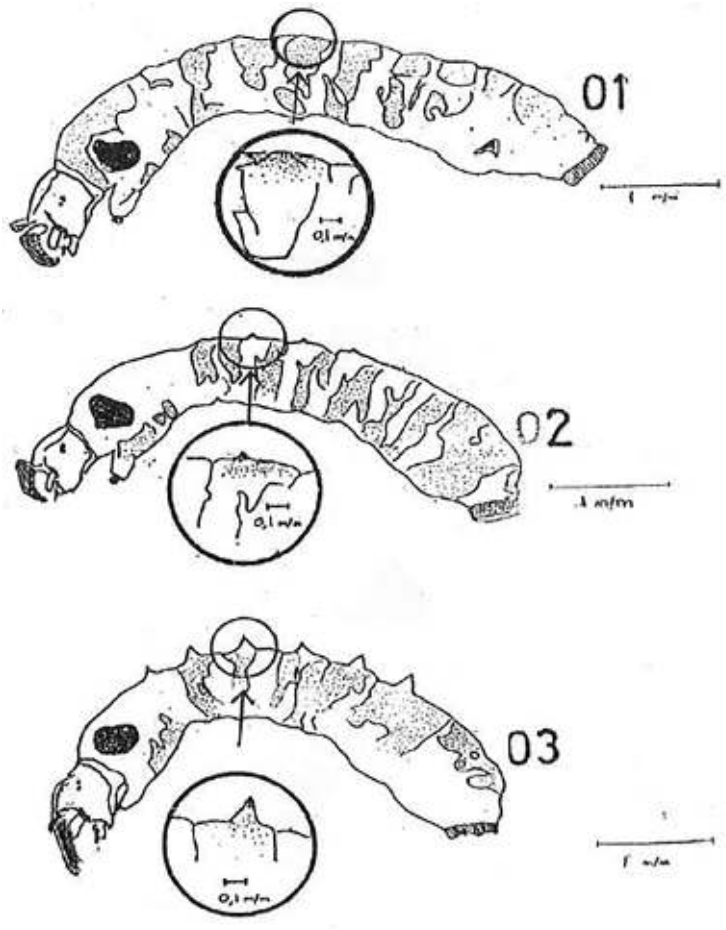


Source: reference *49*

(d) Use of larval dorsal tubercles for identification of cytospecies of *S. damnosum s.l.*

Under certain circumstances, variation in the morphology of the dorsal tubercles has been found useful for identifying some groups of taxa within the subgenus *Edwardsellum*. Fig. 4.6 shows these variants in larvae of *S. sanctipauli/soubrense, S. sirbanum/damnosum s.s.* and *S. squamosum/yahense* groups. This information applies to the West African species, but some taxa, e.g. *S. sanctipauli/soubrense,* cannot be recognized solely by the abdominal tubercles, as other widespread species (e.g. *S. cervicornutum*) may exhibit a similar character. Similar or convergent variants can also be found among East African cytospecies.

Fig. 4.6. WHO OCP classification of *S. damnosum* complex larvae according to variations in the morphology of dorsal tubercles. 01, *S. sanctipauli/soubrense*; 02, *S. sirbanum/damnosums.s.*; 03, *S. squamosum/yahense*.



The following key has been prepared to identify later-stage larvae of African *Simulium* spp. according to Crosskey *(51)*.

Key to later-stage larvae of the principal subgenera of mainland Afrotropical *Simulium*, with anthropophilic species, groups and complexes highlighted

Assessment of some of the characters requires additional slide preparations and use of a compound microscope. There is no universal larval key to all African black fly species.

1. Larvae attached to crabs. Hypostomium with a relatively even row of 13 apical teeth and shaped much as in Fig. 4.7A: ***S. neavei* group (subgenus *Lewisellum*)**

   Larvae not attached to crabs, or hypostomium not the same as in Fig. 4.7A: Go to **2**.

2. Thorax, including proleg and abdomen, with scales or scale-like setae (Fig. 4.5). First five abdominal segments with paired dorsolateral subconical tubercles are particularly covered with setae, making them conspicuous even when smaller than normal: ***S. damnosum* complex (subgenus *Edwardsellum*)**

   Body cuticle usually without such vestiture. If present (*S. albivirgulatum*) with conspicuous simple scales, these do not extend to the proleg. Abdomen without tubercles or slightly produced but without covering of setae: Go to **3**.

3. Larvae attached to mayfly nymphs or river prawns. Hypostomium sometimes of unique form, as in Fig. 4.7B: **subgenus *Phoretomyia*; includes occasional man-biting *S. dukei***

   Larvae not attached to phoretic hosts. Hypostomium not of this form (Fig. 4.7C, D): Go to **4**.

4. Thoracic and abdominal cuticle with one type of flattened scale-like setae (Fig. 4.7E). Postgenal cleft elongated, subelliptical and not strongly rounded (Fig. 4.7F). Vector in Congo Basin: ***S. albivirgulatum* (subgenus *Metomphalus*)**

   Cuticle bare or with different vestiture; if abundant setae present on cuticle of both thorax and abdomen, then minute hair-like or fan-shaped. Postgenal cleft different: Go to **5**.

5. Dorsal cuticle with minute, erect fan-shaped scales **and** simple spine-like setae, and abdomen without ventral papillae: **subgenus *Byssodon*; includes occasional man-biting species, *S. griseicolle***

   Cuticle either without deeply divided fan-shaped setae, or, if present, lying mainly recumbent **and** abdomen with ventral papillae: Go to **6**.

6. Ventral papillae present, sometimes small and rounded: Go to **7**.

   Ventral papillae absent: Go to **9**.

7. Head capsule with distinct positive pattern of dark head spots: **Subgenus *Nevermannia***

   Head pattern negative with pale head spots surrounded by variable darker pigmentation, or, if with dark head spots, abdomen without ventral papillae: Go to **8**.

8. Postgenal cleft large and rounded. Ventral papillae sometimes rounded and small, relatively inconspicuous: **subgenus *Meilloniellum*; includes occasional man-biting species, *S. adersi***

   Postgenal cleft not of this form. Ventral papillae subconical, well developed: **subgenus *Pomeroyellum***

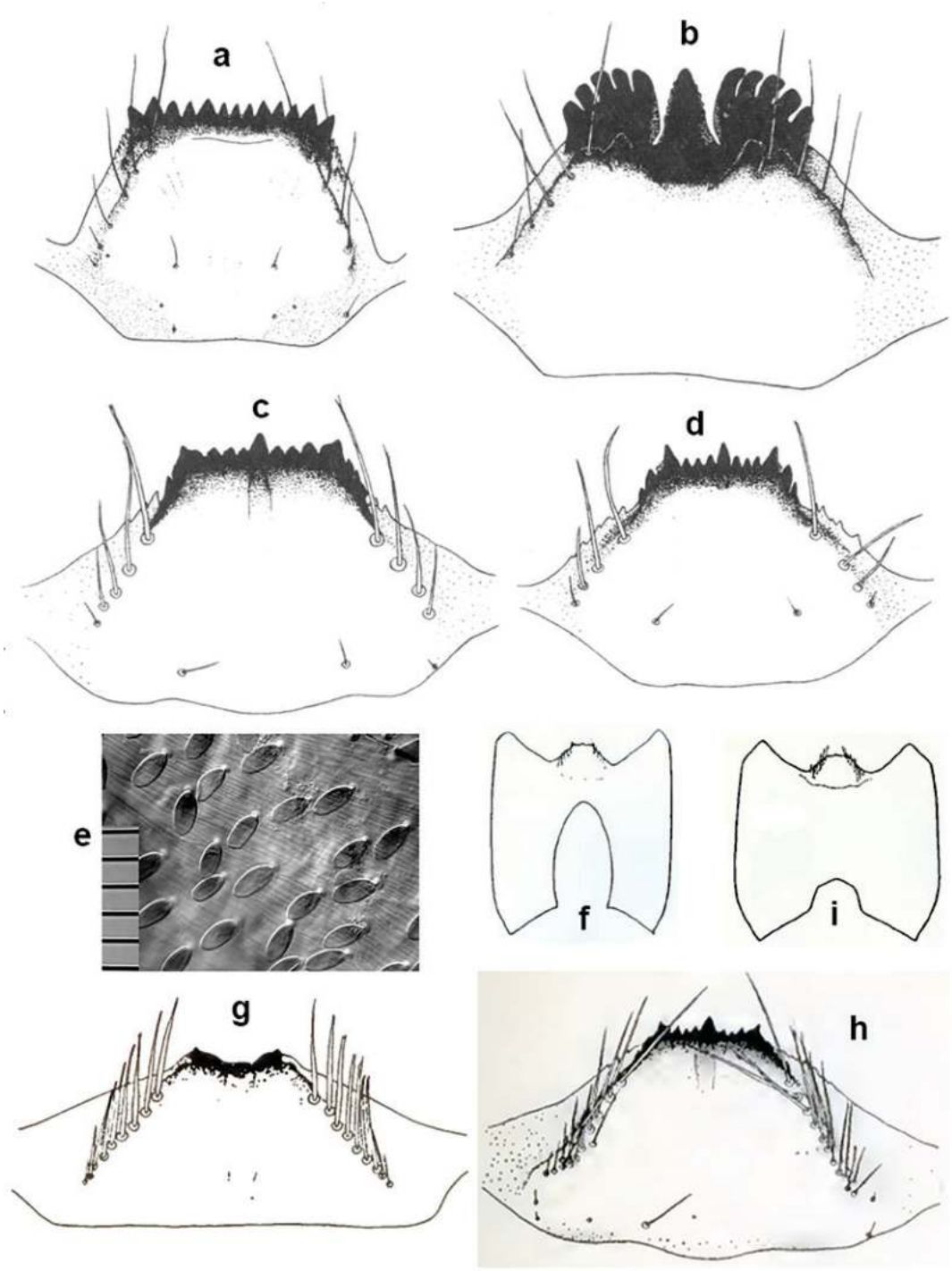
9. Abdominal cuticle bare. Sides of hypostomium (outside the rows of setae) broadly expanded and hypostomial teeth very reduced (Fig. 4.7G): **subgenus *Freemanellum***

   Abdominal cuticle, at least posterodorsally, with small scales or minute, scattered, simple setae. Sides of hypostomium less widely dilated, hypostomial teeth less reduced (Fig. 4.7H): Go to **10**.

10. Postgenal cleft small, much shorter than postgenal bridge and forming a subquadrate notch (Fig. 4.7i). Rectal gills always with many secondary lobes: **subgenus *Anasolen*; includes occasional man-biting species, *S. dentulosum***

    Postgenal cleft large and postgenal bridge shorter than the cleft. Rectal gills simple or compound: **subgenus *Metomphalus*** (excluding *S. albivirgulatum*); **includes occasional man-biting species, *S. bovis***

Fig. 4.7. Distinguishing characteristics of three structures of African *Simulium* larvae used in identification. A, the type of hypostomium found only in *S. neavei* and its allies. B, the unique hypostomium of *S. berneri* (subgenus *Phoretomyia*). C and D, the type of hypostomium in the vast majority of *Simulium* species. E and F, cuticular scales and postgenal cleft of *S. albivirgulatum*. G, hypostomium of subgenus *Freemanellum*. H and I, hypostomium and postgenal cleft of subgenus *Anasolen*.



Sources: A–D and F–I, references *50*, *51*; E, photograph by A. Krueger

Note: Images of unknown larvae (or pupae) made under a dissecting microscope with the camera of a smartphone or digital camera provide a permanent record for a local inventory of collected material. Such images may also be sent electronically to specialists for further evaluation and possible identification. Photographic equipment should fit closely onto the eyepiece of a dissecting scope and a series of images – lateral, dorsal, ventral – made for each unknown specimen.

4.3.2 Specific identification of *Simulium* pupae

The length and shape, number and branches or arrangement of the pupal respiratory filaments (gills) are characteristic of many species and provide useful taxonomic criteria for identification (Table 4.2).

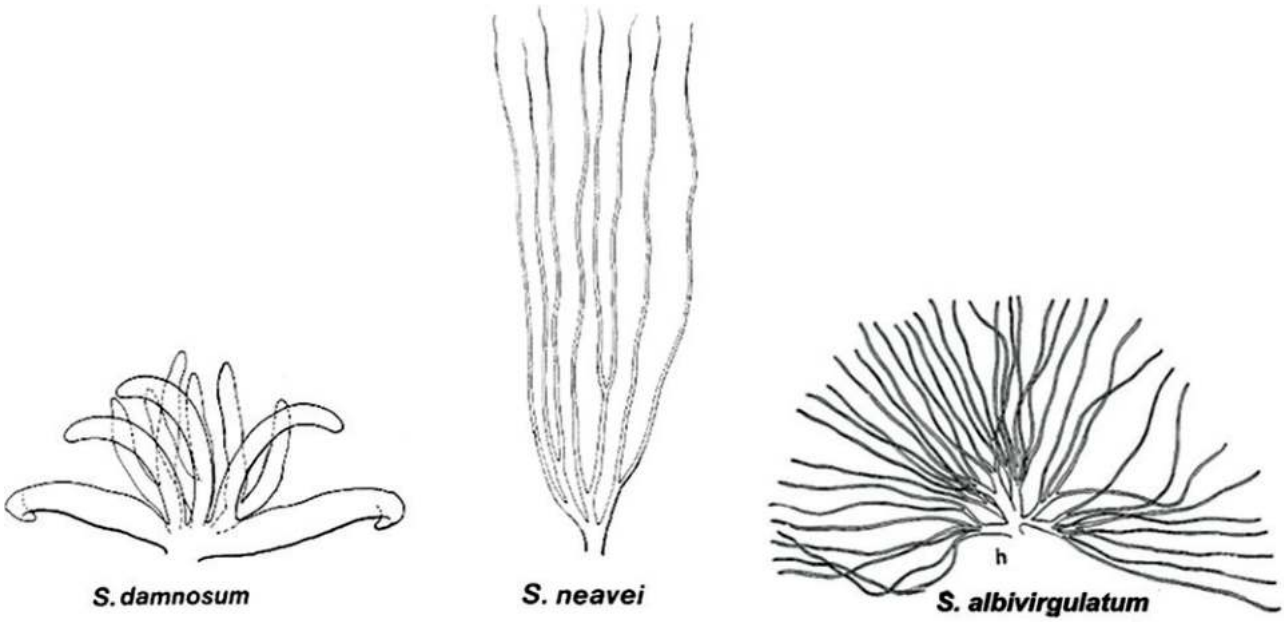


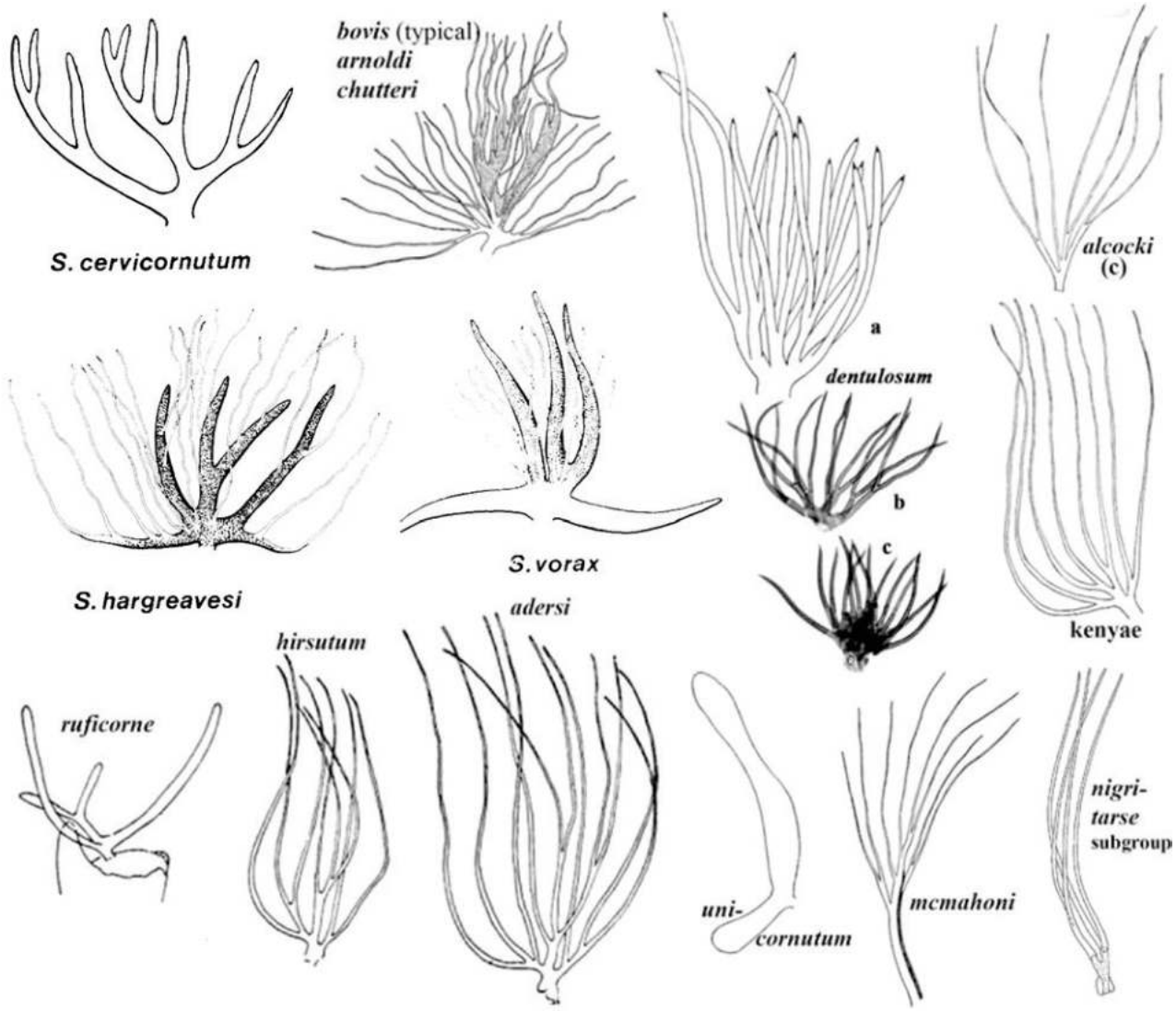
Table 4.2. Key biological features and geographical distribution of pupae of African *Simulium* species that are vectors of *O. volvulus*.

Number and order of gill filaments	11: 1 pair of broad, thin-walled, basal arms; secondary series of six upright filaments (outer three banana-shaped; inner three each with a tertiary branch)	Eight long, thin filaments	25–40: in groups of four or eight, each group with a short common stalk
Geography	Sub-Saharan Africa	Mainly forests in East and Central Africa	Congo Basin and southern Africa
Habitat	Attached on trailing vegetation	Phoretic on crabs	Attached on trailing vegetation

Source: reference *51*

Fig. 4.8 shows examples of pupal gills of common non-vector species mentioned in the larval taxonomic key. A key to pupae of all Afrotropical species of *Simulium* in onchocerciasis-endemic countries can be found in Annex1. Illustrations of African simuliid pupal respiratory filaments (gills) are provided in Annex 2.

Fig. 4.8. Examples of pupal gills of other common non-vector Afrotropical *Simulium* species.



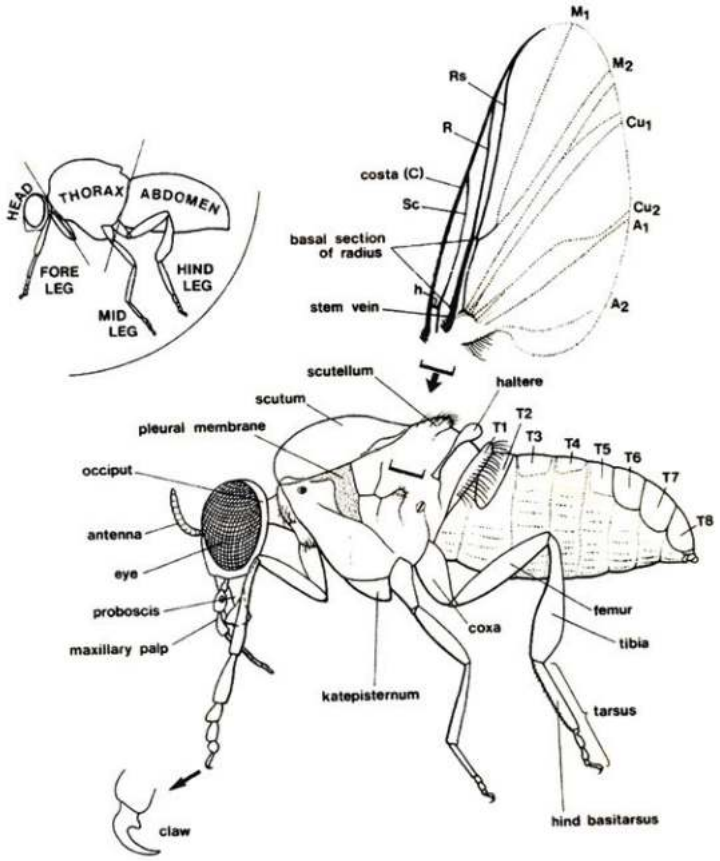
Sources: Illustrations have been re-drawn from various sources.

4.3.3 Morphological characteristics used to identify adult female black flies attracted to humans in Africa

(a) Adult morphological features used in the identification of adult female *Simulium* spp.

Figs 4.9–4.13 illustrate the general morphological features of adult female flies used to identify *Simulium* spp., including *S. damnosum s.l. (50).*

Fig. 4.9. General morphological features of an adult, female black fly.



Wing veins are C, costa; Sc, subcostal; R, radius; Rs, radius sector; M_1, medius anterior; M_2, medius posterior; Cu_1, cubitus anterior; Cu_2, cubitus posterior; A_1, anal anterior; A_2, anal posterior

Figs 4.10–4.13. Illustrations are reproduced with permission from several sources, particularly references *1*, *50*, *51* and *53*.

Fig. 4.10. Lateral view of adult female *S. damnosum s.l.* showing swollen foretarsi (FT), banded hind basitarsus (BT), clumps of scales on sides of abdomen (SA), and position of the pleural membrane (PM) and basal section of vein R (VR).

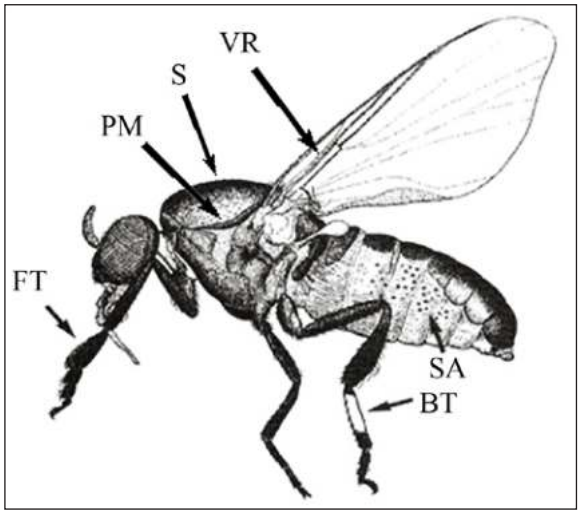


Fig. 4.11a. Front leg of *S. damnosum s.l.* showing swollen foretarsi in relation to three other species.

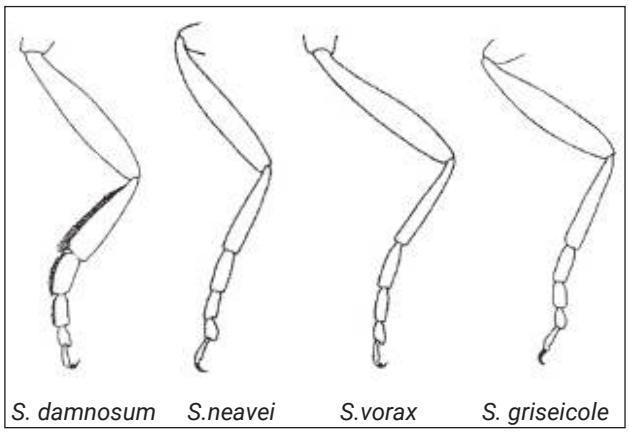


Fig. 4.11b. Examples of three-lined patterns on thoracic scutum (dorsal view with anterior end at top).

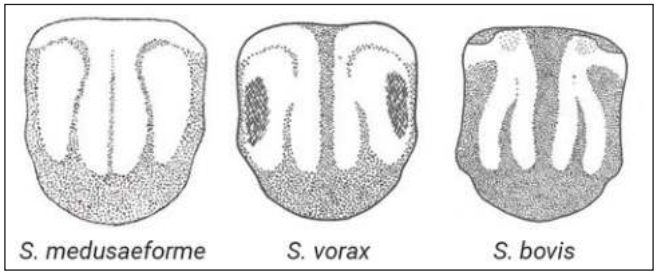


Fig. 4.12. Showing basal section of Radius (vein R).

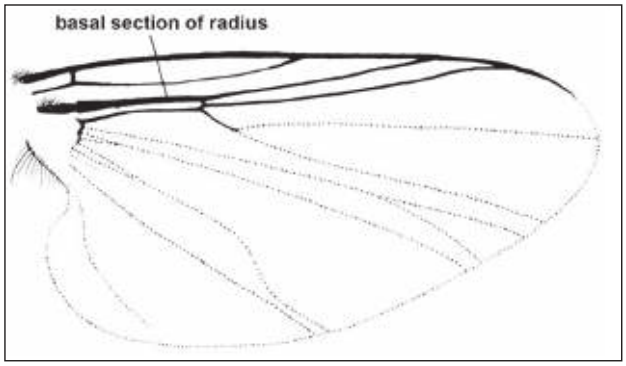
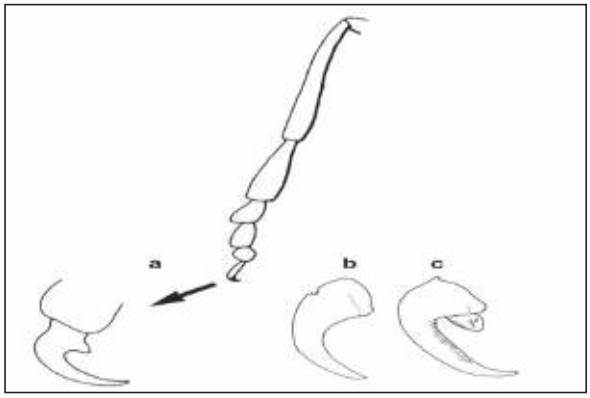


Fig. 4.13. Position of claw at end of leg (a), and size of basal tooth.a – Basal tooth small (*S. damnosum s.l.*). b – Basal tooth absent (*S. hargreavesi*). c - Large basal tooth (*S. hirsutum*).



(b) *Simulium* species in African countries in which onchocerciasis is endemic

As adult female black flies represent the most relevant life stage for an onchocerciasis programme, the user of this manual should first be able to recognize those species that are known vectors of *O. volvulus*, i.e. members of the *S. damnosum* complex, the *S. neavei* group and *S. albivirgulatum*. Table 4.3 summarizes the species commonly attracted to humans in Africa, followed by an identification key.

Table 4.3. Species of *Simulium* commonly attracted to humans in Africa.

Subgenus	Morphospecies	Notes on anthropophily and distribution
Edwardsellum	*S. damnosum* complex	Comprises at least 55 cytospecies and cytoforms, most of which do not bite humans
Lewisellum	*S. neavei* group	Contains three vector species, *S. neavei* (Uganda, eastern Democratic Republic of the Congo), *S. woodi* (United Republic of Tanzania) and *S. ethiopiense* (Ethiopia)
	S. ovazzae	Mostly in Cameroon savannah
Anasolen	*S. dentulosum*	Anthropophilic in Democratic Republic of the Congo, Ethiopia and Rwanda (possibly a vector in Haute Ituri focus in the Democratic Republic of the Congo)
	S. shoae	Ethiopia
Byssodon	*S. griseicolle*	Anthropophilic but rarely bites in northern Nigeria, South Sudan and Sudan
Meilloniellum	*S. adersi*	Mainly in northern savannah of West Africa
Metomphalus	*S. albivirgulatum*	Only regularly anthropophilic in Zambia and in central Democratic Republic of the Congo (where it is a vector)
	S. bovis	Only recorded as regularly anthropophilic in central Nigeria, northern Uganda and northwest Ethiopia
	S. chutteri	Anthropophilic in South Africa, but does not bite. Not in onchocerciasis-endemic countries
	S. gibense	Ethiopia
	S. vorax	In association with cattle, generally in the Haute Ituri focus in the Democratic Republic of the Congo
	S. wellmanni	Angola
Phoretomyia	*S. dukei*	Cameroon forest

Key to *Simulium* spp. females commonly attracted to humans in tropical Africa

In most circumstances, it is impossible to identify adults of some species in the field, because some of the character assessments require use of a dissection microscope. For a complete key to all African species, see Freeman & de Meillon *(53)*.

1. All legs with entirely black cuticle (although there may be some silver or gold scales), except for the hind basitarsus, which has a very conspicuous creamy-white broad band. Also, foretarsi broadly expanded and flattened (Fig. 4.10) and silvery lateral abdominal scales in clumps: ***S. damnosum s.l.***

   Legs not like this (except subgenus *Phoretomyia*, which may have expanded foretarsi) and abdominal scales not clumped. Go to **2**.

2. Scutum of thorax with three dark longitudinal lines on cuticle (which may be obscured by scales in some young specimens and are best seen under different directions of lighting as the fly is turned) (Fig. 4.11B). Go to **3**.

   Cuticle of scutum without three-lined pattern (usually without any obvious pattern, although there may be some coloured scales or hairs). Go to **8**.

3. Wing with basal section of vein R finely haired along its length (see Fig. 4.12) and claws without basal tooth (Fig. 4.13A). Go to **4**.

   Basal section of wing vein R b are and claws with a basal tooth (Fig. 4.13C): ***S. griseicolle***

4. Pleural membrane with hairs (often a small group in the anterior dorsal corner, which can be rubbed off in older specimens) (see Fig. 4.9). Go to **5**.

   Pleural membrane without hairs. Go to **6**.

5. Scutum grey with black, lyre-shaped, narrow three-lined pattern with narrow lines (see Fig. 4.11A). Found primarily in the Congo basin: ***S. albivirgulatum***

   Scutum grey with black three-lined pattern with broad lines (see Fig. 4.11C). Occurs in Ethiopia: ***S. gibense***

6. Antennae and legs entirely black: ***S. wellmanni***

   Base of antennae paler than black, and legs not entirely black. Go to **7**.

7. Three-lined pattern on scutum with oval brownish areas lateral to outer black stripes (Fig. 4.11B), large species (wing length > 2.5 mm: ***S. vorax***

   Usually without lateral brown patches (Fig. 4.11C), small species (wing length < 2.5 mm): ***S. bovis***

8. Pleural membrane with hairs (maybe a small group in the anterior dorsal corner, which can be rubbed off in older specimens) (see Fig. 4.9). Go to **9**.

   Pleural membrane without hairs. Go to **10**.

9. Claw with large basal tooth (Fig. 4.13C): ***S. adersi***

   Claw with small or minute basal tooth (Fig. 4.13B): ***S. dentulosum*** (and ***S. shoa*** in Ethiopia)

10. Abdomen with hairs mostly black, yellow only at base and/or on mid-lateral parts. Legs entirely black. Go to **11**.

    Abdomen with hairs mostly silvery yellow or golden (sometimes coppery or bronze on middle segments). Legs black or dark brown with basal parts of hind basitarsus dingy or reddish yellow (and some other parts sometimes faintly paler). Go to **12**.

11. Very dark species with hair of frons, clypeus, scutellum and hind part of scutum black or bronze–black. Abdomen with yellow hair only on first two segments: ***S. dukei***

    Paler species with hair of frons, clypeus, much of scutellum and all of scutum silvery yellow to pale golden. Abdomen with yellow hair on first two segments and mid-laterally (sides on segments 3–6): ***S. ovazzæ***

12. Abdominal hairs unicoloured silvery yellow or golden. Go to **13**.

    Abdominal hair not unicoloured silvery yellow or golden, darker coppery or bronze on middle segments: ***S. woodi***

13. Legs black except for indistinct dingy yellow bases to hind basitarsi (and these not definitely pale-banded): ***S. neavei***

    Legs clearly not uniformly black, mid part of each tibia and hind femoral/tibial "knees" dingy reddish orange or brownish; hind basitarsi distinctly reddish yellow on basal two thirds or so (and fairly definitely pale-banded): ***S. ethiopiense***

Source: Updated in part from reference *1*

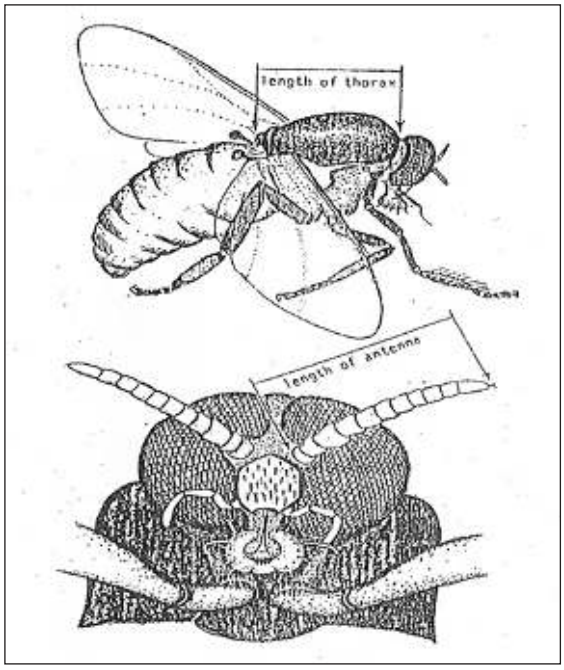
(c) Identification of different cytospecies of female adult *Simulium damnosum s.l.*

Once *S. damnosum s.l.* has been identified with the above key, common *S. damnosum* cytospecies can be identified by assessing:

- the ratio of the length of thorax and antenna and other antennal morphological features,
- the colour of the wing tufts (and post-cranial hairs and coxae) and
- the morphology of the forelegs.

(i) *Use of antennal characters in West Africa:* Features of the antennae (such as the colour of segments, length relative to the length of the thorax; see Fig. 4.14) and compression of the antennal segments vary among the West African cytospecies sub-complexes (Table 4.4).

Fig. 4.14. Practical method for measuring the length of the thorax and antenna of *S. damnosum s.l.*



Source: reference *54*

Table 4.4. Features of antennae for identification of adult *S. damnosum* complex in West Africa.

Character	*S. damnosum/sirbanum*	*S. squamosum/yahense*	*S. soubrense/sanctipauli*
Compression of antennal segments	 Segments 4 and 5 always compressed, often with other segments	 Segment 4 (and 5) distinctly compressed	 Very seldom compressed
Colour of antennae	First four segments usually pale	First two or three segments pale	All segments dark
Antennae:thorax ratio	2.1–2.4 (median, 2.3)	2.04–2.2 (median, 2.15)	1.8–2.04 (median, 1.96)

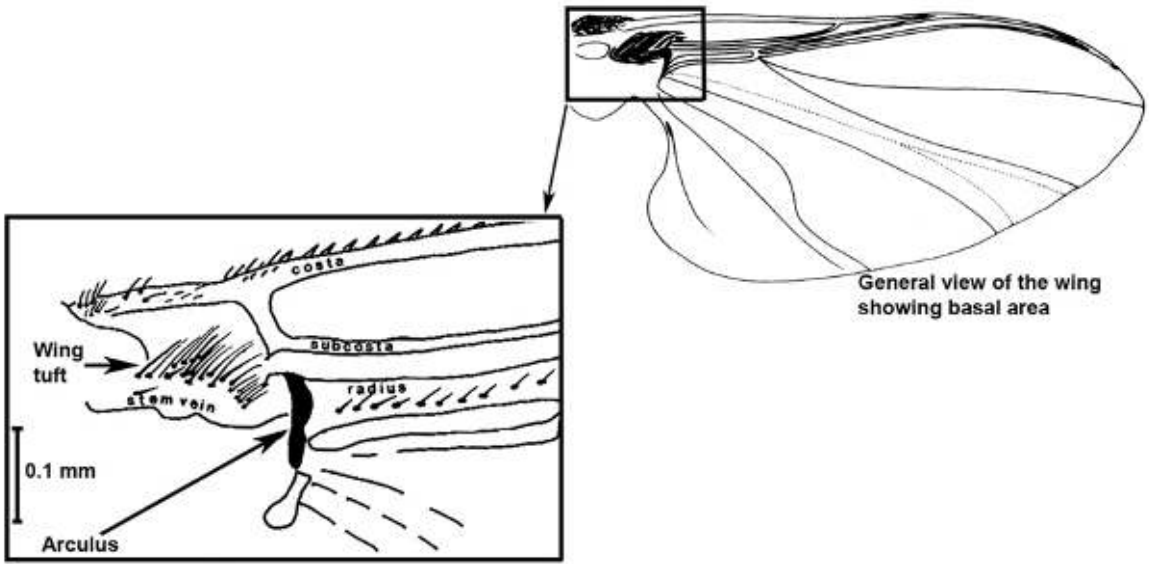
Source: reference *54*

In order to measure the lengths of antennae and thoraxes under a standard stereo-microscope, an ocular (eyepiece) micrometre should be used.

(ii) Morphological identification of females of S. damnosum s.l. only by colour characters: The OCP developed a simplified system for identification of the cytospecies of adult female *S. damnosum s.l.* in West Africa that remains useful. The system is based on the colours of various external structures and does not require difficult measurements (such as the antennal:thorax ratio described in this section) or subjective assessment of the shape of antennal segments. Although limited to the area covered by the former OCP and partially outdated, the pictorial keys provided by Dang & Peterson *(55)* are useful for handling specimens. Morphological identification requires examination of dry specimens under a stereo-microscope (dissecting microscope) at no less than 40x magnification, for the colour of the:

- setae of the wing tuft (see Fig. 4.15),
- antenna,
- first article of the foreleg (the forecoxa),
- arculus (near the base of wing; see figures below),
- setae of the scutellum and
- the 9th abdominal tergite.

Fig. 4.15. Position of the wing tufts and arculus in adult *S. damnosum s.l.*



Source: reference *54*

Each hair in the tuft is classified as pale or dark, and the tuft is classified as: 01, all hairs pale; 02, ≤ 5 dark hairs; 03, mixed; 04, ≤ 5 pale hairs; 05, all hairs dark. The arculus is the dark structure shown just distal to the stem vein (i.e. to the right of the wing tuft in the diagram).

The key identification criteria are as follows.

Savannah group (*S. damnosum s.s., S. sirbanum*):

- forecoxa paler than the thorax,
- pale antennae,
- pale wing tufts (categories 01 and 02),
- pale arculus,

- setae on the scutellum pale and
- setae of the 9th abdominal tergite pale.

Forest group I (*S. yahense*):

- forecoxa as dark as the thorax,
- antenna dark but first article generally pale,
- dark wing tufts (categories 04 and 05),
- dark arculus,
- dark setae of the scutellum and
- dark setae of the 9th abdominal tergite.

Forest group II (*S. squamosum, S. sanctipauli, S. soubrense*)

- forecoxa as dark as the thorax,
- dark antenna, but articles 1 to 9 generally pale,
- dark (categories 04 and 05), but sometimes pale or mixed wing tufts in *S. squamosum* (categories 01–05),
- brown arculus,
- pale or mixed setae on the scutellum and
- pale or mixed setae of the 9th abdominal tergite.

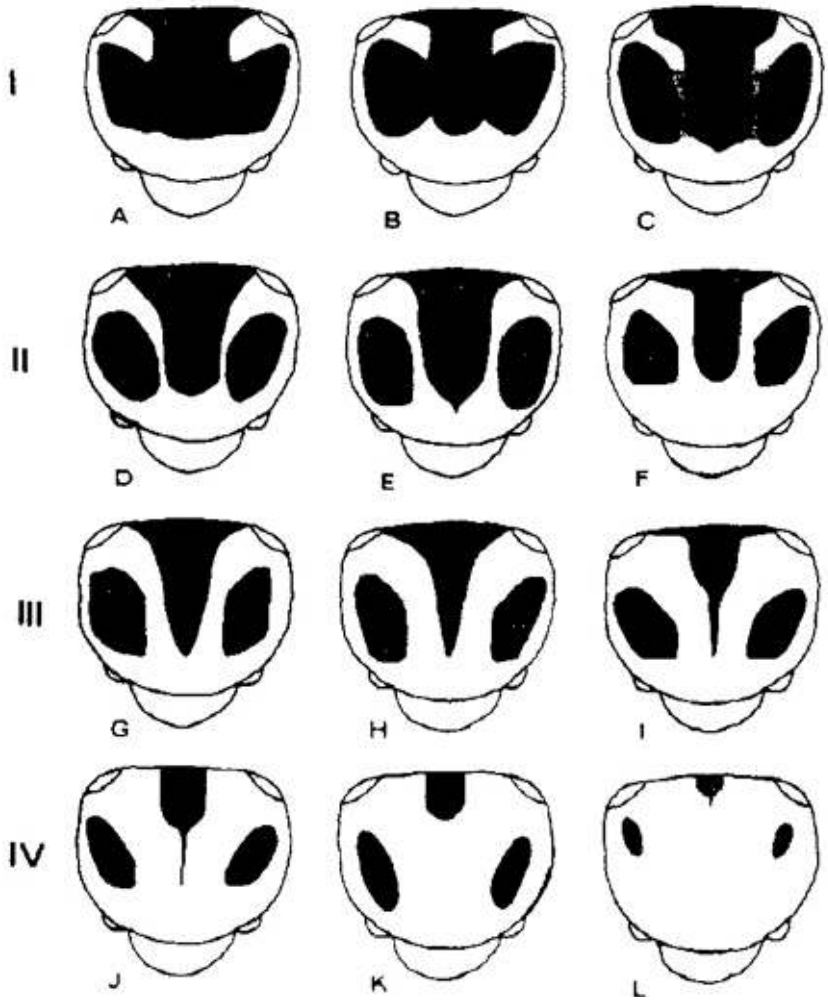
Morphometry sometimes makes it possible to further identify specimens into cytospecies (see *56*).

- In the *damnosum* sub-complex*, S. damnosum s.s.* and *S. sirbanum* may be distinguished from one another in 80–90% of the cases by comparing measurements of the thorax, antenna, basitarsi and femur.
- In the forest group, *S. squamosum s.s.* may be distinguished from the species of the *sanctipauli* sub-complex in 96–100% of the cases by comparing the measurements of the antenna and the tibia.
- In the *sanctipauli* sub-complex, *S. sanctipauli* may be distinguished from the other forms of the complex in 85–90% of the cases by comparing the measurements of the femur, the tibia, the basitarsus, the antenna and the thorax.

(iii) S. damnosum s.l. in East Africa: Females of most of the East African cytospecies have pale wing tufts (categories 01–02), except a few cytospecies which have dark, mixed or variable wing tufts *(57)*.

(iv) The mesonotal pattern of S. damnosum s.l. males: Under certain circumstances, this character has been found useful for characterizing and separating some cytospecies. Males reared from pupae in both West and East Africa can sometimes be identified by a combination of the mesonotal pattern (Fig. 4.16) and wing tuft colour *(57, 58)*. While there is usually little point in trying to identify males, this can help to identify the cytotype of species breeding at a particular location if cytotaxonomic identification of larvae cannot be obtained.

Fig. 4.16. Examples of the mesonotal patterns of male *S. damnosum s.l.*, separated into four major types, each with three representative variants.



Source: reference *58*

Chapter 5.

Selection of study sites and community mobilization for entomological evaluation

Learning outcomes for Chapter 5

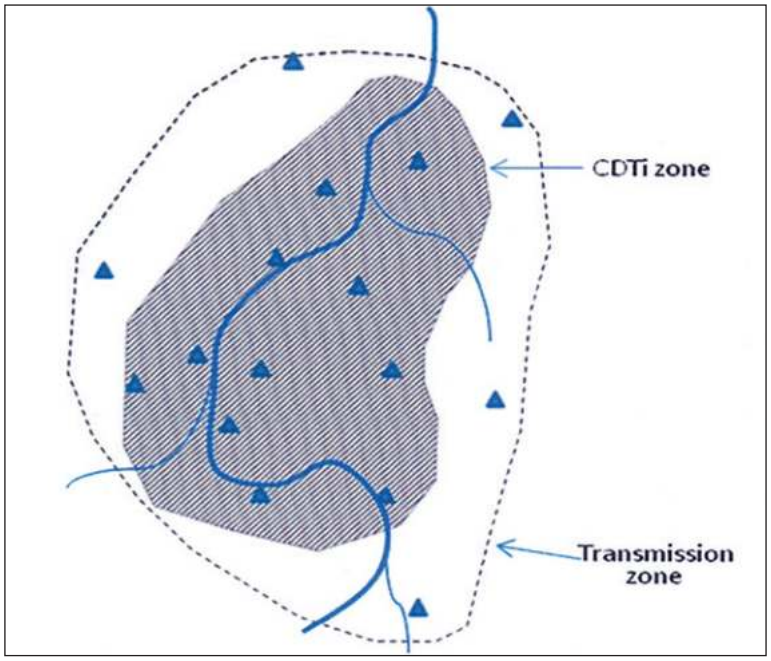
By the end of this chapter, the reader should be able to:

- review the results of any previous entomological evaluations;
- determine the team composition for selection of entomological sites;
- properly select entomological evaluation sites;
- know the critical aspects of promoting community mobilization; and
- appreciate community-directed distributors (CDDs) as an outcome of community mobilization.

5.1 Introduction

In order to determine where *O. volvulus* transmission has been interrupted or eliminated, the APOC introduced the concept of a transmission zone, defined as a ''geographical area where transmission of *Onchocerca volvulus* occurs by locally breeding vectors which can be regarded as a natural ecological and epidemiological unit for intervention". According to APOC, the core of a typical transmission zone (Fig. 5.1) is a river with vector breeding sites, endemic communities located close to the river and levels of infection falling with distance from the breeding sites until they become negligible or overlap with those in another transmission zone.

Fig. 5.1. Scheme of an onchocerciasis transmission zone.

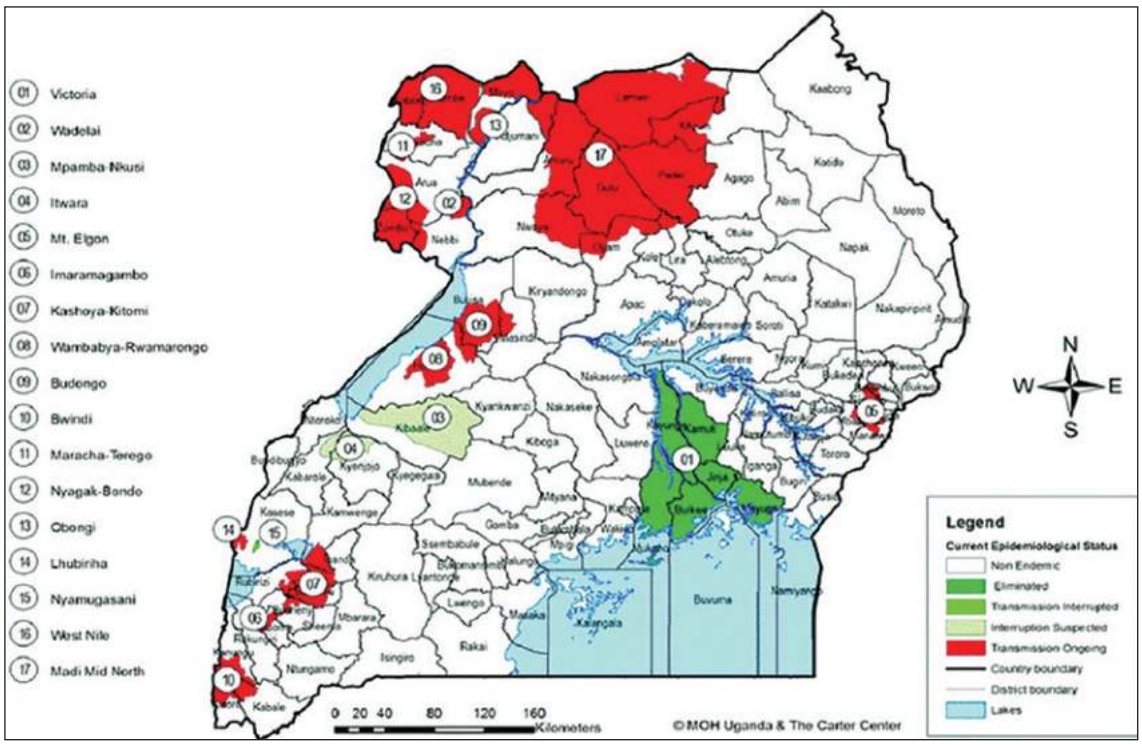


Source: reference *33*

Note: During the control era with ivermectin MDA, the treatment zone (CDTi zone) did not always cover the entire transmission zone. In the elimination era, because of the often extreme flight capability of *Simulium* vectors, the goal is to treat everyone in the transmission zone. The blue triangles represent villages.

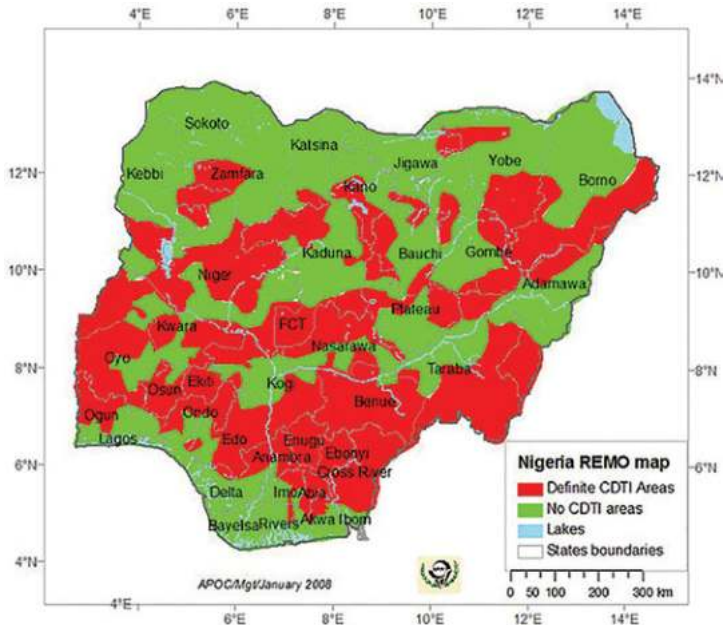
In some countries, such as Uganda (Fig. 5.2), onchocerciasis is clearly distributed in distinct, discontinuous transmission zones, with gaps where transmission of the parasite is absent (buffer zones).

Fig. 5.2. Map of Uganda showing clear distribution of discontinuous onchocerciasis transmission zones (TZ) with gaps between them where *O. volvulus* transmission is absent (buffer zones).



In other countries, such as Nigeria (Fig. 5.3), onchocerciasis is distributed continuously over wide areas because of the common occurrence of contiguous larval habitats. In these cases, strict separation of transmission zones is challenging, and river basins that represent political boundaries may be used as surrogates for transmission zones. Transmission is considered to be widespread rather than limited by local ecological conditions. When political boundaries are used to define the scope of a transmission zone, the programme must account for the possibility of parasite transmission due to movement of vectors across borders. Therefore, in defining a transmission zone in an endemic country, the extent to which parasites move from one area to another by both migrating vectors and infected humans must be considered.

Fig. 5.3. Map of Nigeria showing continuous distribution of onchocerciasis (in red) over very wide areas.



CDTI, community-directed treatment with ivermectin

The intensity and distribution of transmission of *O. volvulus* depend on the ecology of the transmission zone (e.g. the location of breeding sites, contacts between breeding sites and human populations) and the behaviour of the black fly vector. Thus, entomological evaluations should be conducted close to breeding sites and not at random locations throughout the transmission zone. Most breeding sites are near rapids or fast-moving water (Chapter 3), although, as discussed in previous chapters, there are exceptions.

This approach to evaluation is based on the fact that the vector flies have highly specific breeding-site requirements, and older parous flies, which are the most important transmitters of the parasite, have a more limited flight range. Use of appropriate topographical maps will help the team to select catching sites and villages most likely to be seriously affected by onchocerciasis.

5.2 Basis for selecting sites for entomological evaluation

Selection of entomological sites consists of:

- identification of river systems,
- delimitation of transmission zones along the river system of interest and
- exclusion of empty and unsuitable areas.

Unsuitable areas include those likely to be free of onchocerciasis because there are no resident human populations or rivers or because they are totally unsuitable for breeding of black fly vectors. Unsuitable areas include deserts, river deltas (because of the slow flow of water), national parks and game reserves and densely forested areas lacking human habitation.

5.3 Team composition

In initial planning for site selection, two to four people with as much relevant knowledge as possible are necessary. Ideally, a site selection team should comprise:

- an entomologist with many years of field experience of *Simulium* vector species,
- an epidemiologist with good understanding of *O. volvulus* transmission dynamics,
- a geographer with good knowledge of the physical features of the study area and
- a local field assistant (guide).

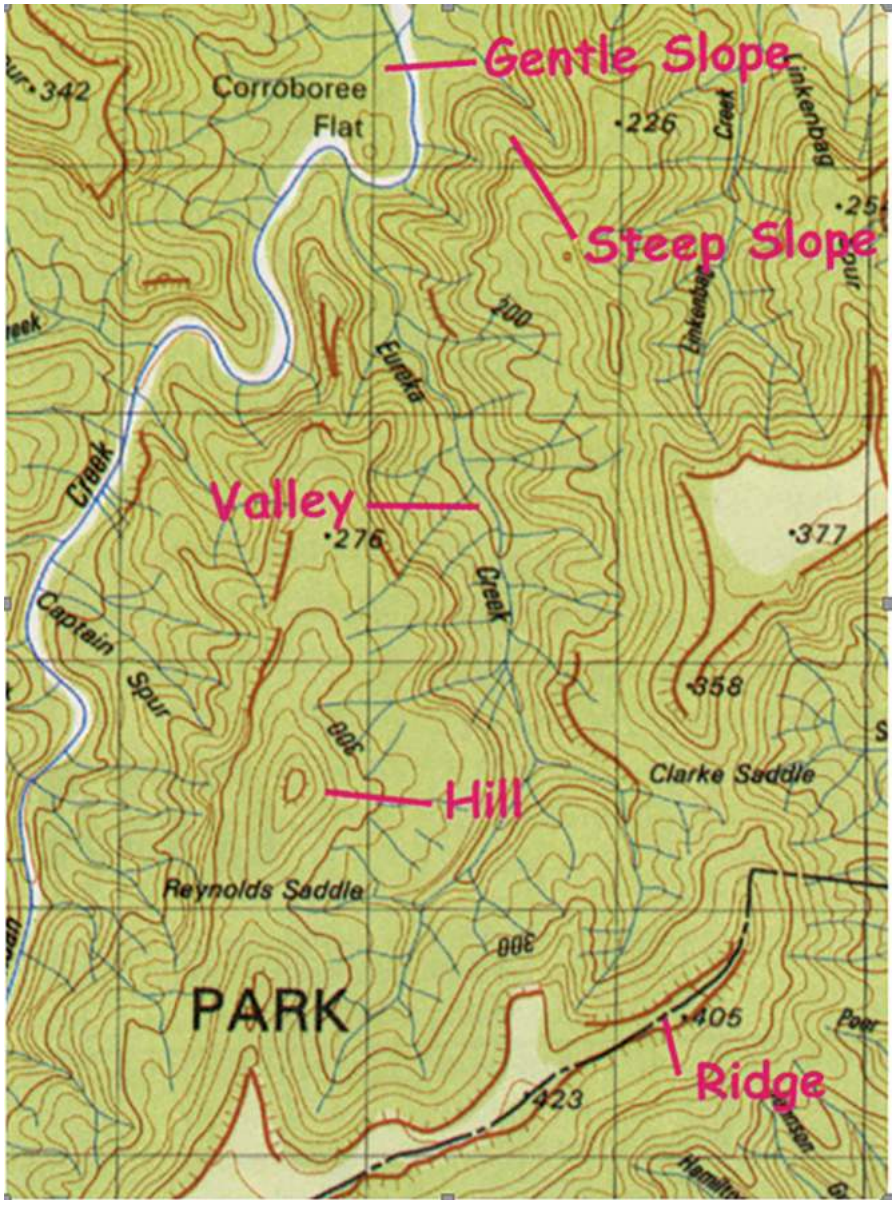
If the programme does not have direct access to such specialists, the programme coordinators should look beyond the ministry of health to local university entomologists and qualified biologists in the programmes of other countries.

For this exercise, the site selection team should use a regional atlas that provides information on hydrology, geology, climate, vegetation and demographics, as well as standard topographical maps.

5.4 Selection of sites for entomological evaluation

The team for selecting entomological sites should work with the best available topographical maps that provide accurate representation of the rivers, the relief of the region and the names of villages and communities. Topographical maps at a scale of 1:200 000 are probably best for this purpose, and such maps are available in most Francophone African countries. In Anglophone African countries, topographical maps at a scale of 1:250 000 or 1:50 000 that convey the necessary information are usually available. Any topographical map that contains the necessary information may be used. Fig. 5.4 shows the detail seen in an elevation map with contour lines. The closer the lines are, the steeper the landscape. Note the general features (gentle slope, steep slope, valley, hill, ridge), which are indicated by changes in contours.

Fig. 5.4. A 1:50 000 scale map. The contour intervals are 20 m.



Source: Australian Intergovernmental Committee on Surveying and Mapping

Fig. 5.5. Example of entomological sites selected on a river system in Plateau State of Nigeria.

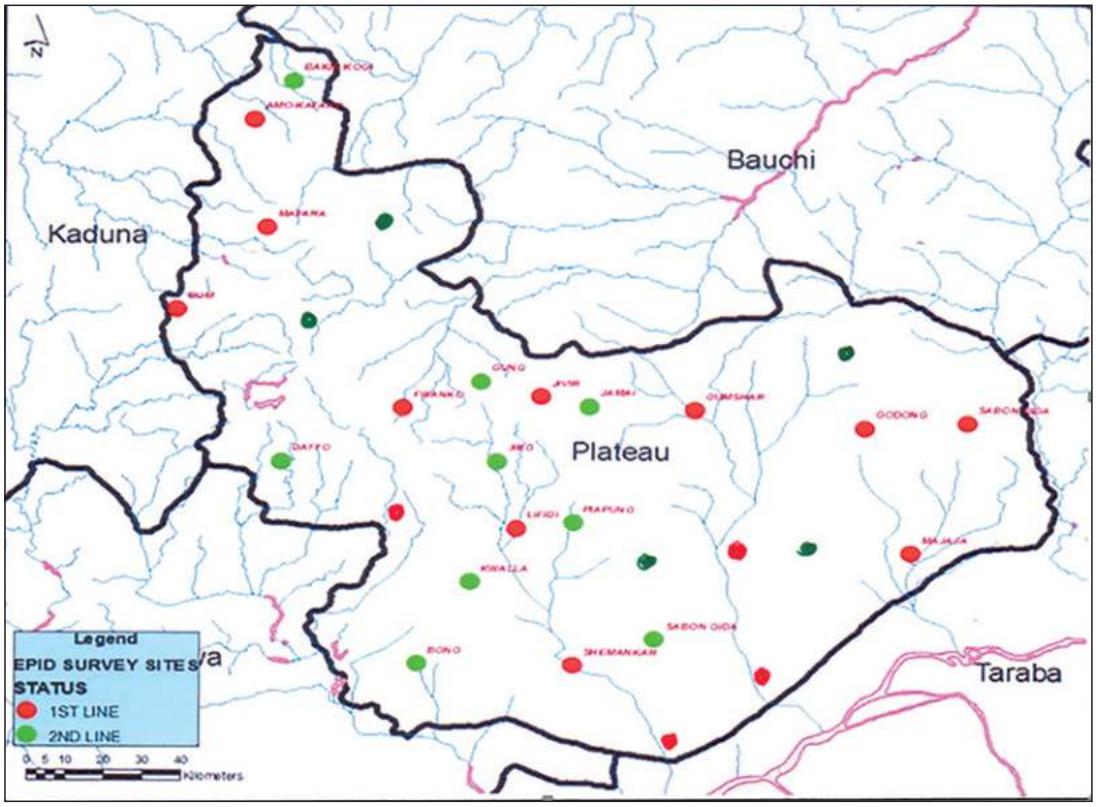


Fig. 5.5 shows survey sites selected on a river system in Nigeria. A first-line village has no settlements between it and the nearest vector breeding site and is hyper- or mesoendemic for onchocerciasis. Second-line villages are located at least 10 km farther from the main breeding site.

As noted earlier, in order to make a good selection of sites, preliminary desk work is required, which includes finding:

- published works on the prevalence and other epidemiology of onchocerciasis in the area;
- known rivers in the area, historical breeding sites and previous catching sites; and
- other important data on the epidemiological and entomological aspects of the disease in the area, such as ABRs and transmission indices.

Practically, for selecting villages and communities, the team should first identify a first-line village, i.e. one that has no other settlements between it and the nearest vector breeding site (≤ 10 km). Then, a second-line village should be identified within a 15–20-km radius to the right and to the left of the first site for further entomological evaluation (both on the main river and on tributaries). In making these selections, it must be remembered that black fly vectors of *O. volvulus* breed mainly in fast-flowing, well-oxygenated rivers and streams. This crucial factor limits the vector population to villages near river banks. In onchocerciasis elimination, the selection of fly-catching sites for xenomonitoring of black flies to determine appropriate parameters for elimination should be influenced by the location of first-line communities.

The important criteria for selecting first-line (high-risk) communities are thus:

- The first-line community should be located close to known breeding sites (< 10 km).
- There should be no human settlement between the first-line community and the riverine breeding site.
- Preference should be given to large, productive breeding sites, which are often close to rapids (marked on topographical maps).

- On the main river, at least one catching point or breeding site should be selected for monitoring transmission every 20–30 km, in line with epidemiological guidelines; additional catching points may be required, depending on the distribution of communities and the length of the river.
- Breeding sites for monitoring transmission should also be selected on tributaries of the main river close to first-line communities.

Seasonality of transmission is a major consideration in understanding the transmission dynamics of *O. volvulus* because of changes in breeding-site productivity (Chapter 3). Knowing when black fly vector populations begin to increase and eventually peak in accordance with rain and temperature is crucial for timing collections. As transmission may be seasonal, identification of sites for evaluation may require visits throughout the year until the full impact of seasonality is determined. In an area in which the team expects to find a breeding site and onchocerciasis is known to occur, repeated visits are required, which may be guided by local knowledge of when black flies bite.

In East Africa, selection of sites for entomological evaluation of *S. neavei* as the vector differs from selection of sites for *S. damnosum s.l.*, as *S. neavei* does not travel long distances in search of a blood meal. For example, in a transmission zone in Uganda, 84% of *S. neavei* females were collected at the edge of a forest near their breeding site and < 1% beyond 1 km. The farthest the flies travelled seeking a blood meal was 4 km *(59)*. In these areas, therefore, breeding sites should be identified in shorter segments than that usually recommended of one site per 20–30 km. The river should be fast flowing with some forest cover and preferably with a rocky bed to support survival of phoretic crabs.

The location of man-made structures such as dams and other structures that alter the flow of water to promote larval breeding should also be considered. Some information is presented in Chapter 3, and the importance of such structures is considered in more detail in Chapter 6.

The choice of sites for entomological evaluation starts at the lowest fast-flowing stretch of the main river and continues upstream. In this exercise, the team should consider the pre-control epidemiological results, the available topographical maps, the location of particularly hyper- and meso-endemic communities, rapids marked on maps, bridges (often built at rocky sites) and other sites of reported biting activity in riverine communities.

In an entomological evaluation, the village or community in which a breeding site has been chosen for catching or trapping flies should also be evaluated epidemiologically in order to synchronize entomological and epidemiological results. The national programme should ensure that all information on transmission and treatment is available for each breeding site and its associated first-line village. This should include baseline, pre-treatment community prevalence, seasonality of black fly breeding, black fly transmission indices (when known), history of MDA (year started, coverage by year), history of vector control activities and results of any previous epidemiological or entomological studies. This information should be collected as soon as the breeding sites are identified.

5.5 Community mobilization

5.5.1 Introduction

Community mobilization is essential for creating partnerships with various sectors of a community in order to address a pressing issue such as an NTD. Mobilization empowers community members and groups to act to facilitate change. Actions include identifying resources, providing information, generating support and fostering cooperation between public and private sectors in the community. Mobilization is often described as “building community or collaborative partnerships”, “community engagement” or “coalition building”.

In public health programmes, community mobilization ensures that the community can address its health needs. This contributes to the provision of primary health care, sustainability, integration in accordance with community priorities and to bridge the gap between the community and centralized health services. An example of large-scale community mobilization is the APOC, which built a network of 472 972 CDDs trained to deliver drugs, educate their peers about health issues and participate in other disease control activities. CDDs were selected by their communities and were thus perceived as honest and trustworthy. As they were volunteers, their motivation was to gain knowledge and contribute to the development of their communities, rather than to receive a financial reward.

The choice of CDDs is not an entomological activity; however, in some countries, such as the United Republic of Tanzania, CDDs (who are usually not paid) are preferentially selected as vector collectors (who are paid) as a form of encouragement. Nonetheless, the CDD system developed by APOC resulted in community engagement in the onchocerciasis programme, contributing to both its success and its sustainability.

5.5.2 Community mobilization for entomological evaluation

Although there is no expectation that CDDs will be a part of an entomological evaluation, the example highlights the importance of community mobilization for the success of public health programmes. Field workers must mobilize endemic communities for successful entomological evaluation in onchocerciasis elimination. In such activities, endemic communities have significant roles to play in at least six areas.

(i) Identification of breeding sites: While the first step in identifying breeding sites is to consult maps, there may have been changes in the names of villages or rivers, diversion or branching of a river or relocation of villages. Sometimes, the characteristics of a river may have changed to such an extent that the local ecology no longer favours breeding of black flies, or the changes may have resulted in more or new breeding sites (e.g. construction of a dam). Villagers can be extremely helpful in guiding vector personnel to productive breeding sites, as they will be aware of the presence, abundance and seasonality of biting black flies.

(ii) The safety of breeding sites: Some river basins are not safe for entomological evaluation, for example, if dangerous wild animals such as crocodiles or hippopotamuses inhabit the river basin (Fig. 5.6) or a selected site hosts the shrine of village gods that forbid visits of foreigners or other groups. Some areas may be insecure because of village conflict. Villagers can inform the entomological team of such situations.

Fig. 5.6. A basking crocodile next to a larval breeding site.

Note how well the animal blends into the background.

Source: photograph by D. Boayke

(iii) Prospection of breeding sites: Local residents can inform the team of the depth of the river for prospection, may assist the team in locating the aquatic stages of black flies or may confirm human biting activity of the female black flies. Villagers might offer to assist in a mock fly human landing catching exercise during early prospection (see below).

(iv) Clearing and establishing capture points: Villagers may help the team in clearing and establishing catching points.

(v) Identification of locations for setting and protecting black fly traps: Villagers may be involved in identifying locations for collecting adult female black flies in traps. In some field experiences, children pulled traps apart and played with them, or traps were removed from sites. Villagers should be mobilized to "own" and protect traps.

(vi) Human landing collection of black flies at designated sites: For human landing collection (HLC) of female black flies, villagers may identify trusted members of the community to be trained as village vector collectors (VVCs). Mobilization and involvement of leaders are necessary to identify members of the community who meet the criteria set by the entomology team to perform the role of VVCs. More detail is provided in Chapter 8.

Communities should be mobilized before the evaluation exercise. In all locations for evaluation, community leaders should be contacted and informed about the survey, including the objectives, the importance to health and the importance of community participation. Feedback from the community should be considered and the survey or prospection adjusted accordingly.

CDDs are usually recognized and respected by the community and should be involved in community mobilization. This should be discussed with village leaders in planning mobilization.

In ivermectin-naive areas, the team should initiate fresh community mobilization with appropriate onchocerciasis education materials, with emphasis on the evaluations to be undertaken in the villages and their purpose.

Chapter 6.

Prospection of breeding sites of black flies

Learning outcomes for Chapter 6

By the end of this chapter, the reader should be able to:

- identify rivers with rapids and white-waterfalls;
- understand how to use maps to locate black fly breeding sites;
- understand prospection methods, including visiting communities and rivers and streams that harbour the aquatic stages of *Simulium* spp;
- identify potential aquatic substrates and search for *Simulium* larvae and pupae;
- preserve larvae and pupae in alcohol or Carnoy's fixative;
- record prospection data with a sampling code;
- complete standard field forms; and
- recognize the major differences in the breeding sites of *S. damnosum s.l.* and *S. neavei.*

6.1 Introduction

"Prospection" refers to the search for something (for example, gold) in a location in which it is suspected to exist. In *Simulium* ecology, prospection is the search for aquatic black fly stages (eggs, larvae, pupae) in potential breeding sites. As described in Chapter 5, topographical maps are used to identify potential breeding sites along river systems. Once the team arrives at a potential site, it must physically wade through the river or stream and locate and examine the substrates (rocks, trailing vegetation, aquatic plants, etc.) found in fast-flowing water for the presence of larvae and pupae (Fig. 6.1). Identification of vector species allows the team to confirm the relevance of the site for breeding. In the context of elimination of onchocerciasis, confirmation of a breeding site allows identification and location of first-line and second-line communities in which epidemiological evaluations or black fly collections should be conducted as part of the national surveillance strategy and in order to meet the WHO criteria for stopping MDA or confirming interruption of transmission through post-treatment surveillance.

Fig. 6.1. Examining aquatic vegetation for the presence of black fly larvae and pupae.

Source: photograph by A. Elaagip

Prospection has an important secondary role to play if programmes implement vector control or elimination as complementary treatment for successful interruption of transmission of *O. volvulus*. For more information on vector control, see Chapter 12.

6.2 Requirements for prospection of black fly vectors

In preparation for prospecting aquatic stages, in addition to logistic arrangements, the following are required:

- map of the river system, showing areas endemic for onchocerciasis;
- lists of potential breeding sites to be prospected (see selection of entomological sites in Chapter 5);
- cold box;
- ice packs;
- knife, pruning shears or large scissors;

- hand magnifying lens;
- dissecting kit with soft forceps;
- zip-lock plastic bags;
- waterproof notebook;
- pencil;
- thermometer;
- exclusion box;
- labelling materials;
- universal (and bijou) tubes with reagents for preservation (70% alcohol) (see Fig. 7.3);
- reagents for preparation of Carnoy's fixative (see Chapter 7);
- GPS device to record the coordinates of each breeding site;
- life jackets for those who will enter water;
- waist belts for those who will enter water;
- clothes appropriate for prospection, particularly footwear that does not slip on rocks;
- artificial larval and pupal substrates (plastic strips, etc.);
- walking stick for wading; and
- first aid kit (in case of injury).

An important aspect of prospection is community involvement, by consulting local villagers for assistance in identifying potential breeding sites and for information on the safely of the river or the area. Do not neglect this important source of information.

Note: Prospection of high-volume rapids is **dangerous** and should not be done without proper training. Entomologists should never work alone. Life jackets and waist belts are not optional equipment.

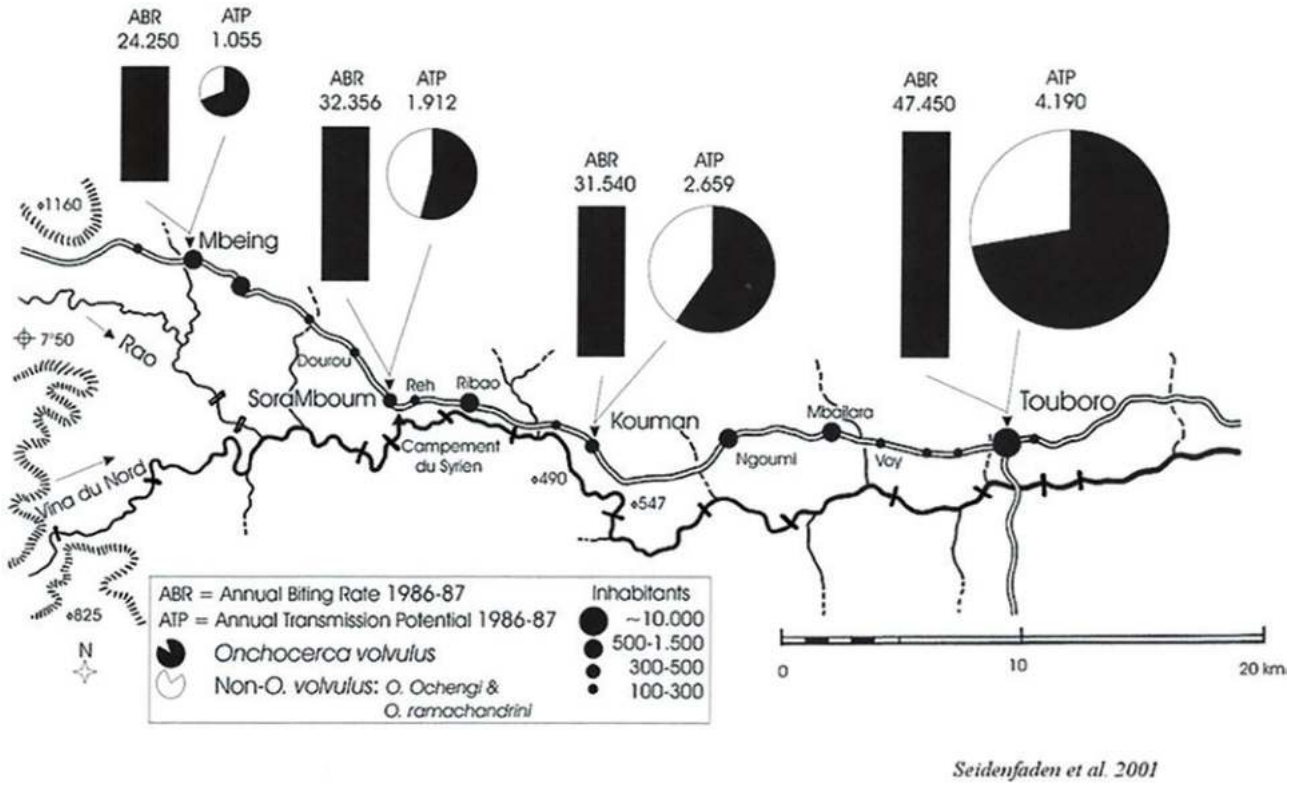
6.3 Mapping *Simulium* spp. breeding sites

A basis for all epidemiological studies and interventions is comprehensive geographical maps of *Simulium* breeding sites, the locations of human populations and areas of contact between human and fly populations. Google satellite maps include aerial photographs and maps; therefore, the evolution of landscape use can be seen by comparing old (from about 1980) and new satellite maps. Breeding sites in large rivers can be detected easily, but smaller sites in tributaries that flow only in the rainy season and those hidden under dense rainforest canopy are not visible (Chapter 3). While these maps may be of historical interest, prospection and identification of the prevailing *Simulium* populations must be done on the ground.

The location of breeding sites is only one component of the ecology of *O. volvulus* transmission. Human settlements are often built near the rivers that serve as their water source, and roads connecting villages may travel along or cross the river. Comprehensive maps of *Simulium* breeding sites should therefore include the location of the sites, roads that come close to the sites and the location of villages exposed to the black flies that breed at the sites. A description of a breeding site should include the size of the site, whether the stream flows seasonally, the size of the surrounding human population and the rate of contact between humans and flies

(e.g. daily or annual biting rate).This information should be added to the map during field prospection. Standard coding is used to display this information on a map (Fig. 6.2).

Fig. 6.2. A, Map of the classic focus of the savannah blinding type of *O. volvulus* in northern Cameroon in villages situated where the road comes close to the river Vina du Nord, which were hyperendemic for frequent blindness.

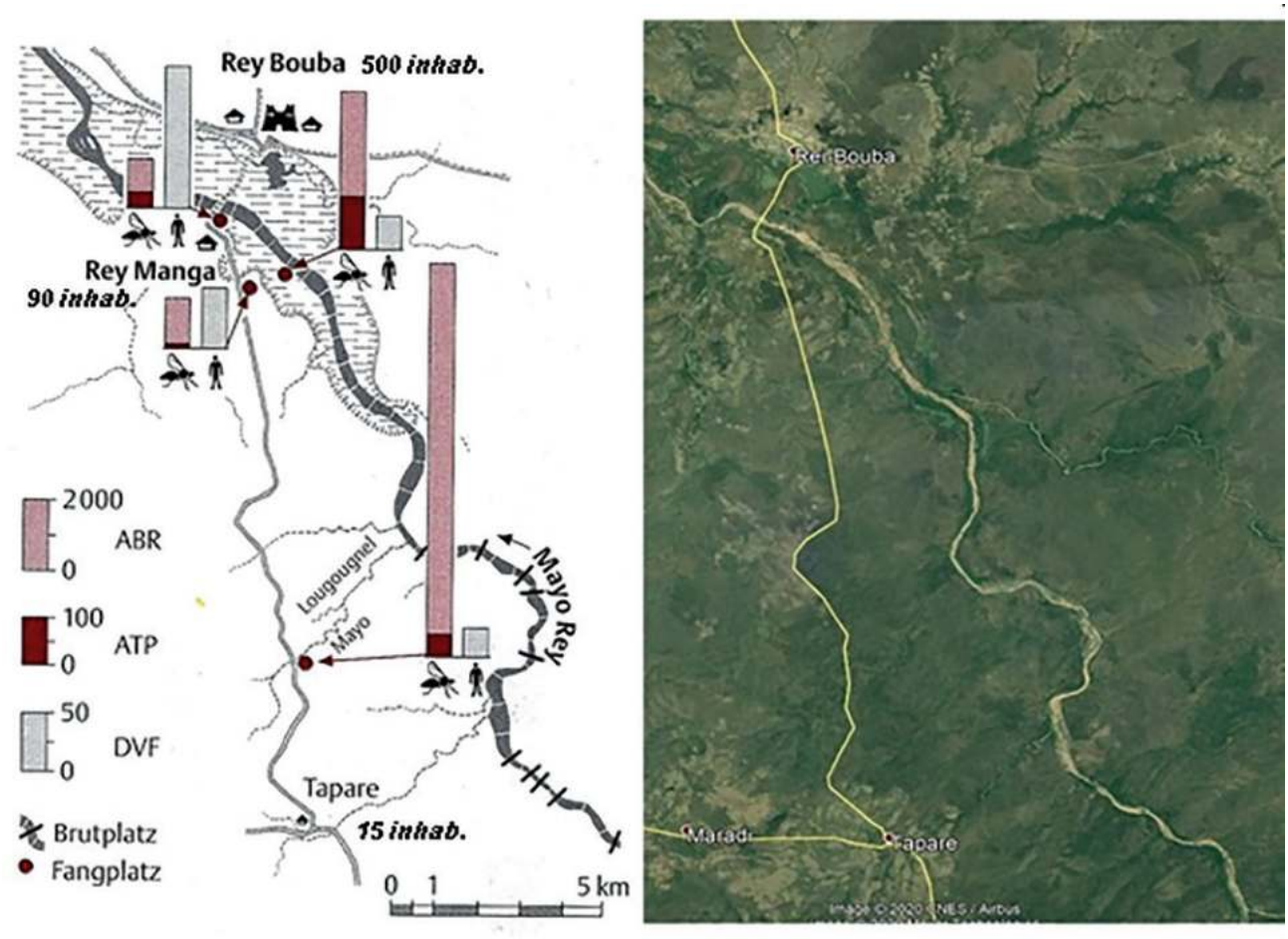


Source: reference *60*

Sampling code notations: Permanent or seasonally flowing water: broken lines indicate where flow may be seasonal, corresponding to the months of no flow; long stretches of unbroken lines indicate that water flows most of the year; short stretches of unbroken lines and long sections of broken lines indicate that water flows for only a few months during the rainy season. *Simulium* breeding sites and their productivity are indicated by strokes perpendicular to the river flow (open squares for rainy season only, filled squares for +/- perennial breeding). The thickness of the stroke indicates the importance of the breeding site. Prevalent *Simulium* spp. are added following prospections.

ABR, annual biting rate; ATP, annual transmission potential

Fig. 6.2. B, Map of *S. damnosum s.l.* breeding sites in the Sudan savannah of Cameroon.

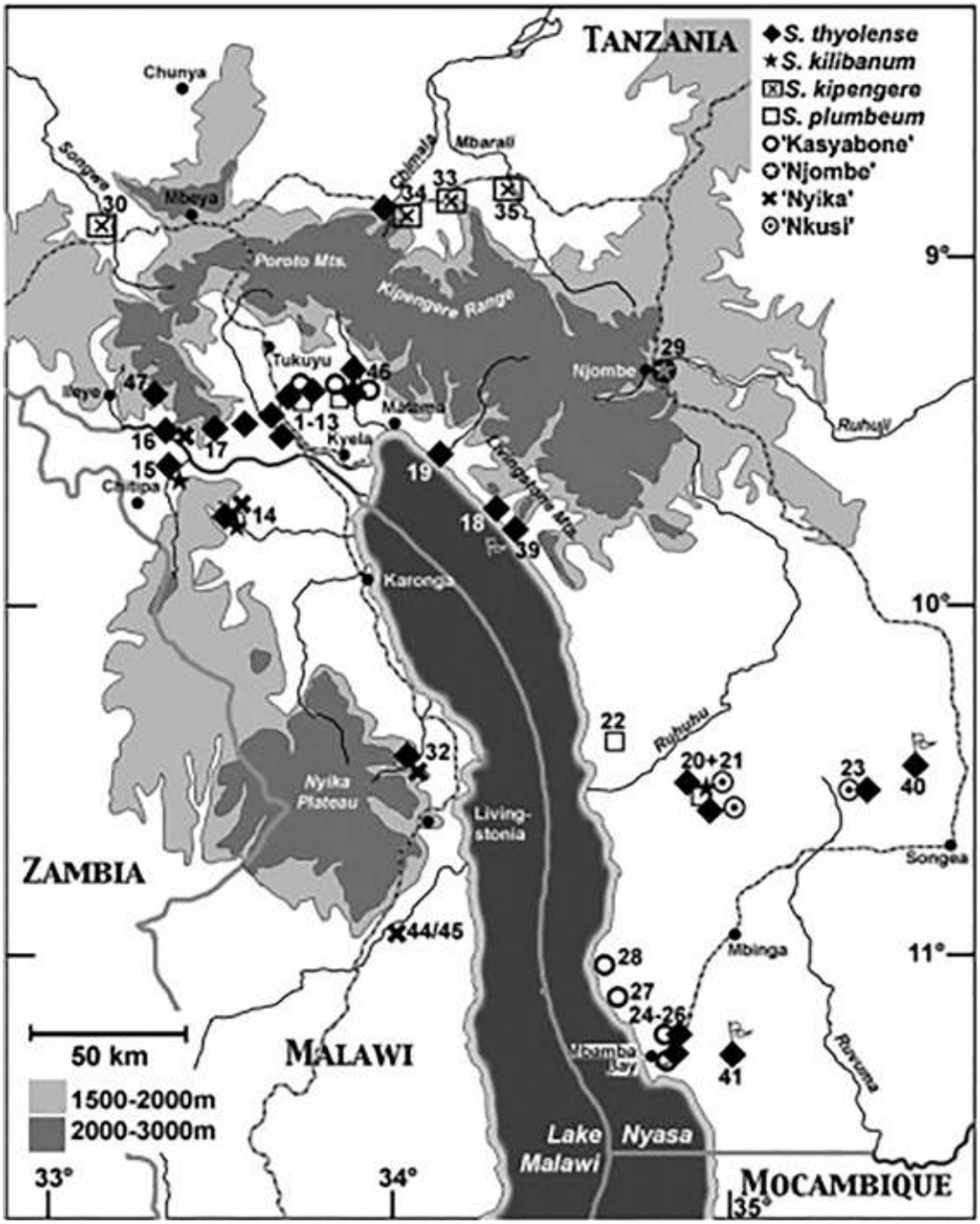


Sources: references *5* and *46*

Section 1 depicts the two major rivers with the modified daily visiting frequency (DVF, man-hours spent at the place per day) near the breeding sites and the resulting annual biting (ABR) and transmission potential (ATP) of *O. volvulus*. Section 2 is a satellite photograph of the two rivers (Mayo Rey on the right and Benoué on the left). Note the differences in the ABR between sites as a function of time spent by the human population at a given site.

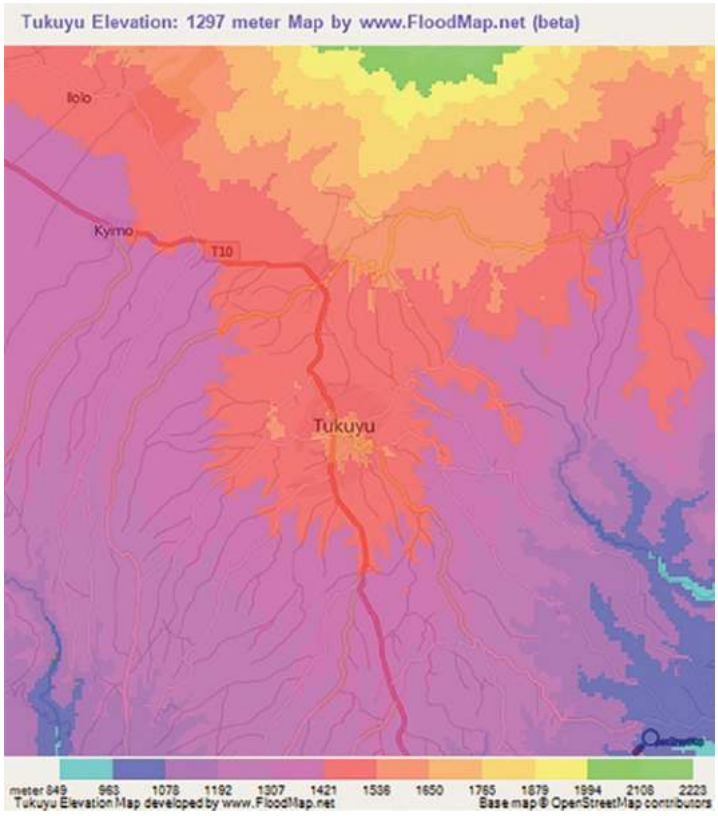
Topographical or relief maps allow location of rivers and streams on the basis of changes in elevation. As seen in Figs 6.3 and 6.4, different elevations may be indicated by shades of black and white or colour, with rivers flowing from higher to lower elevations. In other topographical maps, three-dimensional changes are depicted by contour lines, which indicate the shape and steepness of the terrain (see Fig. 5.4). The map's scale indicates how detailed a map is. For example, a 1:24000 scale indicates that 1 cm on the map = 24000 cm or 0.24 km on the ground.

Fig. 6.3. Relief map of the Tukuyu (United Republic of Tanzania) focus of onchocerciasis. Note the distribution of *S. damnosum* cytoforms at different altitudes.



Source: reference *61*

Fig. 6.4. Colour-coded elevation map. Tukuyu, United Republic of Tanzania.



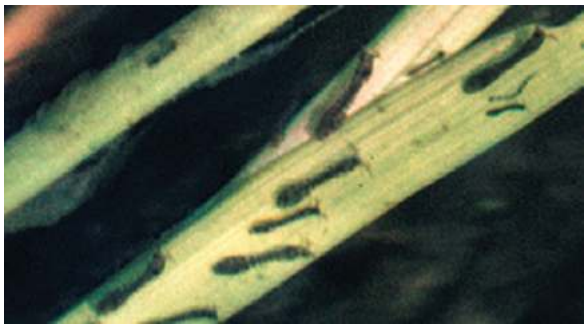
Source: Base map © OpenStreetMap contributors

Despite the importance of maps for prospection, a field investigation is conducted on the ground, by searching for breeding substrates, collecting *Simulium* larvae and pupae and identifying them in the laboratory.

6.4 Methods of prospecting for black flies

Reaching a prospection site may require travel by road, boat or foot. Black fly aquatic stages at a site may be prospected by boat or by wading through the rivers or streams. When wading in streams, appropriate safety precautions must be taken, such as wearing appropriate clothes, life vests and waist belts and never prospecting alone. Prospection involves careful inspection of substrates (plants, rocks, twigs, grasses, bridge piers, etc.) at the breeding site to observe and take samples of pre-imaginal stages (larvae, pupae) (Fig. 6.5).

Fig. 6.5. Black fly larvae on grass stems under water.



Source: photograph by R. Post

Both aquatic stages can be collected with forceps. GPS coordinates should be recorded at all inspected breeding sites, with all the digits shown on the instrument recorded for each coordinate. After collection, larvae and pupae should be maintained in a cold box with ice packs or fixed in 70–80% alcohol for sorting and identification in the laboratory. Samples may also be placed in Carnoy's fixative for cytotaxonomic studies (Chapter 7). Larvae and pupae can be identified through a hand lens in the field or under a binocular (dissecting) microscope in the laboratory, with appropriate identification keys (see Chapter 4).

After collection, the material may be classified into one of four categories for entry onto the prospection form: young larvae, old larvae, pupae and empty cocoons. The densities of the pre-imaginal stages are then coded as:

- (–), negative breeding site (no larvae and no pupae were found on substrates);
- (+), ≤ 10 larvae or pupae;
- (++), 11–50 larvae or pupae; or
- (+++), > 50 larvae or pupae.

The results of prospection are recorded on a field form (see below), with the following additional information in notes: river basin, name of river, breeding site, GPS coordinates of the breeding site, density of the aquatic stages and water velocity and temperature. If eggs are found, this information should also be included.

Prospection field form

Basin	River	Breeding site	Coordinates	Black fly species				Notes		
				YL	OL	P	EC	Velocity	Temp	Eggs

YL, young larvae; OL, old larvae; P, pupae; EC, empty cocoons

Name of collector: _____ Date of collection _____

6.5 Specific methods of prospecting for *Simulium neavei* group

The immature stages of *S. neavei* group live mainly in phoretic association with freshwater crab species belonging to the genus *Potamonautes* (see Chapter 2). The most common species of freshwater crabs reported in East and Central Africa are *P. aloysiisabaudiae*, *P. niloticus*, *P. antheus*, *P. pseudoperlatus*, *P. granviki* and *P. johnstoni (62)*. In order to assess the level of *S. neavei* infestation in a river system, freshwater crabs must be collected with a trapping tool (funnel basket trap, Fig. 6.6) and then inspected for black fly larvae and pupae (Fig. 6.7). This is the main difference in prospection methods from those for *S. damnosum s.l.*

Fig. 6.6. Funnel basket traps being prepared for trapping freshwater crabs to prospect for the immature stages of *S. neavei* in a river in Uganda.

Source: photograph by A. Krueger

Fig. 6.7. *S. neavei* larvae and pupae attached to the legs and carapace of a freshwater crab. Note the larvae and pupal cases on the legs and lower carapace.

Source: photograph by R. Garms

Field procedures for use of a funnel basket trap for river crabs

1. Cut fresh meat into small pieces, and tie them each with a rope about 50 cm long.
2. Insert the meat into the trap at the narrow end, allowing it to hang inside, then block the opening with grass or well-shaped leaves. Make sure that the blockage is tight.
3. To ensure that the piece of meat is hanging correctly, observe through a small hole in the funnel side.
4. When you have prepared all the traps with the meat bait, go to the river and look for a suitable place, preferably one that is rocky, at the side or in the middle of the river where the current is not too strong to sweep away the trap.

5. Place the trap in the water, ensuring that it is immersed, and then cover it with grass or leaves as camouflage. Place the funnel side of the trap in the direction of the flow of water.
6. Anchor the traps with long ropes to objects inside the river or at the bank.
7. Once all the traps are in the water, record the time, and leave them for 1 h. In rare circumstances when there are very few crabs, traps may be left in the water overnight *(63).*
8. After 1 h, remove all the traps, and empty the crabs into a container. Add more water to ensure that the crabs are clean before counting the attached immature stages. Record this information on a standardized form (see below).
9. Examine the crabs one by one, and measure the size of the carapace with a vernier caliper, recording male and female crabs separately.
10. Immediately after the crabs have been assessed for infestation, they should be returned to the river or stream.
11. GPS readings should be made at all sites where crabs have been caught.
12. Prospections are continued until the river or stream is fully evaluated, and the results are then plotted on a map to plan ground larviciding or any vector control activity.

Crab catching record form for *S. neavei*

District, local government area, state: _____ County: _____

Focus or transmission zone: _____ Village: _____

River: _____ Site: _____

Coordinates of site: _____ pH: _____ Dissolved O_2 (mg/L): _____

Conductivity: _____ Temp: _____ Discharge (m^3/s): _____

Name of field team											
1.						3.					
2.						4.					
Date						Date					
No.	Crab size (mm)		*S. neavei* immature stages			No.	Crab size (mm)		*S. neavei* immature stages		
	♀	♂	L	P	PCs		♀	♂	L	P	PCs

L, larva; P, pupa; PC, pupal case

Comments: _____

Source: National Onchocerciasis Control Programme, Uganda

Chapter 7.

Collection and preservation of black fly larvae for cytotaxonomic identification

Learning outcomes for Chapter 7

By the end of this chapter, the reader should be able to:

- understand the procedures for collecting and preserving black fly larvae for cytotaxonomy;
- know the difference between a sample and a specimen;
- know how to prepare Carnoy's fixative;
- recognize suitable larvae for collection and preservation;
- collect and preserve larvae;
- label and document collections;
- store larvae properly after collection; and
- prepare materials and reagents for the collection and preservation of larvae.

Warning 1: This protocol requires handling of Carnoy's fixative and acetic acid, both of which are corrosive and must not be used without proper training in health and safety.

Warning 2: This protocol requires the entomologist to enter river rapids to collect black fly larvae. This is hazardous and must not be done without proper training in health and safety.

7.1 Introduction

Cytotaxonomy is the practice of taxonomy by observation of differences between the cells of organisms; most are chromosomal differences. All cytotaxonomic studies of black flies involve microscopic examination of special (giant) chromosomes, "polytene" chromosomes, which are found in the salivary glands of larvae. As polytene chromosomes are difficult to see in black fly adults and pupae, the best stage for cytotaxonomy is larvae collected from breeding sites (Chapter 6). Occasionally, for particular research projects, larvae of vector species can be raised in the laboratory for cytotaxonomy, but this is not routine and is outside the scope of this manual.

The reasons for using cytotaxonomy are explained in Chapter 4. Cytotaxonomic studies are required only to identify members of the *S. damnosum* species complex. Other man-biting and vector species (such as members of the *S. neavei* group and those in the Americas) can be identified by examination of morphological characters under a microscope (see Chapter 4) and do not require chromosome examination.

The quality of collection and preservation is very important for successful preparation of larval polytene chromosomes. Therefore, cytotaxonomic studies of black flies require specialized methods and skills for larval collection and preservation to ensure good preparations.

A single larva collected at a breeding site is a "specimen". A number of larvae collected from the same site at the same time is a "sample" (of the local population of wild larvae) and consists of all the specimens collected at that place at that time. A "collection" from one site at one time is the same as a sample, but a collection in a museum or in a laboratory usually consists of many samples from different places and many samples taken at different times.

7.2 Finding and choosing black fly larvae for cytotaxonomy

Cytotaxonomy is used only for members of the *S. damnosum* complex, and therefore larvae for cytotaxonomy are collected routinely only from *S. damnosum s.l.* breeding sites. It is relatively easy to recognize black fly larvae in the field because they orientate themselves with the flow of the water while clinging to vegetation, rocks and other objects (Fig. 2.4); however, it is often difficult to identify the species of black fly larvae in the field precisely. The larger, often darker specimens collected from typical *S. damnosum s.l.* breeding sites, from the fastest parts of a river, are nevertheless usually members of this species complex. A few specimens of other species may be included accidentally in a sample; however, these can be separated and disposed of later in the laboratory.

The polytene chromosomes investigated for cytotaxonomy occur in the salivary glands of larvae. The polytene chromosomes grow as the larva grows, and cytotaxonomy is possible only with the largest chromosomes in the last instar (7th instar) or the penultimate instar larvae (6th instar) (Fig. 2.3). The function of the larval salivary glands is to make silk, and, because pupae do not make silk, the salivary glands begin to change at the end of the final (7th) instar, just before pupation. Therefore, the oldest larvae (with black pupal histoblasts) do not make the best chromosome preparations, and last instar larvae with white pupal histoblasts (or 6th instar larvae) are preferred (Fig. 7.1). These distinctions are, however, difficult to make during fieldwork, and most people simply collect "large" larvae (see Fig. 4.2A).

Fig. 7.1. Late instars of *S. damnosum* larvae. The 6th and early 7th instars are preferred for cytotaxonomic studies.

Source: photograph by R.J. Post

7.3 Collection and preservation of black fly larvae

7.3.1 Background

Larvae are routinely fixed on the riverbank immediately after they have been collected. In exceptional circumstances, plastic bags can be inflated with air and kept on ice in a cold box for a few hours so that the larvae can be transported and fixed in the laboratory. There is, however, a danger that the larvae will become suffocated, stressed and die during the journey, and, under these circumstances, they may yield poor chromosome preparations.

For cytotaxonomic studies, larvae must be collected alive and undamaged. Soft, flexible forceps are ideal for handling live *Simulium* larvae; however, with practice, watchmakers' forceps or other hard forceps can be used (Fig. 7.2).

Fig. 7.2. Suitable forceps for handling live larvae (watchmakers' forceps above, soft flexible forceps below).

Source: photograph by R.J. Post

The standard, acceptable fixative for cytotaxonomy is Carnoy's fixative, and larvae must be dropped alive (or at least freshly dissected, see below) into the fixative, which will kill them within seconds. Carnoy's fixative is a mixture of three parts of ethanol to one part of acetic acid, without water (i.e. 3:1 absolute ethanol to glacial acetic acid). Some older recipes include chloroform in the mixture, but this is unnecessary and outdated. It is important that the reagents be free from water: the acetic acid should be "glacial" and the ethanol "absolute'" (or at least 98%).

Carnoy's fixative does not keep and must be made freshly for each sample or small batch on the riverbank (see below).

Black fly larvae can be preserved for cytotaxonomy in a number of ways. The methods differ mainly in how the larvae are handled after removal from the plastic collection bag, on the riverbank, before fixation. Excess water may be removed, they may be kept on ice, or they may be dissected before fixation. The method described below is for fixing whole intact larvae. It is the commonest, easiest method.

Materials and reagents required for collection and preservation of black fly larvae for cytotaxonomy

Chemicals: glacial acetic acid, absolute ethanol

Materials and equipment: forceps, penknife, dissecting needles, 10-mL syringe, glass "bijou" bottles, glass "universal" bottles (Fig. 7.3), refrigerator for preservation, cool box, freezer packs or ice blocks, No. 4 Whatman's filter paper (any size).

Materials and specimens are usually preserved in a functioning refrigerator. If a constant power supply cannot be guaranteed by the local or national supplier, a stand-by electric generator is essential.

Fig. 7.3. "Universal" (left) and "bijou" (right) bottles for containing and mixing reagents to prepare Carnoy's fixative.

Source: photograph by R.J. Post

7.3.2 Protocol for preparation of Carnoy's fixative and collection of larvae for cytotaxonomy

The method described below is for fixing whole intact larvae and is the commonest and easiest method.

- In the laboratory, use a 10-mL syringe to put 21 mL of absolute alcohol into 10 of the 28-mL universal bottles.
- Put ice or freezer packs into the icebox with the 10 bottles containing absolute alcohol. Label the bottles appropriately. In the absence of ice, put the bottles in cool water.
- Using another 10-mL syringe or a suitable measuring device, put 7 mL of acetic acid into 8-mL bijou bottles, and label appropriately. Do not put these bottles on ice (because they will freeze); however, they can be carried in the cool box if they are insulated against freezing (by wrapping them in a towel for example).
- Transport all the bottles of absolute alcohol and acetic acid to the breeding site chosen for collection of larvae.
- At the edge of the breeding site (on the riverbank), open a Universal bottle containing 21 mL absolute alcohol retrieved from the cool box and add to it 7 mL of acetic acid from the bijou bottle, to obtain a final volume of 28 ml of Carnoy's fixative. **Note:** Gloves should be worn during this procedure to avoid contact with the final mixture.
- Close the cap tightly, and mix by shaking to create a homogeneous solution.
- Enter the river (wearing suitable clothing and safety equipment and taking forceps and a fairly large transparent plastic bag), and go to points where you expect to find larvae. Carefully remove the substrates (mostly pieces of vegetation such as twigs and leaves, but also stones) with a penknife from the breeding site and select those with the most late-instar *S. damnosum s.l.* larvae attached.
- Larvae of *S. damnosum s.l.* are usually larger and often darker than those of other species, and they usually recoil into a U-shape as soon as the substrate is out of the water. Larvae of other species of *Simulium* often continue to move about. Place the substrates with the larvae into the plastic bag. Pour out excess water, and continue searching. If substrates cannot be removed (for example rocks), the larvae can be scraped off into the plastic bag. When you have collected sufficient larvae, return to the riverbank.
- Gently sponge the larvae on the substrate with blotting or filter paper to avoid diluting the Carnoy's.
- Remove the larvae with forceps, and drop them into the bottle of Carnoy's fixative. Then, start again with another substrate until the bottle contains approximately 50 larvae. If there are more than 50 larvae, use a second bottle.
- Place a sample label with the name of the river, place name, date and GPS coordinates written in pencil inside the bottle, and close the bottle.
- Prepare a second bottle of Carnoy's fixative. Pour away the old Carnoy's from the larval sample, and replace it with fresh fixative.
- Put the bottle back in the icebox. Do not let water enter the bottle of fixative.
- Transport the collected larvae in Carnoy's solution to the laboratory in the cool box.
- In the laboratory, prepare a third bottle of Carnoy's fixative. Pour away the old Carnoy's from the sample of black flies, and replace it with the fresh solution.
- Store all the specimens in a refrigerator. Under this condition, the larvae should have readable chromosomes even after 2 years of preservation.

If a refrigerator is temporarily unavailable, the Carnoy's solution should be changed once a week. Specimens in Carnoy's fixative can nevertheless be maintained for short periods at ambient temperature (a few days for transport for example). It is always best to keep them cold.

Stored material may be airmailed anywhere in the world, but the courier should be consulted to make sure that there are no restrictions on material preserved in Carnoy's fixative. Bijou bottles are excellent containers for shipment as they are almost impossible to break. It may be necessary to pour away excess Carnoy's before final packaging. The package should be clearly labelled: "FRAGILE. Preserved insects for scientific study. Of no commercial value."

7.3.3 Optional variations in the collection protocol

Use of ice in transporting larvae for cytotaxonomy (after the method of the OCP)

Ice can be used for transferring living larvae from the field to the laboratory, where they can be fixed in Carnoy's. Carriage of living larvae is sometimes problematic because, in warm weather or warm climates, larvae may be adversely affected shortly after they are removed from running water, and their giant chromosomes begin to deteriorate. This problem can be largely overcome by immobilizing the larvae on ice immediately after collection.

A variety of ingenious ways have been found. For example, where there is a limited amount of ice or not too much weight can be carried, wide-mouth vacuum (Thermos) flasks can be filled with crushed ice to hold larvae for a day. Larvae are first collected into a vial, which is then capped and placed in the ice in the flask. The larvae should not be so crowded that they are buried away from the air in the vial, nor should there be any water other than that adhering to the larvae.

Another way is to use a large, insulated box with adequate drainage. Several days' supply of ice can be carried in a vehicle in this way. Speed in immobilizing the larvae is still essential. Some field workers use polyethylene bags. Sticks, leaves and other substrates to which larvae are attached, with a quantity of air, are sealed in the bags with an elastic band. If the collection site is a considerable distance from the ice box, a wide-mouth vacuum flask is useful.

Larvae immobilized on ice frequently show no movement and may appear to be dead. They usually form a shallow S-shaped curve. However, if they are allowed to warm up gently for a few minutes, they soon become quite lively. It is not known how long larvae may be kept immobilized on ice, but *S. damnosum* (Sanje form) stored in this way for 3 days has produced good slides.

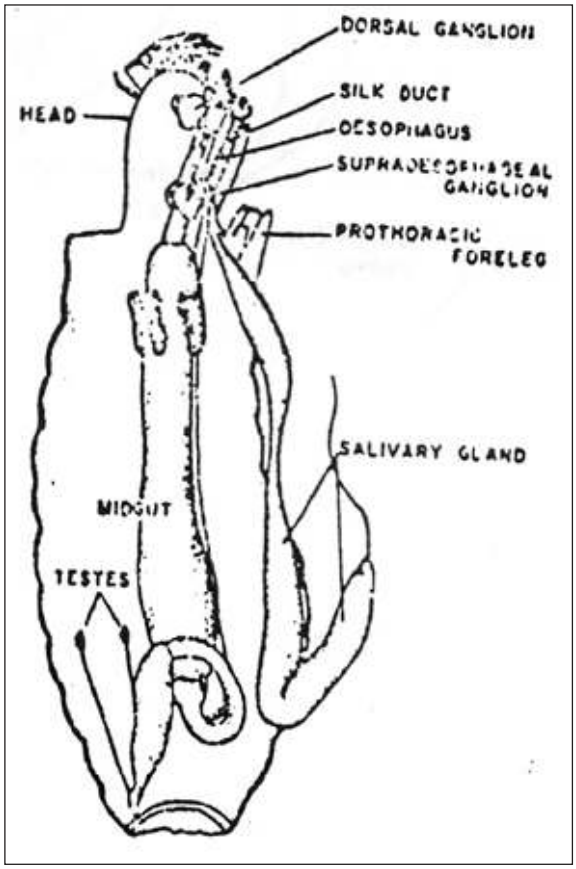
The prime advantage of this method of collection is consistent production of very good-quality chromosome slides. As metabolic activity appears to be almost completely arrested, the larvae can be examined at a more suitable location, i.e. in the laboratory. It is no problem then to take time to remove unwanted specimens (such as non-*damnosum* complex larvae or larvae of the wrong size) while they are in a pool of melting ice or even on an ice block, without impairing the quality of the subsequent slides. Immobilized larvae remain viable and may also be induced to continue developing in laboratory rearing systems. This approach is, however, rarely used because other methods usually give good enough chromosomes and are much more convenient.

Dissection of larvae in the field before fixation

In this method, larvae are fixed in the field as quickly as possible to prevent deterioration of the polytene chromosomes, by allowing very rapid penetration of the larval body by Carnoy's fixative. To achieve this, larvae are dissected on the riverbank before fixation. This method is rarely used because others usually give good enough chromosome preparations and are much more convenient.

The paired salivary glands (silk glands) are extremely large, stretching over two thirds or more of the length of the body and then doubling back (Fig. 7.4). Dissection to expose them is easier under a low-power binocular dissecting microscope. The larvae should first be placed on a suitable surface (for example, a slide, or the back of a Petri dish) one or a few at a time. Excess water adhering to the larvae should be removed by gentle blotting.

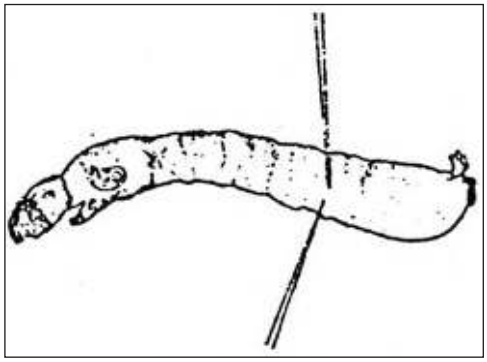
Fig. 7.4. Lateral dissection of a *Simulium* larva showing the salivary glands.



Source: reference *64*

The distal third of a larval body is torn open by inserting two sharp needles or forceps near the top of the bulge and pulling them apart to make a hole in the body wall (Fig. 7.5). The salivary glands, and sometimes other organs, then usually "pop out". The entire larva is then rapidly placed in fixative. If the larvae have been stored on ice, it is recommended that the dissection be done in relatively cool surroundings, for example, on a cooled surface (such as an ice block), or in the evening, or both, and that the fixative is also cold.

Fig. 7.5. Larva prepared for dissection by tearing open the distal third if its body. Two sharp needles or forceps are inserted near the top of the bulge to tear a hole in the body wall.



Chapter 8.

Human landing collection and trapping of female black flies for entomological evaluation

Learning outcomes for Chapter 8

By the end of this chapter, the reader should be able to:

- understand the importance of human landing collections (HLC) in entomological surveillance;
- select suitable community members for HLC;
- organize collection activities in the field;
- understand use of the Esperanza Window Trap (EWT) in verification of onchocerciasis elimination;
- deploy and operate EWTs in the field; and
- complete all field forms for safe-keeping and data analysis.

8.1 Human landing collection

The HLC technique (previously called the "human bait technique") is the global gold standard for black fly collection. The fly collectors are usually referred to as "village vector collectors" (VVCs) or human attractants (Chapter 5). The HLC method typically gives information on the relative density of the populations of biting females of *Simulium* spp. vectors per person in a given place and period *(65)*. This approach involves collection of female black flies attracted to humans before they are able to take a blood meal from exposed human skin *(66, 67)*. VVCs are required to sit down (normally) or stand up along the bank of the river or a designated area in close proximity to breeding sites to attract and collect flies that land on their exposed legs or arms. HLC is a direct approach to the collection of anthropophilic black flies and thus makes it easy to estimate a fundamental component of vectorial capacity, i.e. biting rate per person. *Onchocerca volvulus* transmission indices can also be determined if the infection rate of L3s in the heads of black flies is known. As black flies are collected, the landing rate measured from the collections is taken as an estimate of the biting rate over a unit of time (daily, monthly, annual), although this approach probably overestimates the true biting rate, as a proportion of the flies landing in a natural setting do not successfully obtain a blood meal.

8.2 Selection of human landing collectors

The selection of human landing collectors and of the optimal sites for black fly collection in a community may be difficult if there is inadequate community discussion and mobilization before starting collection. Collection by fly collectors can be limited by factors such as skin colour, body odour, work ethic and ability *(68)*. To minimize the effects of such differences among collectors, the entomologist should select individuals who are representative of the local endemic community. To address these confounding issues (and avoid position bias), collectors and positions should be rotated daily.

To maximize community involvement in the work, the entomologist should ensure the following.

- Communities are properly informed about the importance of the entomological evaluation and study protocol.
- Community and opinion leaders can nominate potential fly collectors.
- Preference is given to residents of the community who are readily available for the task, which usually ensures that the residents have a sense of ownership and readiness to accept the outcome of the evaluation. This is also one of the ways to promote security in the field.
- Where feasible, preference is given to young, able, agile individuals; literacy is an added advantage.
- The honorarium to be given to each vector collector should not be disclosed to the community leader(s) until selection has been completed.
- Fly collectors should be paid in instalments, according to local custom, to ensure that they remain motivated while conducting the work.
- All collectors should receive Mectizan® in accordance with the regular community mass drug treatment regimen or at least 1 week before collecting.

8.3 Selection of catching sites for human landing collection

Catching sites for HLC are usually selected in one of two ways. Established breeding sites are used for routine entomological surveillance, or collection is based on spot catches. Spot catches should be made when there is little previous knowledge about black fly breeding sites or the biting activity in an area. It is a method of rapid surveillance for vector black flies.

8.3.1 Use of established breeding sites

Catching points for black flies can be setup along rivers that are productive for the breeding of *Simulium* vectors (see Chapter 5). The following criteria should be considered in selecting a catching point for routine entomological surveillance in accordance with standard procedure *(65).* The catching point should:

- be shaded, with little or no wind disturbance;
- not be a place for human gatherings, in order to prevent "dilution "of biting (whereby flies have access to people who are not part of the fly-catching team) and disturbance of human landing collectors;
- allow collectors to be readily visible to questing flies; and
- secure and accessible all year round.

8.3.2 Spot catches

Spot catches involve random sampling of potential collection sites without prior knowledge of the biting density of black flies in the areas. Human landing collectors are posted for a few hours at a single location and then moved to another site. Spot catches are thus used to establish whether black flies bite in an area and, if so, which location would be best for long-term surveillance.

8.4 Procedures for human landing collection

8.4.1 Equipment and materials required

In addition to logistical arrangements, the following are necessary for collecting flies:

- catching tubes, usually 70 x 12 mm polypropylene, with caps;
- cotton bags for storing tubes containing flies during the day;
- cotton wool;
- Bijou or universal bottles (moulded glass; Essco glass ampoules and vials; see Chapter 7);
- 80% alcohol;

- a pipette or similar device to decant alcohol;
- a cold box with ice pack (if ice packs are not available, a damp towel can be used);
- indelible pencil or marker; and
- recording forms.

8.4.2 Types of human landing collection

The two basic types of HLC are normalized and bulk catching.

(i) Normalized catches or standardized collection

Normalized catching is the standard method of fly collection for estimating transmission indices of *O. volvulus* in a focus *(66)*. Normalized catches can be conducted daily, twice a week or twice a month, depending on the study protocol and its general purpose. This type of catch is no longer done as frequently as bulk catches but was used extensively in early control programmes to monitor progress. As used originally, normalized catches provided data on biting rates and parasite infection rates to calculate transmission potential per unit time (see Chapters 9 and 11).

The procedure is as follows.

- Train at least two VVCs in the community;
- The two VVCs at the designated catching point (about 10 m away from an established vector breeding site) collect flies alternately (rotationally) every hour between 07:00 and 18:00 every catching day. If collector A starts at 07:00 h on day 1, then collector B should start at 07:00 h on day 2 (in addition to the hourly rotations). Otherwise, the estimates from hourly collections may be biased by differences in collector attractiveness to black flies. In some countries in the Americas, collection periods were timed for 50 or 40 min before taking a 10- or 20-min break.
- The VVC exposes the lower part of his or her legs and collects any black fly landing on the exposed skin. The collector should preferably wear short knickers during catching hours (Fig. 8.1).
- A black fly landing on exposed skin is caught before feeding by inverting a small plastic tube (catching tube) over it and replacing the cap on the tube immediately to prevent the fly from escaping. An aspirator may also be used.
- Catching tubes containing black flies are kept in a cold box with an icepack before being transported to a local laboratory for analysis. Alternatively, in remote areas where ice packs are difficult to obtain, the tubes can be wrapped in a damp towel, which should be wetted again before the tubes containing the flies are transported to the laboratory. Care should be taken to avoid condensation of the water inside the tubes. The tubes should be transported to a local laboratory within a few hours of collection. Depending on the protocol, black flies are best dissected in the field or in the local laboratory for parity and infectivity shortly after collection (Chapter 9).
- When flies are being collected for PCR, they should be transferred into bijou bottles containing at least 80% alcohol (80 mL of absolute alcohol plus 20 mL of distilled water) for further analysis.
- Once in the laboratory, tubes containing black flies should be kept at 4 ºC in a refrigerator or cooler with wet paper towels until use.

Fig. 8.1. Collection of *S. damnosum s.l.* by the HLC technique (A) and captured flies in a collection tube (B).

A B

Source: photographs by M. Adeleke

(ii) Bulk catches

The purpose of bulk catches (also called "mock catches") is to collect as many female black flies as possible in an area at a particular time for later evaluation by PCR (Chapter 10). This approach is used to catch enough flies to meet the WHO criteria for stopping MDA and for post-treatment surveillance. Some programmes do not perform bulk catches but use the normalized methods for PCR only (no dissection). This may depend on the number of flies caught as determined by historical knowledge of the breeding site's productivity.

The procedure for bulk catches is similar to that for normalized catches, with some differences.

- Instead of one person collecting flies every hour, at least four people are positioned to attract and collect flies between 07:00 h and 12:00 h, and another team (comprising at least four people) resumes at 12:00 h and collects until 18:00 h. The collectors are usually placed 20–50 m apart. Collectors may work individually, as in Africa (Fig. 8.1), or as a team of two (attractant and collector), as in the Americas.
- Flies collected by this method are usually preserved in bijou bottles containing 80% alcohol, which are kept at 4 °C in a refrigerator until use.

8.5 Esperanza window traps for collecting black flies

The use of a trap – the EWT – to replace VVCs for collecting black flies seeking a blood meal is being evaluated in several African countries. While the initial results are very encouraging, this technology is still under development, and there may be improvements to the EWT or to the suggested protocols. Programmes in which EWTs have not been used should consult programmes with such experience and look for updates from WHO.

8.5.1 Development of the Esperanza window trap

The HLC technique, a fundamental monitoring procedure for several vector-borne diseases, has been criticized for both its limitations and the associated ethical risks *(69)*. Major considerations are the fact that the HLC is time-consuming and VVCs may be at risk of receiving large numbers of bites. Further, collection of at least 6000 black flies, which is required for verification of transmission interruption, may be difficult to achieve in some areas.

Although several attempts have been made to develop traps for black flies *(70, 71)*, the outcomes were limited in both application and adaptability to various ecological zones. The EWT was developed and optimized for the collection of host-seeking *S. ochraceum* in 2012 and 2013 in southern Mexico *(69)*. The trap consisted of a combination of olfactory cues and visual attractants to lure flies onto a sticky surface, where they were immobilized and trapped. The trap, made of blue fabric (1 m x 1 m), was baited with organically produced carbon dioxide generated from a mixture of sugar, yeast (*Saccharomyces cerevisiae*) and water, with BG-Lure™, a commercially available attractant consisting of a mixture of compounds found in human skin secretions, such as ammonia, lactic acid and caproic acid (Biogents AG, Regensburg, Germany), or a previously worn shirt. The trap collected similar numbers of female *S. ochraceum s.l.* (a dominant anthropophilic vector in Mesoamerica) as with the HLC method. In Mexico, the EWT was usually suspended on a tree at a height comparable to the upper part of human body, as *S. ochraceum s.l.* bites preferentially above the waist in Latin America.

Because of differences in the ecology and host-selection behaviour of black flies in Mesoamerica and in Africa, Toé et al. *(72)* modified the EWT for use in Burkina Faso to collect *S. damnosum s.s.* and *S. sirbanum*. They showed that EWTs made with a black background and a blue strip in the middle and placed about 1.5 cm above the ground (as *S. damnosum s.l.* females prefer biting the lower part of the body) worked well when baited with organically produced carbon dioxide and worn trousers. This modification caught a similar number of black flies as a team of VVCs, and in a few instances, outperformed the HLC technique. The modified design was named "EWT (African version)" (Fig. 8.2).

Various trials to further optimize this version of the EWT in several parts of Africa have shown promising results for its use as a complementary tool to HLCs for sampling *Simulium* vectors in the context of an elimination agenda *(68, 73)*. These trials also showed that the trap is biased for exclusive collection of host-seeking females of *Simulium* spp. vectors, providing an opportunity for direct comparison of results with those of HLC.

Further studies have been conducted to improve the performance of the EWT by identifying and using human-derived attractants for *S. damnosum s.s.* in Uganda *(74)*. Two of these compounds (naphthalene and *tert-*

hexadecyl mercaptan) were highly attractive to *S. damnosum s.s.*, and traps baited with these compounds outperformed those baited with the current standard bait of worn socks. The optimized trap design with these two new compounds can potentially collect large numbers of flies, i.e. up to 10 times more vector black flies than an HLC. Evaluation of EWTs is ongoing, and countries wishing to use the trap for bulk collection should first evaluate its performance for local vector cytospecies, as the performance has varied. Currently, in-depth data are lacking for the forest cytotypes of *S. damnosum*, *S. kilibanum*, *S. albivirgulatum* and *S. neavei*. A central technical issue is also availability of an alternative glue to that (Tanglefoot) currently being used.

Fig. 8.2. A. Esperanza window trap (original African version), which was black with a wide blue strip in the middle. The yellow 5-L plastic container holds a yeast, sugar and water mixture for organic production of carbon dioxide. The original lure (red object in the centre of the trap) was a commercial product (BG-Lure®). B. 2020 version of the EWT being used in Uganda. Note that the median black strip is smaller, and the lure most commonly used with carbon dioxide is a worn sock or trousers. Note also that the trap is raised slightly above the ground.

A

B

Source: photographs by M. Adeleke (A) and T. Unnasch (B)

The African version of the EWT is easy to construct, and all its components can be locally sourced in any part of Africa, except for Tanglefoot glue. Products similar to Tanglefoot adhesive, such as Temmen Insektenleim (http://www.temmen.de/produkte/insektenleim.htm), could be used if Tanglefoot is difficult to obtain. If acquisition of Tanglefoot or Temmen is problematic because of the high cost of importation, an alternative adhesive might be locally sourced.

There is no simple, standardized way of calibrating EWT catches with HLC biting rates, although models could be developed to relate HLC results to EWT collections *(73)*. Such models, however, would require parallel collection of data on actual biting rates on VVCs. Thus, for the foreseeable future, the EWT is best used for bulk catching to collect the number of flies necessary to meet the WHO criterion of testing a minimum of 6000 flies per transmission zone to verify suppression or interruption of transmission. Alternatively, traps could be deployed at the same time as limited VVCs to estimate the biting rate, thereby improving estimates of the annual transmission potential in areas in which fly biting densities are low.

8.6 Procedures for deploying the Esperanza window trap in the field

8.6.1 Equipment and consumables required

- yeast (*Saccharomyces cerevisiae*);
- brown sugar (containing molasses);
- water;
- polymer trap 1m x 1m (blue with black strip in the middle);
- wooden or metal frame and pegs;
- Tanglefoot glue, Temmen or local substitute;
- brush;
- rubber gloves;
- rope;
- scissors;
- copper wire;
- 5-L plastic container;
- 2.5-L plastic container;
- tubing;
- forceps;
- bijou bottles;
- white mineral spirits (or kerosene);
- isopropanol;
- human scent lure consisting of used, unwashed trousers or socks. Hendy et al. *(68)* found a pair of worn socks to be an effective odour bait. They may also be easier to acquire than worn trousers. Communities could be offered pairs of new socks to replace their worn socks, which they often welcome once they understand the reason for their use.
- commercially available compounds (naphthalene and *tert*-hexadecyl mercaptan) plus plastic aroma beads (Bitter Creek Candle Supply, www.candlesupply.com) placed in 50-mL conical centrifuge tubes; 40 g of beads impregnated with either compound cost about US$ 4.86.
- field recording book or form; and
- lead pencil (not an ink pen).

8.6.2 Field deployment of an Esperanza window trap

The current procedure for deployment of an EWT (African version) is based on earlier publications by Rodríguez-Pérez et al. *(75)* and Toé et al. *(72)*, with some modifications. The modified protocol is given below.

(i) Preparation: Preparation of organically generated carbon dioxide:

- Weigh 30 g of dried yeast.
- Weigh 500 g of sugar.
- Measure 2.5 L of clean water.
- Mix all the constituents in a 5-L plastic container.

Note: The organic carbon dioxide should be prepared at least 2 h before starting trapping, and the sugar–yeast mixture should be changed daily.

(ii) Field deployment of the trap: The steps in setting up the traps for entomological evaluation are as follows.

- Dig a few centimetres into the ground to erect the two pegs of the trap 1 m apart at the selected trapping site.
- Hook the trap firmly to the pegs at both ends with copper wire or rope, ensuring that the trap is 15–20 cm above the ground.
- Sandwich or coat the two surfaces of the trap with Tanglefoot adhesive or Temmen with the brush or spoon.
- Place the tubing connected to the plastic bottle containing the yeast–sugar mixture to release carbon dioxide to the trap. The tubing should extend to the middle of the trap, as shown in Fig. 8.2B and should be connected securely to the side or top of the trap.
- Baiting the trap with sweat-soaked trousers worn for several days (in addition to carbon dioxide) enhances catches, and sweat-soaked socks worn by a villager are also effective for luring black flies *(68)*. Worn socks were more effective as a bait than a synthetic mixture (BG-Lure).
- The worn trousers or socks lure should be removed daily at the end of trapping.
- Bait composed of plastic aroma beads impregnated with naphthalene and *tert*-hexadecyl mercaptan is also effective. The compounds should first be dissolved in mineral oil at a 1/100 concentration (w/v); this solution can be used to prepare two 10-fold dilutions in mineral oil. Aliquots of 25 g of plastic aroma beads are then placed in 50-mL conical centrifuge tubes, and the tubes are filled with the solutions. Place the tubes on a tube rotor, and allow the beads to absorb the solutions for 2 days. Decant the excess solution from the beads, and seal the tubes *(74)*. The bait should be replaced weekly.
- Ensure that the traps are set perpendicular to the breeding sites in partially shaded or slightly exposed areas, as shown. **Placement of the traps in totally shaded areas or excessively exposed sites may result in poor catches**.
- Tanglefoot or Temen is effective for up to 1 month and should then be replaced by removing the adhesive with kerosene and applying fresh Tanglefoot or a similar compound.
- Traps should be kept clean before trapping by removing any insects or leaves trapped overnight with forceps.
- During the rainy season, traps can be protected by wrapping them in white polymer plastic (Fig. 8.3).

Note: Tanglefoot can be diluted with kerosene to thin it so that it is easy to transfer to the trap. Only a thin coating is required, and over-application should be avoided. After application, the trap should be allowed to stand for at least 24 h before it is used for collection, allowing the kerosene to evaporate completely. If Temen is used, it may be diluted with white spirit. Other glues may require different procedures and solvents.

Fig. 8.3. Protection of an older Esperanza window trap with a plastic covering during rainfall.

Source: photograph by M. Adeleke

(iii) Recovery of flies from the trap: The steps in recovery of flies from the traps are:

- Recover trapped *S. damnosum* once (at 17:00) or twice (at 12.00 and 17:00) daily using forceps.
- Put the black flies in a plastic container or kidney tray containing white mineral spirits or kerosene.
- Allow the sample to remain in the solvent for a few minutes; then, turn it slightly to remove all Tanglefoot.
- Count the number of the black flies trapped, and transfer them to a bijou bottle containing 80% alcohol.
- Remove all other insects from the trap every hour to keep it clean and before starting daily trapping.

Note: One person can operate two or three traps effectively each day.

8.7 Sorting and identification of flies

Experience in the field has shown that flies other than *S. damnosum s.l.* are often collected by VVCs and EWTs. While these are usually sweat bees, other Dipterans and some species of Simuliidae have been recovered in EWT catches. Sorting and proper identification of the flies collected by VVCs and EWT is therefore necessary to ensure that only female *S. damnosum s.l.* are tested in a PCR pool screening assay. The flies collected should be identified under a dissecting binocular microscope with appropriate identification keys (see Chapter 4).

8.8 Completion of field records and forms

Accurate recording of activities is important for the integrity of data obtained in the field. Proper training of entomology technicians and VVCs in field recording should be conducted prior to the commencement of activities. It is desirable that at least one of the VVCs be literate to ensure proper completion of the forms. Below are examples of forms used to record entomological activities in Nigeria.

Form 8.1. Entomological field form 1A: Human landing catches

State ..... District/Province ..... Village (H/S) .....

Catching site ..... Code .....

River (M/T) .....

Name of collector A ..... Name of collector B .....

Date .....

Hours	**No. of *S. damnosum s.l.* and non-*S. damnosum* collected***	**Remarks, weather conditions**
07:00–08:00		
08:00–09:00		
09:00–10:00		
10:00–11:00		
11:00–12:00		
12:00–13:00		
13:00–14:00		
14:00–15:00		
15:00–16:00		
16:00–17:00		
17:00–18:00		
Total for the day		

H, high-risk village; S, second-line village; M, main river; T, tributary

*For comparison only

Form 8.2. Recording form for Esperanza window trap catches

State ..... Local government area ..... Village (H/S) .....

Catching site ..... Code .....

River (M/T) .....

Name of collector .....

Date .....

Day/week	Time of collection	No. of *S. damnosum s.l.* and non-*S. damnosum* collected*	Remarks, weather conditions
1			
2			
3			
4			
5			
6			

*For comparison only

Form 8.3. Entomological summary field form for monthly supervision and data recovery

State/Province ..... Transmission zone .....

Number of catching points ..... No. of traps .....

Number of human landing collectors .....

Month	Week	Number of *S. damnosum s.l.* collected			Remarks
		Human bait	Traps	Total	

Name of state or provincial coordinator .....

Signature ..... Date .....

Chapter 9.

Dissection of female black flies to determine parity and infectivity

Learning outcomes for Chapter 9

By the end of this chapter, the reader should be able to:

- understand the reasons for dissecting female *Simulium* spp. after capture;
- identify the fly before dissection;
- dissect a female black fly;
- determine the physiological age of female black flies (nulliparous and parous flies);
- determine the infectivity of parous flies with *O. volvulus* (when possible); and
- summarize and analyse the results of entomological assessments after dissection.

9.1 Introduction

This chapter describes important aspects of the dissection of female black flies to determine parity and infectivity, with particular reference to species found in Africa. In the absence of PCR, the easiest way to determine key transmission indices of *O. volvulus* is to dissect and analyse wild-caught adult female *Simulium* vector species. In the past, the results of dissection were used for deciding on vector control operations and evaluating their progress.

Dissection of adult female black flies caught with a human landing method or trap (Chapter 8) allows determination of the physiological age of the flies, possible infection and intensity of infection with *O. volvulus* and, crucially, infectivity (flies harbouring *O. volvulus* L3s) at the location of capture. Dissection also indicates whether the flies originate from local breeding sites or are influxes of migrating flies.

9.2 Identification of flies to be dissected

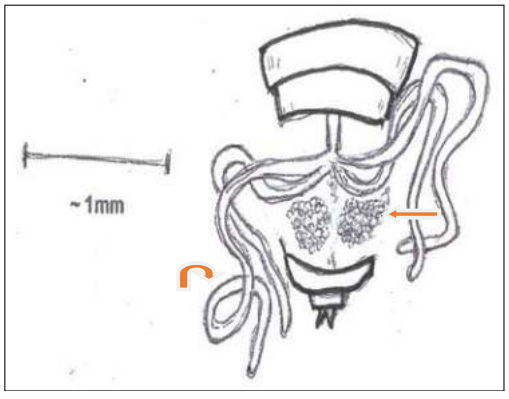
Before dissecting a female black fly, the first action is to ensure that the fly is a vector species of *Simulium* and that it is a female. Identification of *Simulium* vector flies and determination of their sex is discussed in Chapters 2 and 4.

9.3 Dissection of female black flies

Female black flies are dissected under a binocular microscope or a field microscope. Before dissection, the flies are anaesthetized with carbon dioxide or by placing them in a cold environment such as a freezer. Flies can also be immobilized by dropping them into a solution of detergent. After immobilization, they can be deposited one at a time in a drop of normal saline on a glass slide.

After examination of the morphological characters to confirm their identify, the tip of the abdomen is examined for the presence of a spermatophore (Chapter 2). If one is present, the fly has recently emerged and has not taken a blood meal. Dissecting needles are then used to split the end of the abdomen in order to withdraw the Malpighian tubules and ovaries to determine parity (Fig. 9.1).

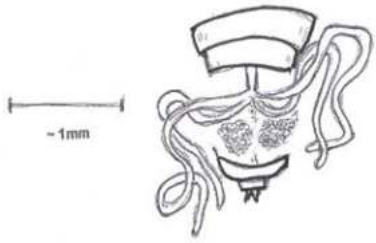
Fig. 9.1. Dissection of a female black fly. The large round structures are the Malpighian tubules (curved arrow, left), and the circular structures are the ovaries (straight arrow, centre).



9.4 Physiological age of female black flies

Females of haematophagous insects such as *Simulium* spp. lay their eggs in batches – one batch after each blood meal. There is thus a cycle of egg development in the ovarioles (the linear tubes of an insect ovary that contains the ovarian follicle and an oocyte). Only flies that have taken a blood meal and then laid at least one batch of eggs before biting again are likely to carry *Onchocerca* spp. parasites. Those insects that have not laid eggs are nulliparous, while those that have laid at least one batch of eggs are parous. These terms refer to what is called the physiological age of the female flies. Only parous flies that could have picked up parasites during their first or subsequent blood meals can harbour *O. volvulus*, and only those that bite for their third or later blood meal can transmit the parasite, because of the time required for development from microfilariae to the infective stage (L3). The features of parous and nulliparous *S. damnosum s.l.* flies are shown in Table 9.1.

Table 9.1. Morphological characteristics of parous and nulliparous female flies of the *S. damnosum* complex.

Dissection		
Differences	Nulliparous flies	Parous flies
Malpighian tubules	 Opaque under transmitted light	 Usually clear under transmitted light
General appearance of ovaries	 Nulliparous ovaries are generally clear, with slightly larger, more spherical, turgid follicles. Voluminous fat body is usually present	 Parous ovaries are yellowish, with a speckled appearance usually due to follicular relics. The follicles are slightly smaller and more ovoid. Fat body is present only occasionally.
Stretched appearance of the ovaries	 Nulliparous ovaries stretched only slightly and abruptly break	 Parous ovaries stretch as much as three times their original length and then tear slowly.
Stretched appearance of the follicles	 The space below the follicle is clear in nulliparous ovaries.	 The space beside the follicle contains a follicular relic (the dark dot) in parous ovaries.
Notes	The presence of a few fully developed retained eggs with undeveloped follicles indicates the parous state. Ovaries are best examined in saline solution (0.45%) under a stereomicroscope with strong light from below and at about x 50 magnification	

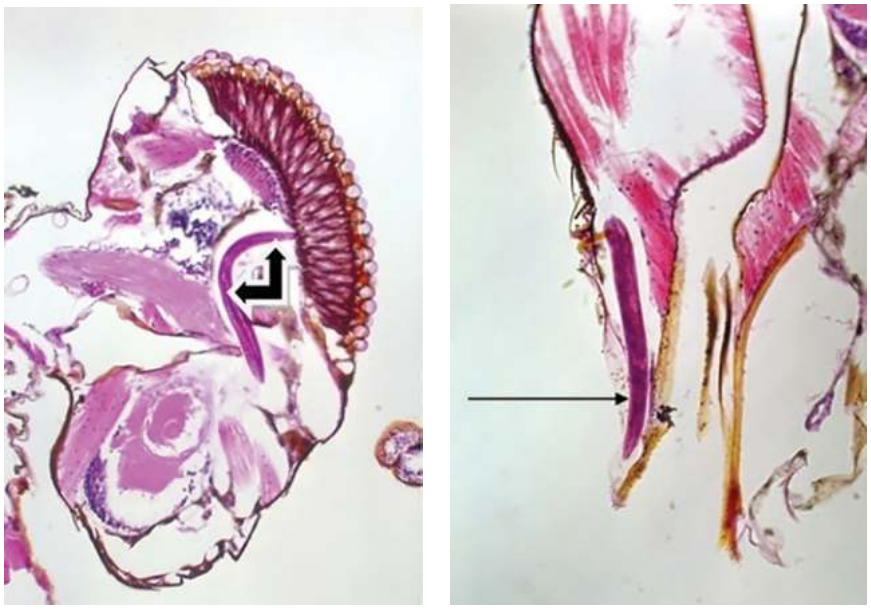
Source: reference *54*

The physiological age can be determined unequivocally by examining the ovaries or by finding developing *Onchocerca* spp. worms (other than microfilariae that may have been inadvertently taken up from a vector collector) within a fly, while other characters, such as the Malpighian tubules and abdominal fat bodies, provide supporting evidence for a decision. Dissection allows separation of the fly sample into nulliparous and parous females.

9.5 Determination of fly infectivity

After physiological age has been determined, the flies can either be dissected fresh or, especially if there are time constraints, preserved in 70–80% alcohol for later staining with Mayer's Haemalum to clearly reveal any parasites, as described by Garms & Cheke *(76)*. A female classified as parous is then separated into head, thorax and abdomen, each of which is later torn apart in a physiological saline drop (or in 25–50% acetic acid with a drop of glycerol for stained specimens) and thoroughly examined for *O. volvulus* larvae. These may be distributed in the head (L3 or infective larvae) (Fig. 9.2), the thorax ("sausage" L1, L2, L3) and the abdomen (all stages possible but usually L3).

Fig. 9.2. Infective stage larvae (L3s) in the head of *S. ochraceum*. A. Black arrows point to a coiled L3 positioned behind the compound eye of a female fly. B. *O. volvulus* L3 (arrow) in the mouthparts (membranous tip of the labrum-epipharynx).



A B

Source: photographs from the archives of the United States Centers for Disease Control and Prevention

In dissection, it is very difficult to separate the infective larval stage of *O. volvulus* from those of non-human origin such as *O. ochengi* because of their close morphological similarities. The current diagnostic procedure for identifying *O. volvulus* larvae to determine fly infectivity is PCR, which has the advantage of specificity and can also be used to estimate the ATP (see Chapter 11). Flies fixed in acetic acid cannot, however, be used for PCR because of the effects of the acid on parasite DNA. Furthermore, pool screening cannot be used to determine parous rates, statistics such as numbers of infective larvae per infective fly or the degree of aggregation of parasites in vector populations. Also, if only black fly heads are used in the PCR analysis early in an MDA campaign, transmission rates based on pool-screening may be underestimates of the full extent of L3s in the vector population. Nevertheless, pool-screening has the major advantage of accurately distinguishing *O. volvulus* from other *Onchocerca* species.

9.6 Other parasites sometimes found in *Simulium* vectors

Other parasites, some of which can be confused with *O. volvulus*, may be encountered when dissecting black flies. In addition to larvae of the bovine species, *O. ochengi*, other *Onchocerca* species such as *O. ramachandrini* and filariae of other helminths, probably including bird parasites, may be found. Furthermore, nulliparous flies are often, and parous flies very rarely, parasitized by mermithid worms that a novice might mistake for *Onchocerca* larvae, although mermithids are much bigger. Other organisms that may be puzzling at first sight include ciliates, hymenopteran planidial larvae, fungi and ectoparasitic water mites attached to the outer surfaces of black flies.

9.7 Dissection record-keeping

Accurate records must be kept of all dissections so that critical epidemiological indicators can be calculated and recorded. This is especially true for data to be used in a country-wide elimination dossier. Strict transmission criteria have been established by WHO, and original records may be audited as part of verification. The type of detail required is summarized in the box below. Given the large number of flies that must be collected and evaluated to meet WHO verification criteria, dissection would not be used to satisfy the entomological aspect of the verification criteria. It can, however, be used for the regular surveillance required by the guidelines, on the understanding that transmission indices may be overestimated for non-*O. volvulus* parasites that are transmitted in the evaluation area.

Example of a summary result sheet for entomological impact assessment

State ..... District ..... Village (H/S) .....

Catching site ..... Code .....

River (M/T) .....

Name of collector .....

Date ..... GPS coordinates .....

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Total
No. of catching days													
No. caught													
MBR													
No. dissected (%)													
No. parous													
% parous													
No. parous infected													
% infection rate													
No. infective flies													
% infective rate of parous													
Total No. head L3													
MBR													
L3 (head)/1000 parous flies													
MTP (head)													

L3, infective larvae; MBR, monthly biting rate; MTR, monthly transmission potential

Chapter 10.

Preparation of female black flies for the polymerase chain reaction pool screening assay and interpretation of results for onchocerciasis elimination

Learning outcomes for Chapter 10

By the end of this chapter, the reader should be able to:

- understand the principle of the PCR pool screening assay;
- prepare black flies for the PCR pool screening assay;
- interpret the results generated by pool screening software; and
- appreciate the importance of the PCR pool screening assay in determining elimination of onchocerciasis.

10.1 Use of the polymerase chain reaction pool screening assay to determine black fly infectivity

The classical method of dissecting adult flies to determine infection has been used for decades to understand the dynamics of infectivity and the magnitude of parasite transmission by a number of vector species. This method was used by WHO upon establishment of the OCP in 1974 to determine the extent of *O. volvulus* transmission and the impact of weekly larviciding on fly infectivity in the control areas of West Africa *(77, 78)*. The dissection method has, however, two critical limitations. First, the method cannot be used to distinguish *O. ochengi* (a cattle parasite) from *O. volvulus,* as the parasites are largely indistinguishable morphologically under a dissecting microscope *(79)*. This has profound implications for calculating transmission indices in a control area (Chapter 11). Secondly, after years of control, the method requires skilled entomologists and technicians to dissect numerous black flies in order to detect the parasite in areas where the infectivity level is extremely low. Given these two limitations, evolving knowledge in molecular biology was used to find a better method for estimating vector infectivity in order to identify *O. volvulus* specifically for calculating parasite prevalence and transmission indices (e.g. ATP). This work led to development of the PCR pool screening assay.

10.2 Principle of the polymerase chain reaction pool screening assay

The PCR involves enzymatic amplification (multiplication) of a particular DNA sequence to produce millions of copies of that DNA. The technique was developed in 1983 by Dr Kary Mullis, who was awarded a Nobel Prize for his discovery. The assay is based on enzymes known as DNA Taq polymerase, which are extracted from the heat-tolerant bacterium *Thermus aquaticus*. The method is highly sensitive and requires only small amounts of sample DNA for detection *(80)*. In 1991, PCR amplification of an *Onchocerca*-specific repeated DNA sequence (the O-150 sequence) was achieved *(81)*. Thus, PCR can be used to test individual flies or, more commonly, pools of the heads or bodies of flies to detect *O. volvulus* DNA.

Katholi et al. *(82)* developed a mathematical model for calculating the prevalence of infection in a black fly population in a pool screening assay. Pool screening (also referred to as "group testing") is designed for estimating the prevalence of a factor in a population *(83)*. Unlike the traditional dissection method, which requires observation of individual flies, the pool screening technique allows researchers to test samples in groups or pools. For example, the O-150 PCR pool screening assay can detect a single infected fly in pools of up to 200 black flies *(84)* and has been used to estimate *O. volvulus* vector infectivity and transmission in various disease foci with different black fly species in Africa and in the Americas *(32, 79, 85–90)*.

10.3 Preparation of black flies for the polymerase chain reaction pool screening assay

10.3.1 Equipment and consumables

- glassware of various sizes,
- 95% alcohol, watch glass,
- 25-mesh brass sieve,
- Eppendorf tubes (1.5 mL) and
- forceps.

10.3.2 Procedures for preparing flies

The infective stage of *O. volvulus* is transmitted mainly from L3s found in the head of the vector. During the initial phase of MDA with ivermectin (Mectizan®), it was found that the DNA of *O. volvulus* larvae could be detected in the head, thorax and abdomen of flies by PCR pool screening; therefore, in early protocols, heads and the remaining body parts were tested separately. As MDA progressed and reduced the presence of microfilariae in transmission zones, however, it became apparent that it was unnecessary to test all body parts. Therefore, to save time and laboratory resources, many mature control programmes moving toward elimination now test only the heads of vectors, and data on vector heads only are used to calculate transmission indices, as stipulated in the WHO guidelines *(27)*. The heads must therefore be separated from the body before PCR analysis.

The procedure is as follows.

1. Use flies that have been preserved in isopropanol or ethanol. Rinse the flies in 95% ethanol, and pour them into a plastic container or onto weighing paper. Allow the ethanol to evaporate until the flies appear to be dry.
2. Divide the flies into pools. Do not combine flies from different breeding sites in the same pool. Each pool can contain up to 200 flies, although PCR may be difficult when more than 100 flies are included in a pool (see DNA extraction). Do not allow the flies to desiccate completely; otherwise, head purification will not work.
3. Place the flies from one pool in a clean, dry 15-mL conical polypropylene centrifuge tube.
4. Place the tubes at –80 °C or on dry ice overnight or in the vapour phase of liquid nitrogen for 30 min.
5. Place individual tubes containing frozen flies into the fingers of a regular latex glove. Five tubes can be placed in one glove (one per finger). Snap off the heads of the flies by holding the sleeve of the glove and pounding the fingers against a bench. The tubes can be prevented from cracking by placing a foam pad (such as an old computer mouse pad) on the bench surface.
6. Collect the flies from the tubes by re-suspending them in 95% ethanol and removing them with a wide-bore pipette, such as a plastic transfer pipette. The amount of ethanol is not important, but as much fly material as possible should be recovered.
7. Pour the ethanol–fly mix through a 25-mesh sieve attached to a collection pan (Fig. 10.1A, B). The bodies will collect on the 25-mesh sieve (Fig. 10.2A), and the heads will pass through and collect in the pan (Fig. 10.2B). Rinse the flies collected on the sieve to ensure that all the heads have passed through.
8. Collect the fly heads from the pan with ethanol (Fig. 10.2). Put the heads in a weigh boat, and remove as much alcohol as possible; then, allow the heads to dry at room temperature.
9. The bodies can be disposed of or stored in ethanol at 4 °C if additional work is planned.

This protocol was designed for efficient separation of heads and bodies from *S. damnosum s.l.* Other vector species may be larger or smaller and may require different-size mesh sieves.

Fig. 10.1. A, Stacked sieve on collection pan with 25-mesh sieve (red arrow) on top. B, Collection pan.

A B

Source: photographs by H. Hassan, University of South Florida School of Public Health

Fig. 10.2. A, Fly bodies on 25-mesh sieve. B, Fly heads (dots) collected in the pan.

A B

Source: photographs by H. Hassan, University of South Florida School of Public Health

10.4 DNA extraction and the pool screening assay

This assay requires a standard molecular biology laboratory and trained personnel. Black fly heads contain large quantities of compounds that inhibit PCR reactions and often co-purify with DNA extracted from the heads. Thus, it is important to carefully choose and validate the DNA extraction method to be used. Silica-based methods of DNA purification, either home-made *(85)* or commercial DNA extractions kits, all work well for black fly heads. Magnetic bead capture of parasite DNA from fly head homogenates has also been shown to be effective *(84)*. Different purification methods vary, however, in their ability to remove inhibitors. Thus, preliminary mixing experiments should be conducted for any method to determine the maximum number of heads that can be processed in a single pool without inhibiting the subsequent PCR assay. It is generally safe to pool 100 fly heads.

O-150 PCR amplification and detection

The current WHO guidelines recommend use of PCR assays that target the O-150 repeat, a tandemly repeated DNA sequence present in the genome of *Onchocerca* parasites. Several protocols for PCR pool screening of black flies that target this sequence have been published, including by Katholi et al. *(82)*, Fischer et al. *(91)*, Guevera et al. *(86)*, Adeleke et al. *(87)*, Gopal et al. *(84)* and Rodríguez-Pérez et al. *(69)*. The most commonly used assay is the O-150 PCR enzyme-linked immunosorbent assay *(92)*, in which PCR amplicons produced from the O-150 repeat are detected by hybridization to the *O. volvulus*-specific oligonucleotide probe OVS2 *(93)*. More recently, several isothermal and real-time PCR assays have been reported for detecting O-150 DNA *(94–97)*. Any of these assays might be adapted to detect the presence of *O. volvulus* DNA in the heads of vector black flies, although, in adapting these assays for this purpose, their ability to distinguish *O. volvulus* from other sympatric *Onchocerca* species, such as *O. ochengi*, must be demonstrated. Alternatively, positive pools can be re-tested with an assay that unambiguously identifies *O. volvulus* to confirm the positive pools as containing *O. volvulus*, such as amplification of portions of the parasite mitochondrial genome, followed by DNA sequence confirmation of the amplicon *(98)*.

10.5 Interpretation of the results of pool screening assays with pool screen software

The prevalence of infection in head pools is usually determined with pool screen software. The software is a computer program developed by Katholi et al. *(82)*, and a version modified by Katholi in 2010 is available from WHO by request. Pool screen software provides an estimated value of the prevalence of infection with 95% confidence intervals (CIs) for the estimate according to the pool size, the number of pools examined and the number of positive pools *(79, 84)*. The software can also provide an estimated ATP for the vector population examined if detailed collection data are available. It is easy to operate and requires minimal training.

The software usually generates three estimates of the point prevalence of flies carrying *O. volvulus* L3s – the maximum likelihood, method of moments and Bayes posterior – and four estimates of the corresponding 95% CIs – likelihood ratio, Cloperson, asymptotic normal and Bayes credibility method. Simulations have shown that the maximum likelihood method of calculating point estimates is the most accurate, while the Bayes credibility interval is the most accurate estimate of the 95% CI. These values should be recorded and reported.

Notes:

- Pool screening of female black flies usually requires PCR analysis of at least 6000 flies per transmission zone according to WHO elimination criteria.
- In a transmission zone in which 6000 flies cannot be collected after 12 months, the ATP or seasonal transmission potential generated by pool screen software can be used.
- The WHO guidelines stipulate that, for a focus to meet the criteria for elimination, the upper bound of the 95% CI of the prevalence of flies carrying the infective parasite stage (L3) in the head must be < 0.1% (< 1/1000) for parous flies or < 0.05% (< 1/2000) for all flies (assuming a parity rate of 50%) *(26)*.

When using ATP criteria, ATP values with a 95% CI of < 20 L3/person per year are required to satisfy the criteria for interruption of transmission *(27)*.

For example, let us assume that entomological evaluations were conducted in two transmission foci to determine the current status of *O. volvulus* transmission after 12 years of repeated ivermectin treatment. Black fly samples were collected and screened with the PCR pool screening method. The result generated by pool screen software is shown in Table 10.1.

Table 10.1. Examples of calculated prevalence of *O. volvulus* in the head pools of black flies from two transmission zones.

Transmission zone	**No. of flies pool screened**	**No. of pools**	**No of pools positive for *O. volvulus***	**Prevalence[a] (% positive)**	**Confidence interval (% positive)[b]**
Owawde	9001	90	0	0.00	0.00; 0.02
Akutipal	7000	70	9	0.14	0.06; 0.2

[a] Maximum likelihood estimated prevalence
[b] Bayes credible interval

Table 10.1 shows that the upper bound interval of prevalence (at 95% CI) is < 0.05% at Owawde but > 0.05% at Akutipal. Therefore, it can be concluded that *O. volvulus* transmission has been interrupted at Owawde (provided that the transmission zone also meets the WHO serological criteria for stopping MDA) while there is continued transmission of the parasite at Akutipal.

Chapter 11.

Determination and interpretation of transmission indices in onchocerciasis elimination

Learning outcomes for Chapter 11

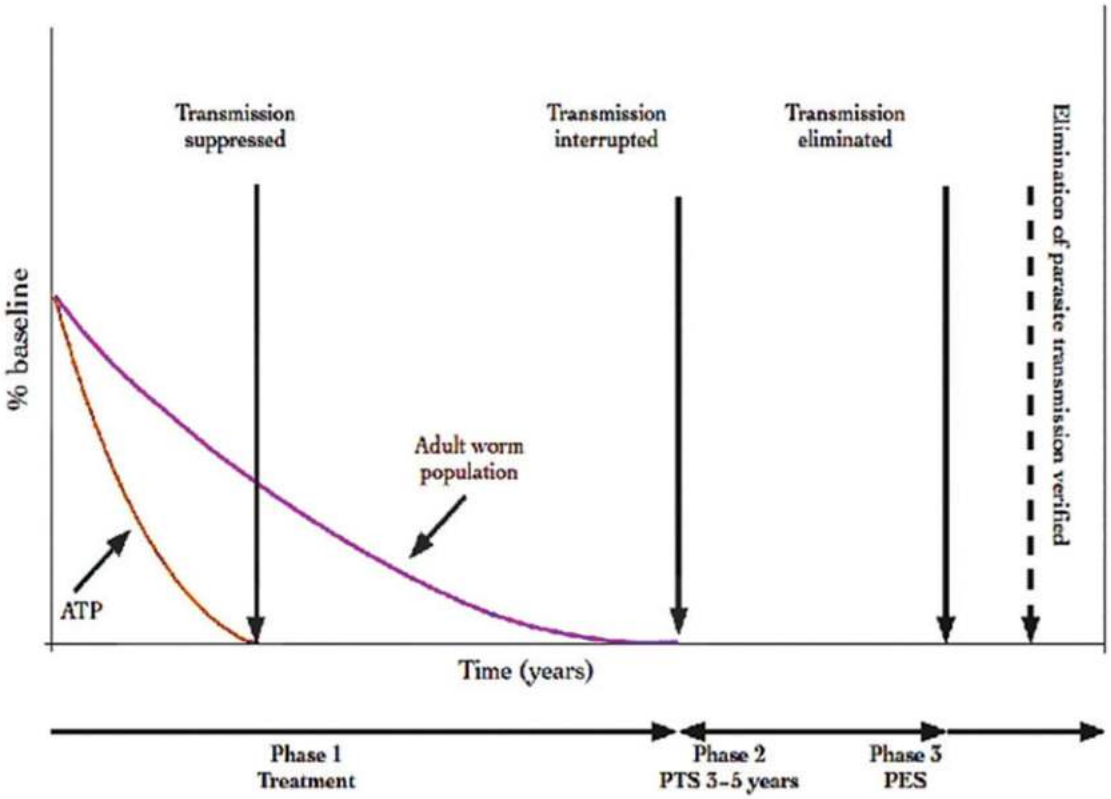
By the end of this chapter, the reader should be able to:

- understand the process of verification of elimination of onchocerciasis;
- calculate the biting rates and transmission potential of black flies in a focus, including MBRs and ABRs; MTP and ATP; the monthly infective biting rate and the number of infective larvae in parous flies; and
- interpret transmission indices in the context of onchocerciasis elimination.

11.1 Onchocerciasis elimination

Onchocerciasis elimination consists of three major phases *(27)* (Fig. 11.1).

Fig. 11.1. Temporal sequence of results of MDA for elimination of *O. volvulus*. Reduction in ATP results in elimination of new infections, followed by the demise of the adult worm population due to the effects of drugs and senescence.



ATP, annual transmission potential; PES, post-elimination surveillance; PTS, post-treatment surveillance

11.1.1 Phase 1

The first phase, the intervention or treatment phase, involves continuous MDA of ivermectin with a minimum coverage of either 65% of the total population or 80% of persons eligible to receive treatment and 100% geographical coverage over 12–15 years or longer (Fig. 11.1). MDA reduces the microfilarial load in the skin, eventually reducing the ATP below that necessary to maintain the parasite population (Fig. 11.1, yellow line), and the adult worm population undergoes complete senescence over time (Fig. 11.1, purple line) *(99)*. The time required to achieve phase 1 will depend on the coverage attained, the frequency of MDA (once, twice or more per year), initial baseline transmission characteristics, vectorial capacity and any vector control interventions. At the end of this phase, both entomological and serological evaluations should be conducted to demonstrate interruption of transmission.

11.1.2 Phase 2

Post-treatment surveillance begins after demonstration that transmission has been interrupted according to standard WHO criteria. All control interventions (e.g. ivermectin MDA and vector control) are stopped to

demonstrate that treatment has been successful. This phase lasts for 3–5 years. At its end, entomological evaluations are conducted to demonstrate elimination of transmission.

11.1.3 Phase 3

Post-elimination surveillance begins after demonstration that transmission has been eliminated. Surveillance is necessary to provide convincing statistical evidence that *O. volvulus* transmission has been permanently interrupted and the parasite population eliminated. Once all programme areas in a country have entered phase 3, the ministry of health may apply for verification of elimination by WHO. Post-elimination phase activities must continue until regional elimination has been verified.

To date, both phase 2 and phase 3 have been based primarily on annual entomological evaluation. As fly populations feed on human hosts over a relatively wide geographical area, they are early indicators of the recrudescence of transmission.

11.2 Transmission indices used before polymerase chain reaction

The concept of quantifying transmission of *O. volvulus* was pioneered by Dr Brian Duke in early studies conducted in Cameroon (for example, *100*). Metrics such as the MBR or ABR and the MTP and ATP of parasite L3s provided the basis for calculating vector infection rates, relating them to levels of disease in the human population *(101)* and, later, assessing the effectiveness of control programmes. For example, biting rates and transmission potentials were the two indices used to evaluate progress in the OCP *(102, 103)*.

To establish a yearly standard, biting rates are used to calculate the ATP, an epidemiological standard in onchocerciasis epidemiology. The formulae are shown below.

11.2.1 Monthly biting rate (MBR) and annual biting rate (ABR)

The MBR is a general estimate of the number of bites that a person exposed to a *Simulium* vector population will receive in a month. It is probably an overestimate, as not all flies that land on a person successfully obtain a blood meal. The MBR is calculated as follows, from human landing catch data (see Chapter 8):

$$\text{MBR} = \frac{\text{Geometric mean no. of flies caught per day per person} \times \text{No. of days in month}}{\text{No. of catching days}}$$

The ABR is an estimate of the number of bites that a person exposed to a *Simulium* vector population will receive in a year. The ABR is calculated as follows, from human landing catch data:

$$\text{ABR} = \frac{\text{No. of flies caught} \times \text{No. of days in year}}{\text{No. of catching days}}$$

Biting rates were of significant importance in earlier control programmes and remain so for evaluating onchocerciasis elimination indices. In the past, many communities in Africa were abandoned because of

intense black fly biting (see Chapter 1); however, during the vector control activities of the OCP, an ABR less than 1000 bites per person in a savannah zone was considered acceptable for re-population of previously abandoned areas *(104)*.

11.2.2 Monthly transmission potential (MTP) and annual transmission potential (ATP)

MTP is an estimate of the number of infective *O. volvulus* larvae (i.e. L3s) that a person will receive if exposed continuously to the local black fly population for a month. The MTP for larvae in the head (H) is calculated as follows:

$$\text{MTP(H)} = \frac{\text{MBR} \times \text{No. flies withL3 larvae in the head}}{\text{No. of flies dissected monthly}}$$

Another version of the MTP formula was used before MDA programmes began, when flies were dissected (Chapter 8) and the *O. volvulus* PCR pool screen was not yet available, which included L3 larvae in the head, thorax and abdomen (A):

$$\text{MTP(A)} = \frac{\text{MBR} \times \text{No. of all L3 larvae}}{\text{No. of flies examined}}$$

In a study to determine the number of *O. volvulus* L3s that escape from infective *S. damnosum* females (a forest cytospecies) during blood-feeding, Duke *(105)* estimated that the highest proportion of infective larvae escaped from the head of the fly and that heavily infected flies shed most of their infective larvae when blood-feeding. His findings suggested that remaining L3s in the abdomen are possibly pushed into the fly's head by hydraulic pressure of the blood meal as the midgut fills. L3s in the thorax were assumed to have just moulted to that stage and could therefore not be transmitted. Consequently, a count of all L3s in the body probably provides an overestimate of transmission potential.

ATP is an estimate of the number of infective *O. volvulus* larvae that a person will receive if exposed to a *Simulium* vector population during a year. The ATP is calculated as follows:

$$\text{ATP(H)} = \frac{\text{ABR} \times \text{No. of flies withL3 larvae in the head}}{\text{No. of flies dissected annually}}$$

$$\text{ATP(A)} = \frac{\text{ABR} \times \text{No. of all L3 larvae}}{\text{No. of flies dissected annually}}$$

MTPs may be summed to determine the ATP.

The MTP and the ATP can also be calculated when PCR pool screen analysis of the heads of flies is used to determine infectivity:

$$\text{MTP} = \text{MBR} \times \text{proportion of infective black flies as determined by pool screen analysis}$$

$$\text{ATP} = \text{ABR} \times \text{proportion of infective black flies as determined by pool screen analysis}$$

These indices are based on the assumption that all flies are examined either by dissection or PCR and might have to be adjusted if only a subsample of captured flies is examined.

MTP and ATP, which are relative estimates, are the standard indicators of the intensity of parasite transmission. During the OCP, an ATP of 100 was accepted as a tolerable limit in savannah areas *(106)*, as that value was below the ATP threshold associated with the onset of blindness. Nevertheless, the parasite remained endemic in many areas of the control territory. Current WHO guidelines accept an ATP < 20 L3/person as an indicator of the interruption of transmission when < 6000 flies can be caught for an entomological survey.

Note that *O. volvulus* PCR pool screen software is capable of calculating both seasonal transmission potential and ATP, with the relevant 95% CIs. See section 10.5.

11.2.3 Monthly parous biting rate

As discussed in Chapter 9, the *Simulium* spp. vector population is composed of both nulliparous and parous individuals. From an epidemiological perspective, the parous portion is the most important, as this group has previously taken a blood meal and is potentially infected with *O. volvulus*. To determine the actual number of infective flies in a population, only parous flies should be evaluated. This often requires examination of a population at a certain time of day (parous *S. damnosum s.l.* often seek hosts most actively in the morning) and in defined locations (usually close to riverine breeding sites) *(5, 107)*.

The monthly parous biting rate is now widely used, particularly near large breeding sites, where a significant proportion of biting flies can be expected to be newly emerged nullipars and in areas of re-invasion, where the proportion of parous flies may be unusually high.

$$\text{Monthly parous biting rate} = \frac{\text{No. of parous flies} \times \text{days in month}}{\text{No. of catching days}}$$

The monthly parous biting rate can be used to adjust the ATP and MTP so that they are specific for parous flies.

11.2.4 Number of infective larvae per 1000 parous flies

This index indicates the efficiency of *O. volvulus* L3 transmission specifically by the parous population and is not affected by the overall number of flies in the population.

$$\text{L3/1000 parous} = \frac{\text{No. of L3 larvae recovered (from the head) in the sample} \times 1000}{\text{No. of parous flies in the sample}}$$

11.3 Transmission indices of black flies in the context of onchocerciasis elimination

Entomological surveillance is conducted to determine changes in the distribution and density of vectors and their infection rate. Early in a programme, these indices reflect the endemicity of onchocerciasis, i.e. the ATP is higher in areas that are hyperendemic than in mesoendemic and hypoendemic areas. Monitoring these indices over time allows the entomologist to evaluate the impact of interventions and to recommend changes to a programme.

In many programmes, the level of parasite transmission over time is not determined; however, all elimination programmes must include measurement of infectivity in order to justify stopping MDA and to demonstrate successful completion of post-treatment surveillance (Fig. 11.1). The current WHO guidelines for stopping drug treatment and for verification of the elimination of human onchocerciasis require that a minimum of 6000 flies in a transmission zone be analysed to demonstrate that the upper bound of the 95% CI of the prevalence of flies carrying infective L3 larvae in the head is < 0.1% (< 1 infective fly/1000 flies) in parous flies or < 0.05% (< 1 infective fly/2000 flies) in all flies (assuming a parity rate of 50%) *(27)*. Infectivity is determined by performing O-150 PCR of the heads of the flies captured and analysing the results with pool screen software. If the minimum number of flies cannot be captured with sufficient effort, demonstration that the upper bound of the 95% CI of the estimated ATP is < 20 L3/person per year is an acceptable alternative.

The method for determining the prevalence of black flies carrying infective larvae of *O. volvulus* has been field dissection, which, however, had several drawbacks.

- Dissection of female flies is expensive, because it requires a trained entomologist and a field microscope, and is time-consuming.
- *S. damnosum s.l.*, the major vector species of *O. volvulus* in Africa, may also be the vector for several zoonotic species of *Onchocerca (108)*. The L3 of several of these species cannot be distinguished morphologically from those of *O. volvulus*, and their inclusion in dissection records yields falsely elevated estimates of transmission.

For this reason, dissection is not used to meet the WHO criteria specified in the 2016 guidelines *(27)*. Dissection may be useful for rapid surveys during longitudinal surveillance and to determine parity and parous biting rates.

Collection of at least 6000 flies has been a challenge in some locations. Programmes have tried to overcome this by increasing the number of collection days and collecting flies for an additional year (as there may be year-to-year variation in the productivity of breeding sites). The Esperanza window trap (see Chapter 8) may increase the yield of fly collections.

Chapter 12.

Black fly control in onchocerciasis elimination programmes

Learning outcomes for Chapter 12

By the end of this chapter, the reader should be able to:

- appreciate the historical background of vector control as a strategy for onchocerciasis elimination;
- determine the information necessary for selecting a sound black fly vector control strategy;
- understand the characteristics of the principal insecticides and larvicides available;
- understand the general method of larviciding, particularly ground larviciding;
- understand the management of resistance to larvicides; and
- understand the basics and potential impact of community-based vector control methods.

12.1 Introduction

12.1.1 Historical perspective

Large-scale black fly control programmes with larvicides have been implemented on several continents. Two early programmes were conducted in Canada and Kenya after identification of DDT as an effective, inexpensive larvicide by Fairchild and Barreda in 1945 *(109)*. Programmes were organized in Saskatchewan, Canada, to control *S. arcticum* and in Kenya to eliminate transmission of *O. volvulus* by *S. neavei*. The Canadian programme, begun in 1948, initially involved aerial application of DDT *(110)*, whereas larviciding in Kenya was conducted by dripping DDT into rivers and streams after extensive mapping of vector distribution *(111)*. As elimination of *S. neavei* occurred over a 9-year period *(112)*, valuable information on the average life span of *O. volvulus* adult females was obtained and served as a baseline for timing the more extensive OCP programme in West Africa (Chapter 1).

Fig. 12.1. A, Aerial application of temephos as a larvicide in a river in West Africa. B, Movement of the larvicide down the river, forming a wide front as it disperses.

A B

Source: photographs by E. Cupp

For about two decades (1974–1990), the OCP relied exclusively on prolonged, regular aerial spraying of environmentally safe larvicides on *S. damnosum s.l.* breeding sites (Fig. 12.1). This was one of the more successful vector control programmes in terms of its impact on local health and economic development; however, despite its benefits, the high costs associated with aerial spraying limited its application and sustainability in other areas.

With the advent of Mectizan® (ivermectin) in 1987 as a new treatment and control strategy, vector control became secondary for containing river blindness. Use of ivermectin in MDA was a sustainable strategy that has led to significant gains in Africa and the Americas (Chapter 1). Despite the overall success of MDA with ivermectin, however, recent epidemiological models suggest that elimination of *O. volvulus* in Africa during the next decade will be difficult in areas where the parasite is or has been highly endemic *(28)*. Interventions to interrupt transmission in these areas will probably include MDA several times a year and vector control. Lakwo et al. *(113)* noted that interruption of transmission was accelerated when vector control was used as a complementary intervention strategy. Garms et al. *(63)* observed earlier in Uganda that ground-level larviciding by well-trained local personnel

in affected communities was a feasible strategy and is more affordable than aerial larviciding; furthermore, its practice could build capacity in endemic countries and accelerate elimination of the disease.

12.1.2 Objectives of the chapter

Chapter 12 is designed to serve as a guide for endemic countries for environmentally safe vector control, where feasible, as an additional or complementary measure to break human–fly contact and accelerate the elimination of onchocerciasis. Vector control by ground larviciding is recommended for treating potential breeding sites of *Simulium* spp. according to former OCP guidelines *(65)* with insecticides that have passed a safety assessment and are prequalified by WHO. APOC released a guide to vector control as a complement to community-directed treatment with ivermectin shortly before its closure. This guide includes consideration of when and how to implement a vector control strategy. It suggested that vector control may be necessary in areas that are hyperendemic, where an MDA programme has failed to make progress, and in areas ineligible for MDA because of co-endemic loaiasis *(114)*. To avoid repetition and to use the best practices recommended by the OCP, parts of Chapter 12 are adapted from the OCP module for training national entomologists in the management and supervision of entomological activities under onchocerciasis control *(65)* and the APOC *Guide for decision making and implementation of vector control as alternative treatment strategies for elimination of onchocerciasis (114)*.

12.2 Information for deciding on implementation and objectives of vector control

Much of the basic information for planning and implementing vector control is available in the earlier (2015) manual *(114)* and is reviewed only briefly here. The information that is necessary is as follows.

Mapping of rivers and potential breeding sites over at least 2 years at the project site (Chapter 5): Maps of river systems and potential breeding places should be obtained to enable the team to assess the general hydrological network and feasibility of vector control in the targeted area.

Impact of seasonality on breeding sites and source of flies (Chapter 3): Information on seasonal variation in rainfall patterns in the country and at the project site should be reviewed. The relation between seasonal variations in rainfall and the productivity of vector breeding sites should be established.

Characterization of *Simulium* species in a transmission zone (Chapter 7): Knowledge of the vector species that occur in intervention areas is very important. The *Simulium* species complex and group should be well characterized and the species determined (see Chapter 4). In areas already being treated with larvicides, knowledge of the *O. volvulus* infectivity level of local black fly populations will help in targeting the breeding sites to be treated, particularly if resource limitations prevent treatment of all potential sites.

12.3 Background information for using larvicides

Once a river basin has been mapped and it is time to start vector control, a trial should be conducted to ascertain the "carry" and the efficacy of the larvicide applied. "Carry" is the length of the river downstream from the point of treatment at which all *Simulium* larvae are killed. It can be measured by sampling aquatic substrates at regular

distances from the point of application to the point at which the insecticide has become diluted and no longer kills larvae. Efficacy is determined by calculating the lowest dose of the larvicide at which 100% of *Simulium* larvae are killed. This value may depend on the type of larvicide formulation (see section 12.5).

12.4 Prerequisites for successful vector control

12.4.1 Establishment of a vector control programme

For proper planning and implementation of vector control activities, the programme should be integrated into the framework of the appropriate section of the ministry of health, which could be the NTD or a vector control programme. Once structural integration is established, vector control activities can be planned at national, regional and district levels. Countries can use any relevant existing structure to coordinate vector control activities without necessarily creating a new one.

To ensure the acceptability of a vector control programme, communities should be informed of the activities and the importance of vector control in order to gain their participation. This will allow establishment of secure insecticide depots and recruitment of local residents for prospection and HLC teams.

12.4.2 Expertise required at national, regional, provincial, district and state levels

Trained professionals are a critical component of a vector control system. At national level, entomologists with formal doctoral training (PhD or equivalent) and field expertise are required. At the subnational level, entomologists with less formal academic training (Bachelor or Master's degree or equivalent) may be required if there is a system for consultation between the national and subnational levels. Entomologists at this level should be relatively independent and able to prepare, implement and assess vector control plans and operations, with guidance from professionals at regional or national level when requested. Although entomologists with the necessary expertise are ideally employed by the ministry of health, it may be necessary to seek such expertise in local and national universities and research institutes.

12.4.3 Trained personnel

Qualified entomologists should be identified at various administrative levels of an onchocerciasis elimination programme, who will be responsible for planning and implementing vector control activities safely and effectively. Their roles include:

- obtaining relevant maps of the project area,
- drawing up an operational plan for mapping,
- identifying suitable field staff,
- compiling a list of field equipment and supplies,
- creating a database for maintaining and analysing data,
- training field staff,

- supervising field staff,
- making technical decisions on vector control and
- preparing technical reports.

12.4.4 Budget

A realistic budget should be drawn up for the plan of action of the vector control project. It should be reasonable and tied directly to core activities. In addition to administrative costs, the main budget lines should include:

- per diem and allowances for personnel;
- fuel for vehicles and motorcycles;
- equipment, including a flowmeter, spray pumps, GPS devices and microscopes;
- field supplies, including personal protective equipment, reagents, chemicals and glassware;
- larvicides; and
- communication equipment.

A number of steps can be taken to optimize the vector control budget while maintaining sound control. The interval between ground larviciding could be reduced to every 2 or 3 months if the density of the vector populations remains relatively low. Experimentation may be required to determine an acceptable level, e.g. a 50%, 80% or 90% reduction in the targeted population, when further treatment could be suspended. Another economy would be to perform larviciding at sites with year-round breeding only during the dry season, so that, at the beginning of the rainy season, when breeding extends to other areas in the transmission zone, the population of flies to repopulate the zone is smaller.

12.5 Characteristics of a good larvicide

Larvicides specifically target the larval stage of an insect. They may be contact or stomach poisons, growth regulators or biological control agents. An insecticide is never dispensed in its pure state (technical grade) but as a formulation, i.e. a combination of various ingredients designed to render the product useful and effective for the purpose claimed and for the envisaged mode of action *(115)*.

Insecticide formulations on the market include suspension concentrates, wettable powders, wettable granules, emulsifiable concentrates and aerosols. Whichever insecticide is used for black fly control, the formulated product should have a lethal effect on 100% of larval populations and a negligible, if not a zero, effect on non-target fauna. Use of WHO-prequalified insecticidal products is preferred, as they have been assessed for efficacy, safety and quality.[1]

Other characteristics of insecticide formulations should include:

- acceptable stability for storage under tropical conditions;
- substantial “carry” after formulation;

[1] https://extranet.who.int/pqweb/vector-control-products/prequalified-product-list

- biodegradable, with very little possibility of build-up in the environment;
- affordability;
- unlikely to promote resistance;
- readily available on the market; and
- easy and safe to apply.

12.6 Suitable larvicides

There are currently two categories of suitable black fly larvicides.

- **organic or synthetic larvicides**, comprising formulations of organophosphate compounds (e.g. temephos or Abate®, chlorphoxim), which are biodegradable and have virtually replaced most of the older organochlorine compounds; and
- **biolarvicides (biological insecticides)**, which include formulations of juvenile hormone mimics of insects (insect growth regulators) and *Bacillus* species (e.g. *B. thuringiensis israelensis* H-14).

12.7 Larviciding techniques

Sites for larviciding should have been fully prospected and identified before application. The productivity and seasonality of each site should be known, and the hydrological characteristics, as specified in Chapter 6, should be defined. Specific parameters to be determined before larviciding are:

- the species identity of the larvae;
- the annual dynamics of adult and aquatic populations and the sensitivity of the local *Simulium* larval population to insecticides (see *116*);
- the river discharge rate and turbidity; and
- baseline assessment of indicator non-target fauna at the targeted breeding sites.

Once these parameters have been determined, insecticide can be sprayed on foot, by boat or by air. The choice of the spraying technique is determined by:

- the type of river (broad, narrow),
- the period (high or low water),
- the surface to be treated and
- the physical properties of the insecticide.

12.8 Ground larviciding of breeding sites

12.8.1 Introduction

Several methods can be used to apply larvicides at ground level. The method should be suitable for sustained, effective treatment of breeding sites and for the physical nature of the river system (e.g. size, flow rate) *(65, 106)*. For larviciding small streams, a simple method is to mix the required dose in a bucket at the water's edge and pour the contents directly into the water course. For larger streams and small rivers, the required dose is mixed with water in a knapsack sprayer or a hand-compression sprayer and applied at the breeding site for 30 min or more *(63)* (Fig. 12.2). In very large rivers, formulated insecticide can be released from a metal drum carried out in a boat and mixed into the water by the boat's propeller.

Fig. 12.2. Examples of ground larviciding with a backpack sprayer. A, treatment of the Namatala River to control *S. neavei* in the Mount Elgon focus, eastern Uganda. B, ground larviciding in the Lanyadyang River to control *S. damnosum s.s.* in the Madi–mid-north focus, northern Uganda.

A B

Source: photographs by T. Lakwo

12.8.2 Larvicides for ground application

Two larvicides are typically used to control black fly larvae: temephos and *B. thuringiensis israelensis* H14 (Table 12.1).

Temephos is an organophosphate that can be used to treat water infested with a number of vector insects, including mosquitoes, midges and black fly larvae. Like other organophosphates, temephos affects the central nervous system of insects by inhibiting cholinesterase I, an enzyme that breaks down the neurotransmitter acetylcholine. In black fly larvae, this results in death before they reach the adult stage. Temephos has extremely low toxicity for mammals and can be applied to potable water to kill water fleas that carry Guinea-worm larvae or mosquito larvae. Its advantages as a black fly larvicide are its relatively low cost, fast insecticidal action and long "carry" after application in natural habitats.

B. thuringiensis israelensis H-14 is a biological insecticide with the advantages of ease of aqueous suspension, high selectivity for nematocerous Diptera (particularly filter-feeding *Simulium* larvae), no risk to non-target fauna when applied at the recommended dose and remarkably high efficacy, i.e. no *Simulium* population has yet been shown to be resistant to it. Its disadvantages as compared with temephos are that a relatively high dose is required and it has relatively low "carry" capacity, thus necessitating more points of application.

Table 12.1. Description and rates of application of temephos and *B. thuringiensis israelensis* H-14.

Larvicide	Formulation	Application rate (amount per L)	Mode of action	Carry (km)[a]
Temephos	Emulsifiable concentrate (EC)	200 g AI/L	Contact and stomach poison	Q 15/m^3/s; Q 100/m^3/s
B. thuringiensis israelensis H-14	Suspension concentrate (SC)	2% (of toxin)	Stomach poison	Q 2–5/m^3/s; Q 15/m^3/s

Source: OCP vector control operations

AI, active ingredient; Q, velocity

[a] Carry (effective "wave" of insecticide) in low water (values on the left) versus high water (values on the right)

12.8.3 Quantity of larvicide to be applied

The quantity of larvicide to be applied depends on the dose and the river discharge. For example, the OCP used both larvicides listed in Table 12.1 in West Africa, but *B. thuringiensis israelensis* H-14 was formulated for application at doses of 0.54–0.73 L/m^3 per s and operational applications of temephos ranged from 0.15 to 0.30 L/m^3 per s *(114)*. These differences in concentration reflect the differential toxicity and carry of the two larvicides.

Abate® is available in two formulations, one of which is not suitable for controlling vector black flies. Abate® 500 E is a liquid concentrate containing 500 g of active ingredient per litre. It is typically used as an emulsifiable concentrate diluted to the correct dose, as indicated in Table 12.1. The 1SG formulation has sand granules as an inert carrier and is not suitable for use as a black fly larvicide. *B. thuringiensis israelensis* H-15 (Teknar) is available in an aqueous formulation and is usually used at a dose of 0.54 L/m^3 per s.

Note: Always read the product label before measuring the quantity of larvicide and dispensing it in natural habitats to ensure the correct dosage.

12.8.4 Importance of hydrology in larviciding

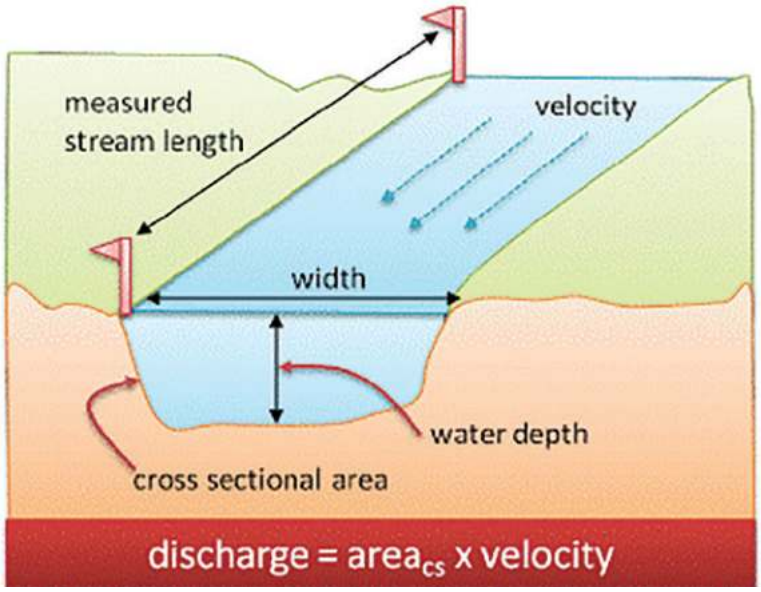
The role played by hydrology (the movement of water in relation to land) in onchocerciasis elimination is significant. The immature stages of black flies are found only in running water (Chapter 2), which makes source reduction by ground larviciding an important complement to MDA. Information on the discharge of the rivers to be treated is therefore essential for the effectiveness of larvicide applications. Under-dosing may be ineffective against the vectors and could have serious epidemiological consequences, such as continued parasite transmission and selection of resistant black fly populations, while overdosing may be detrimental to the environment.

12.8.5 Direct measurement of the discharge of a river

The discharge of the river must be determined precisely in order to calculate the appropriate dosing regimen. Discharge is the volume of water that runs through a river per second. It is related to the height of water in the river, the velocity of the water and the width of the river. To determine the quantity of larvicide required to treat a river section, the formula to be used is:

Quantity of larvicide (L) = Dose of larvicide (L/m^3 per s) × discharge (m^3/s).

Fig. 12.3. Three characteristics required to calculate river discharge.



Source: Dr Carsten Kessler

Direct calculation of the discharge of a river requires determination of three parameters – the width, the average depth and the current velocity (Fig. 12.3). Measurements should be made at a point on the river that can be forded, where the flow is uniform and the bottom is even.

On rivers on which there is a river gauge near the insecticide application point, it is recommended that it be used to determine the depth. A river gauge consists of a series of vertical metal piles with graduated plates. Each plate measures 1 m and is divided into units of 1 cm.

Two methods can be used to measure current speed (velocity) – the micromoulinet method and the float method. The micromoulinet is an apparatus (Fig. 12.4) used to measure the current velocity at any point on a river. Unfortunately, its cost and fragility under field conditions are major disadvantages. The float method consists of estimating current velocity from a float (usually a plastic container or a "pilullier" partially filled with water), which is left adrift in the river.

Measurements are made as follows:

- Choose a portion of the river of known length (L) in meters.
- Determine the time (T1) in seconds for the float to travel the known length (L).
- Repeat the second step twice to determine T2 and T3.

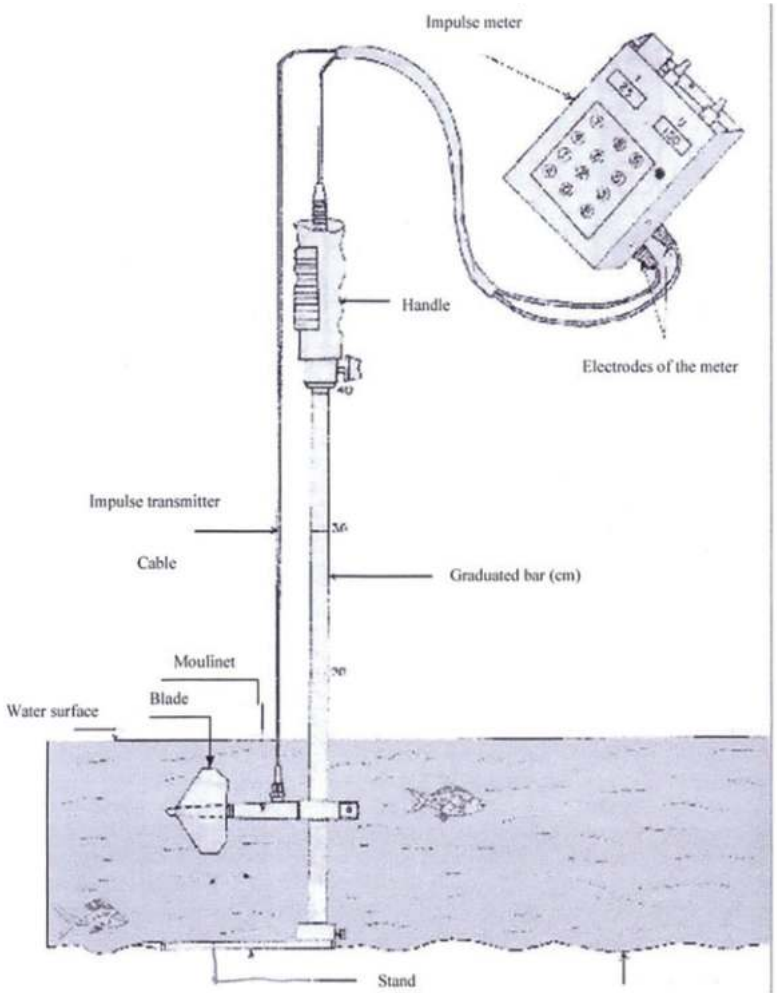
Surface speed (Vs) is derived from the formula: Vs (m/s) = L (m) × 3 ⁄(T1 +T2 + T3) (s).

As the current is faster on the surface, use a coefficient of correction to determine the mean velocity (Vm), i.e. Vm = Vs × 0.8.

The average width and depth of the river must be measured at the same location at which velocity was determined, as follows. Stretch a graduated rope across the river, perpendicular to the current, and measure the width (W) in meters. Do not include the edges, where the current is insignificant. Cross the river, measuring the depth (D) in meters four times, and take the average to determine the average depth (Da): Da (m) =(D1 + D2 + D3 + D4)/4.

Flow (Q) is determined from the mean velocity (Vm), the average depth (Da) and the width (W) of the river, according to the formula: Q (m^3/s) = Vm (m/s) × Da (m) × W (m).

Fig. 12.4. Illustration of a Micromoulinet apparatus.



Source: reference *65*

12.8.6 Applying larvicides

The method used to apply a larvicide depends on the formulation used, the size of the river and the configuration of the larval breeding sites. If the insecticide selected is *B. thuringiensis israelensis* H-14, it must be applied immediately upstream of the first substrates on which larvae are attached, because it has a low carry rate. Temephos has a much higher carry rate.

If the river is narrow and not very deep, the insecticide can be applied starting from the banks or when fording the river upstream of the breeding site. If the river is broad and deep, a boat or dugout is required.

Application on foot starting from the banks: A pressurized spraying machine (Fig. 12.2) consisting of a small tank, a pump and a flexible hose connection with a trigger, a rigid blade and a tube is used. This apparatus makes it possible to spray the insecticide under pressure in several rounds and to cover the entire width of the river. The maximum range of the jet of the apparatus is 10 m. In the absence of a sprayer and when treating a shallow stream or river, a watering can or a bucket can be used to pour the insecticide while crossing the river. **Always** pour the insecticide downstream from where you are standing. When applying *B. thuringiensis israelensis* H-14, the crossing must be made without stirring up mud, because muddy water decreases its efficacy.

Treatment from a boat: When breeding sites occur in large rivers, treatment by boat is required. Care should be taken to use a reliable boat and **always** to wear a life jacket. The insecticide can be sprayed with a spraying machine or from a watering can if the quantity of the mixture is < 10 L. If a larger quantity is used, a barrel or drum should be used, with a tap and a length of pipe with a cut-off valve that makes it possible to regulate the flow of the insecticide. Applications must be uniform, continuous and cover the entire width of the river. A trial with an equivalent quantity of water is often useful for better regulation of spraying of the insecticide.

12.8.7 Selection of spraying points

The location of larval breeding sites on a map should be reviewed for proper selection of spraying points. In the absence of a map, prospection is necessary (Chapter 6) to locate the breeding sites and to create a map for future use. Each breeding site beyond the carry of upstream treatment will require a new application of insecticide. Accurate determination of the carry and the spacing of the application points is important to avoid wasting material.

In seasonal streams during the dry season, larval breeding sites are usually isolated rock thresholds, separated by large basins in which the water is stationary. Each threshold should be treated individually with a full dose of larvicide because the carry of the insecticide (its movement as a wave) is almost zero.

12.8.8 Frequency of treatments

Black flies in African rivers usually complete their larval development within 8–10 days or less (5 days) when the water temperature is higher than 35 ºC. If vector elimination is the goal, treatment is given weekly. When larviciding is used to complement MDA, the goal is to reduce the ABR to a point at which the parasite population cannot be maintained (Chapter 11). Experimentation will be required to determine how often the breeding sites should be treated to achieve the desired reduction in biting rates. Modelling suggests that, for *O. volvulus* to remain endemic, an ABR ≥ 730 is required, but, as the microfilarial reservoir shrinks due to MDA, the biting rate must increase to compensate for this reduction *(99)*.

The results of manual treatments may be recorded on a form such as that below, adapted from OCP forms.

Week/date River system _____ Area _____

Date	River	Stretch	Larvicide	Dose	Points	Remarks

12.8.9 Evaluation of the efficacy of larviciding

The operational efficacy of larviciding should be evaluated to ensure that the money invested in the programme is well spent. Before initiating a larviciding programme, the distribution and density of larvae should be determined, and any changes should be monitored regularly to determine the impact of the treatment on the vector population. The density of the aquatic stages and their distribution into age categories (young larvae, old

larvae and pupae) should be coded at the time of prospection and regularly after treatment. If the larval density and distribution fail to change after treatment, an evaluation should be conducted to determine the cause of the failure (e.g. inadequate dosing or insecticide resistance).

12.8.10 Safe application of larvicides

Larvicides should be applied in such a way as to minimize exposure of the applicators. They should be given proper training in low-risk handling of larvicides and disposal of waste or empty containers and provided with suitable personal protective equipment *(115)*.

12.9 Monitoring resistance of larvae to insecticides

12.9.1 Introduction

Long term, large-scale use of insecticides for public health purposes has resulted in the emergence of insect populations that are resistant to a variety of insecticides, leading to failure of certain vector control campaigns. The risk of resistance as a result of prolonged, intensive use of a single insecticide should therefore be considered when planning vector control.

If treatment fails, several questions should be asked.

- Was the larvicide quality-assured?
- Was the larvicide applied at the recommended rate and frequency?
- Was the formulation of the larvicide unsuitable or too diluted?
- Did the insecticide pass through the breeding sites, or were the spraying points poorly selected?
- Has the target black fly population become physiologically resistant to the insecticide?

Resistance is the capacity to tolerate normally toxic amounts of substances that were hitherto lethal for the majority of susceptible individuals in a population. Resistance has a hereditary basis, emerging due to selection under natural conditions at population level. The various forms of resistance are:

- physiological resistance, which results from the selection of individuals in a population that are equipped physiologically to detoxify and overcome the effect of the insecticide at the usual lethal dose;
- cross-resistance, which is due to selection of one or more detoxifying mechanisms that operate against insecticides of a particular chemical group (e.g. general resistance to organophosphorus insecticides); or
- behavioural resistance, which is selection for changes in behaviour through which the insect avoids contact with or absorption of a lethal amount of insecticide.

In order to mitigate its development, insecticide resistance should be monitored regularly in field populations of vector species and the type of insecticide changed to a replacement or alternative insecticide in another chemical group with an unrelated mode of action. This will also avoid the possibility of cross-resistance. In selecting alternative insecticides, account should be taken of their toxic effects on non-target aquatic fauna, their cost–effectiveness and the degree of sensitivity of the *Simulium* population.

12.9.2 Evaluation of the susceptibility of *S. damnosum s.l.* larvae to insecticides

The occurrence of resistance in a *Simulium* control campaign may require revision of the entire strategy and, in particular, replacement of the larvicide that has become ineffective. The initial sensitivity of the larvae of *S. damnosum s.l.* should therefore be measured and followed continuously, and the frequency and intensity of resistance of *Simulium* species to larvicides should be monitored. Precise, standardized methods are available for measuring the susceptibility and resistance of target insects. In susceptibility tests:

- expose several batches of field-collected *Simulium* larvae to serial concentrations of the active ingredient of larvicide in a laboratory;
- establish the dose–response curve; and
- determine the concentrations of insecticide that are lethal to 50%, 95% or 99% (LC_{50}, LC_{95}, LC_{99}) of the exposed larval populations.

The LC_{50} is the most important value; it represents the basic susceptibility of the tested population. The type of test performed to measure the LC_{50} will depend on the mode of action of the insecticide under investigation. For example, the Mouchet test is used to evaluate the lethality of contact with the insecticide (organophosphate compounds) *(65)*. Another test is used to evaluate the susceptibility of larvae to chemicals after ingestion (for example, *B. thuringiensis israelensis*). In the latter, black fly larvae are exposed to various serial concentrations of *B. thuringiensis israelensis* H14 by placing them in vertical glass containers with magnetic stirrers to create movement of water through their larval cephalic fans. In this way, larvae ingest the spores and become infected.

12.10 Community-based vector control

12.10.1 An example in Uganda

In northern Uganda, communities have provided support in black fly larviciding, e.g. by safely storing chemicals, pumps, measuring cylinders and other materials such as personal protective equipment. Technical staff nevertheless remain responsible for measuring river discharge and ensuring that the quantity of insecticide (temephos) is correct and is applied in the appropriate locations. Partnerships with communities have reduced the cost of operations as compared with locations in which only health ministry staff were involved.

Members of some Ugandan communities have been trained to participate in indoor residual spraying and larval source management for mosquitoes. Operational research could be conducted to determine whether they could also safely implement black fly control activities that involve ground larviciding.

12.10.2 Emerging approaches to black fly vector control

There are two emerging approaches to vector control that do not involve insecticides and are thus amenable to community-based programmes.

Traps

Initial findings in Mexico indicate that the EWT could significantly reduce personal biting rates by *S. ochraceum* in and around households and schools *(117)*. This observation is particularly important because this vector species is highly anthropophilic. Recent findings in Uganda indicate that a modified version of the trap has the

potential to attract and capture large numbers of *S. damnosum s.s.* in and around villages *(118)*. Placement of traps in open classrooms and in agricultural fields resulted in a 90% reduction in biting in a school. In field settings, results were variable, the traps reducing biting by about 50% in one situation and no significant reduction as compared with HLC in another. These results suggest that trap placement may be critical in more open areas where fewer people are congregated. Operational research was underway at the time this manual was written.

Slash and clear

A second community-based approach to the control of *Simulium* species involves environmental alterations that affect fly development. For example, Buckley *(119)* completely removed undergrowth along the margins of the Riana River and partially removed trees overhanging the areas in which *S. neavei* bred, resulting in temporary elimination of the local vector population. More recently, in Uganda, community-directed removal of trailing aquatic vegetation ("slash and clear") dramatically reduced larval breeding sites, resulting in a significant reduction in the biting density of *S. damnosum s.s. (120, 121)*. When slash-and-clear interventions were conducted at 34 *S. damnosum s.s.* breeding sites located within 2 km of affected communities, vector biting was reduced by 95% (Fig. 12.5). When slash-and-clear was extended farther than 2 km, there was no further decrease in biting rate, indicating that the biting black fly population bred locally. Further, when a single intervention was conducted in the first half of the rainy season, there was a 97% reduction in biting rate, while a slash-and-clear intervention at the end of the rainy season resulted in a 94% reduction. The impact of slash-and-clear was great enough that the number of vectors in the intervention villages had not fully recovered by the start of the next rainy season. Community members were motivated to participate in vegetation removal because of the reduction in the biting rate and the likelihood that slash-and-clear practised once or twice a year would bring significant relief. Evaluation of these results in a novel mathematical model of the effect of slash-and-clear on seasonal black fly biting rates combined with a population dynamics model of *O. volvulus* transmission indicated that supplementing annual MDA of ivermectin with slash-and-clear could significantly accelerate elimination of onchocerciasis *(122)*. Further trials elsewhere are awaited.

Fig. 12.5. Removal of trailing vegetation at a *S. damnosum s.s.* breeding site in Uganda.

Source: photograph by T. Unnasch

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Annex 1. Taxonomic keys to African simuliid pupae

AI.1. Key to pupae of Afrotropical species of *simulium* in onchocerciasis-endemic countries

This key includes 102 species. It is modified from Freeman & de Meillon *(1)* to accommodate all post-1953 descriptions and name changes as of 2021 *(2)*, except cytospecies of the *Simulium damnosum* complex (which are indistinguishable by means of pupal characters) and those that belong to the subgenus *Afrosimulium* (one species), as well as the mayfly-nymph associated species of subgenera *Lewisellum* (one species) and *Phoretomyia* (13 species). For ease of use as a "field key", the post-1953 described members of the *S. nigritarse* subgroup are also excluded, but are treated in a separate key (A1.2), which requires the analysis of microscopic slide preparations. The corresponding plates (I–VIII) illustrating African simuliid pupal respiratory filaments (gills) are provided in Annex 2.

Species without geographical reference are known to have a wide distribution range.

1. Respiratory filaments of two types, large thin-walled primary filaments with narrower secondary filaments arising from them (Plates I–III) **2**

   - Filaments of one type only, usually filamentous, though in some species carried on a long stalk (Plate V) **21**

2(1). Large, thin-walled, horizontal primary filaments present, often more or less clasping head and thorax **3**

   - Primary filaments all more or less erect **17**

3(2). Only two upwardly directed filaments (Plate I); Sierra Leone, Liberia ..... **blacklocki** Edwards Liberia

   - More than two upwardly directed branches **4**

4(3). The three outer, stiff upright primaries are simple unbranched **5**

   - These primaries each with one or more branches **6**

5(4). With three simple inner filaments (Plate I); Uganda (Mt. Elgon; may be extinct?) ..... **cavum** Gibbins

   - With three forked inner filaments (Plate I) ..... **damnosum (complex)** Theobald

6(4). Stiff upright primaries with one branch or secondary each **7**

   - These primaries with two or four secondaries each **15**

7(6). One or other or both of the horizontal primaries with a short secondary at its apex or medially and directed downward **8**

   - Neither with a short secondary at the apex **12**

8(7). Whole organ very bulky, (Plate I) .....

   a) **colasbelcouri** Grenier & Ovazza

   b) **akouense** Fain & Elsen; Cameroon

   c) **danense** Gouteux; Côte d'Ivoire

   - Organ not like this **9**

9(8). Horizontal primaries (one or both) with short secondaries at the apex **10**

- One horizontal primary with a short secondary medially and directed downward **11**

10(9). Each horizontal primary with a short secondary at the apex (Plate I); southern Africa..... **letabum** de Meillon

- Only the anterior one with a secondary at the apex and that longer (Plate I) ..... **taylori** Gibbins

11(9). Short secondary on horizontal primary branching in the basal half (Plate I); Cameroon **tondewandouense** Fain & Elsen (+**crosskeyi** Lewis & Disney)

- Short secondary on horizontal primary branching in apical half (Plate I) ..... **ngouense** Fain & Elsen

12(7). All primaries exaggerated, inner secondaries reduced (Plate I) **ruandae** Fain

- Primaries smaller **13**

13(12). At least one inner secondary forked (Plate II):

a) West Africa: Primaries and secondaries cirriform ..... **futaense** Garms & Post

b) Democratic Republic of the Congo: Organ formed deer-antlers-like ..... **kingundense** Fain & Elsen

- None of the inner secondaries is forked **14**

14(13). The secondaries are slender and filamentous (Plate II) ..... **vorax** Pomeroy

- Inner secondaries expanded, branches of upright primaries short and thick (Plate II); Uganda, Kenya ..... **touffeum** Gibbins

15(6). Three outer, upright filaments each with two branches, eight inner simple ones of similar diameter (Plate II); Malawi ..... **zombaense** Freeman & de Meillon

- Main upright trunks much stouter than any inner filaments **16**

16(15). Central primary simple (Plate II; general organ shape very variable!)) ..... **medusaeforme** Pomeroy

- This primary forked:

a) Upright primaries straight and tapered or more or less incurved (Plate II)..... **hargreavesi** Gibbins

b) Primaries short and rounded with a honeycomb cuticular pattern (Plate II); Uganda, Kenya ..... **africanum** Gibbins

17(2). Organ formed of a long main trunk:

a) With 16 thin secondaries (Plate II) ..... **hissetteum** Gibbins

b) With 11 thin secondaries (Plate II)..... **oguama** Lewis & Disney

- Organ formed of three or five main trunks with usually more than 16 secondaries **18**

18(17). Organ formed of five rather slim main trunks, splitting into a 2,2,4,4,4 arrangement (Plate II); Ethiopia **jimmaense** Uemoto, Ogata & Mebrahtu

- Organ formed of three main trunks with at least 25 secondaries..... **19**

19(18). Respiratory organ more spread out and with four basal secondary filaments anteriorly (Plate III) .....

(a) The three erect main trunks distinctly inflated, much bigger than the numerous secondaries.....

(1) 31 secondaries: **arnoldi** Gibbins; **bovis** de Meillon (Plate III); **chutteri** Lewis (southern Africa)

(2) 36 secondaries (Plate III)..... **S. dawaense** Uemoto, Ogata & Mebrahtu; Ethiopia

(3) 25 secondaries (Plate III) ..... **S. fragai** Abreu; Angola, Namibia

(4) about 40 secondaries (Plate III) ..... **S. gibense** Uemoto, Ogata & Mebrahtu; Ethiopia

(b) Main trunks reduced to thick common stalks (Plate III); Democratic Republic of the Congo ..... **kwangoense** Fain & Elsen

- Organ more erect with only two basal anterior secondary filaments..... **20**

20(19). With long stem and 24 rather slim filaments (Plate III)..... **wellmanni** Roubaud; **janzi** Abreu (Angola)

- Without common stem; two distinct types of filaments, 15 strong externals, 10 slim internals (Plate III); Cameroon ..... **eouzani** Germain & Grenier

21(1). Respiratory organ of 25–40 filaments, abdomen without strong terminal hooks..... **22**

- Respiratory organ of 22 or less filaments **23**

22(21). Filaments arranged in groups of four to eight on short stems (Plate III); not living in association with mayfly nymphs (Ephemeroptera)..... **albivirgulatum** Wanson & Henrard

- Filaments not in groups of four to eight; associated with mayfly nymphs .....subgenus *Phoretomyia*

23(21). Filaments short and thick with black tips **24**

- Filaments without black tips **28**

24(23). Some filaments very much shorter than others, or one filament reduced to a basal stump **25**

- All filaments of much the same size **26**

25(24). Eight filaments, four long and four short (Plate IV); Uganda ..... **octospicae** Gibbins

- Nine long equal filaments and one stump (Plate IV)..... **kauntzeum** Gibbins

- 15 filaments, six of them much shorter (Plate IV); Côte d'Ivoire..... **voltae** Grenier, Ovazza & Valade

26(24). Filaments 14 in number, cuticle without black nodules (Plate IV) ..... **nili** Gibbins

- If 14 filaments present, then cuticle covered with black nodules..... **27**

27(26). 18 filaments (Plate IV)..... **bisnovem** Gibbins

- 17 filaments (Plate IV); Ethiopia..... **shoae** Grenier & Ovazza

- 14 filaments (Plate IV; very variable!)..... **dentulosum (group)** Roubaud

- 12 filaments (Plate IV); Rwanda..... **ngabogei** Fain

- 11 filaments (Plate IV); Zimbabwe ..... **rhodesiense** de Meillon

- 10 filaments (Plate IV); Uganda ..... **masabae** Gibbins

- Seven filaments (Plate IV), Democratic Republic of the Congo..... **heptaspicae** Gouteux

- Four filaments (Plate IV) ..... **berghei** Fain; **spinulicorne** Fain & Elsen

28(23). Respiratory organ composed of four filaments only **29**

- Filaments not four in number **36**

29(28). Filaments divergent **30**

- Filaments may diverge basally, but apically at least three are together **33**

30(29). Filaments either narrow filamentous and tapered, or broad basally and sharply tapered or drawn out terminally, one or two may project backwards **31**

- Filaments broad, of even width or slightly tapered, with blunt or rounded apices, all projecting more or less upwards

  (a) with superficial reticulations (Plate V) ..... **duboisi** Fain

  (b) without reticulations (Plate V) ..... **ruficorne** Marquart

31(30). Filaments evenly tapered, two projecting forwards and two backwards (Plate V) ..... **katangae** Fain

- Some at least sharply tapered, usually one projects backwards (Plate V) **32**

32(31). Filament surface reticulated; Rwanda ..... **fuscicorne** Fain

- Filament surface not reticulated ..... **buckleyi** de Meillon

33(29). Filaments short and thick, anterior one separated from others basally (Plate V), with prominent cross striation **34**

- Filaments longer and not so thick **35**

34(33). Posterior filament parallel to neighboring one basally (Plate V); Tanzania ..... **hirsutilateralis** de Meillon

- Posterior filament divergent basally (Plate V) ..... **manense** Elsen & Escaffre

35(33). Anterior filament strongly bowed (Plate V), trichomes long and sinuous ..... **rutherfoordi** de Meillon

- Anterior filament usually not bowed (Plate V); when it is, usually not so strongly (see A1.2), trichomes short and nearly straight ..... **nigritarse (subgroup)** Coquillet

36(28). Filaments carried on a long common stalk **37**

- Filaments not carried on a long common stalk **42**

37(36). Stem broad and not tapering (Plate V), with an internal strengthening rod **38**

- Stem narrower or tapered **39**

38(37). Eight ultimate filaments (Plate V) ..... **mcmahoni** de Meillon

- 12 ultimate filaments (Plate V); Ethiopia, Botswana ..... **awashense** Uemoto, Ogata & Mebrahtu

39(37). Stem tapered (Plate V) and ornamented with short dark lines ..... **bequaerti** Gibbins

- Stem of even width and not so ornamented **40**

40(39). Ten ultimate filaments arranged 3,2,3,2 (Plate V) ..... **coalitum** Pomeroy

- Eight ultimate filaments **41**

41(40). Filaments arranged in pairs (Plate V) ..... **schoutedeni** Wanson

- Filaments arranged 2,1,1,2,2 (Plate V); Cameroon ..... **audreyae** Garms & Disney

42(36). Respiratory with one, two, or three filaments of divisions **43**

- Respiratory organ with more than three filaments or divisions **50**

43(42). A single, long, tapered filament attached basally to the pupa (Plate V); southern Africa..... **harrisoni** Freeman & de Meillon

- Respiratory organ not like this **44**

44(43). Respiratory organ tubular or barrel-shaped attached about 1/3 along its length to pupa **45**

- Respiratory organ with two or three branches or divisions **47**

45(44). Respiratory organ barrel-shaped (Plate V) ..... **rotundum** Gibbins

- Respiratory organ – tubular-elongate **46**

46(45). Respiratory organ a simple crescentic tube (Plate V) ..... **unicornutum** Pomeroy

- Respiratory organ with constrictions along its length (Plate V)..... **palmeri** Pomeroy

47(44). Respiratory organ of two short upright cylindrical elements (Plate V) ..... **bifila** Freeman & de Meillon

- Respiratory organ with three branches or divisions **48**

48(47). Filaments flattened, leaf-like, with irregular edge, held pointing forwards, rather bulky (Plate VI, a+b) **griseicolle** Becker

- Filaments not like this **49**

49(48). Filaments short, cylindrical, and stiff (Plate VI); tubercles on thorax replaced by long pointed spicules ..... **tridens** Freeman & de Meillon

- Respiratory organ in the form of three spheres (Plate VI) with or without apical filaments; Democratic Republic of the Congo ..... **trisphaerae** Wanson & Henrard

50(42). Respiratory organ in form of seven cylinders carried on narrow stems and each with one or two fine filaments apically (Plate VI); only one pupa known (Democratic Republic of the Congo) ..... **schwetzi** Wanson

- Respiratory organ not like this **51**

51(50). Respiratory organ stiff, erect, branches held in antero-posterior plane, often like a deer's antler **52**

- Respiratory organ composed of more elongate, sinuous filaments, usually not held in one plane **54**

52(51). Whole organ stumpy, branches reduced:

a) Branches rounded at their apices, organ erect (Plate VI); Democratic Republic of the Congo ..... **vangilsi** Wanson

b) Branches bent down, pseudo-annulated (Plate VI); Cameroon..... **leberrei** Grenier, Germain & Mouchet

- Respiratory organ with branches more elongate..... **53**

53(52). Eight or nine ultimate branches, each branch single (Plate VI) ..... **cervicornutum** Pomeroy

- With each branch bifid apically (Plate VI), Rwanda ..... **aureliani** Fain
- With stout trunk, widening at base and 2 main branches pointing into 8: anterior with three distinct filaments, posterior with two primaries, which are distally forked (3,2) and reaching far back over the abdomen (Plate VI); Cameroon ..... **ekomei** Lewis & Disney

54(51). Respiratory organ of 20 filaments (Plate VI) ..... **tentaculum** Gibbins

- Less than 20 filaments present **55**

55(54). Six filaments present **56**

- More than six filaments **57**

56(55). Cocoon simple, without neck. Six filaments sub-parallel (Plate VI) ..... **sexiens** de Meillon

- Cocoon with well-developed neck. Six filaments partially spread out .... **loutetense** Grenier & Ovazza

57(55). Respiratory organ of seven filaments (Plate VIc) ..... **alcocki (group)** Pomeroy

- More than seven filaments **58**

58(57). Respiratory organ of eight filaments **59**

- More than eight filaments **64**

59(58). Filaments arising in pairs from quite a long common stalk:

a) Cf. to couplet 36 (Plate V) ..... **schoutedeni** Wanson

b) Cf. to couplet 57 (Plate VI c+d) ..... **alcocki (group)** Pomeroy

- Filaments with a shorter stalk **60**

60(59). Filaments arranged in four pairs on short stems **61**

- Filaments not with this arrangement **62**

61(60). One pair placed on the inner side (Plate VI), cocoon simple; Rwanda, Democratic Republic of the Congo ..... **rodhaini** Fain; **nyaense** Gouteux

- All arranged more or less in the same plane (Plate VI), cocoon with neck..... **kenyae** de Meillon

62(60). Filaments branching 3, 3, 2..... **63**

- Filaments branching 2, 4, 2 (no figure)..... **alcocki (group)** Pomeroy

63(62). Pupae living in association with freshwater crabs (Plate VII) ..... **neavei group** Roubaud

- Pupae not with this habit. 8 filaments (Plate VII) ..... **hirsutum** Pomeroy; **urundiense** Fain (Burundi)

64(58). With 10 filaments **65**

- With more than 10 filaments **67**

65(64). Second series of bifurcations taking place beyond middle of whole organ (Plate VII).... **garmsi** Crosskey

- Second series of bifurcations more basal **66**

66(65). Filaments often of different sizes (Plate VII), whole organ not particularly spread out..... **djallonense** Roubaud & Grenier; **johannae** Wanson

- Filaments always of approximately equal size (Plate VII).....

a) Whole organ rather spread out:

a. Widespread: **impukane** de Meillon

b. Democratic Republic of the Congo, Uganda: **ituriense** Fain

c. Liberia, Côte d'Ivoire: **liberiense** Garms

b) Organ less spread out:

a. Democratic Republic of the Congo: **bayakorum** Fain & Elsen; **mayumbense** Fain & Elsen; **multiclavatum** Fain, Elsen & Dujardin

b. United Republic of Tanzania, Ethiopia: **geigyi** Garms & Häusermann; **weyeri** Garms & Häusermann

67(64). 11 filaments present (Plate VIII).....**adersi** Pomeroy

- More than 11filaments.....**68**

68(67). 12 filaments present.....**69**

- More than 12 filaments present **70**

69(68). Filaments arranged in pairs with short stems (Plate VIII) ..... **duodecimum** Gibbins

- Filaments arranged 3,2,3,4 (Plate VIII), stems longer; Democratic Republic of the Congo.....**allaeri** Wanson

70(68). 15 filaments present (Plate VIII);Congo.....**evillense** Fain, Hallot & Bafort

- 19 filaments (Plate VIII); Democratic Republic of the Congo.....**gilleti** Fain & Hallot

Not included from the mainland Afrotropical region:

S. gariepense de Meillon (1953); only southern Africa (monotype for subgenus *Afrosimulium*)

S. atyophilum Lewis & Disney (1969); on mayfly nymphs (*Lewisellum*)

S. afronuri Lewis & Disney (1970); only Cameroon, on mayfly nymphs (*Phoretomyia*)

S. baetiphilum Lewis & Disney (1972); only Cameroon, on mayfly nymphs (*Phoretomyia*)

S. berneri Freeman (1954); on mayfly nymphs (*Phoretomyia*)

S. copleyi Gibbins (1941); East Africa, on mayfly nymphs (*Phoretomyia*)

S. diceros Freeman & de Meillon (1953); only Democratic Republic of the Congo, on mayfly nymphs (*Phoretomyia*)

S. dukei Lewis, Disney & Crosskey (1969); only Cameroon, on mayfly nymphs (*Phoretomyia*)

S. kumboense Grenier, Germain & Mouchet (1966); only Cameroon, on mayfly nymphs (*Phoretomyia*)

S. lumbwanum de Meillon (1944); on mayfly nymphs (*Phoretomyia*)

S. marlieri Grenier (1950); only Democratic Republic of the Congo, on mayfly nymphs (*Phoretomyia*)

S. melanocephalum Gouteux (1978); only Democratic Republic of the Congo, on mayfly nymphs (*Phoretomyia*)

S. moucheti Gouteux (1977); only Democratic Republic of the Congo, on mayfly nymphs (*Phoretomyia*)

S. rickenbachi Germain, Grenier & Mouchet (1966); only Cameroon, on mayfly nymphs (*Phoretomyia*)

S. zairense Gouteux (1977); only Democratic Republic of the Congo, on mayfly nymphs (*Phoretomyia*)

A1.2. *Simulium nigritarse* subgroup (from references *3* and *4*)

1. Walls of filaments with a strongly raised reticulum (type 1) visible even at low magnification. **2**

   - Filaments without such a strongly raised reticulum. An incomplete and slightly raised reticulum is however present in *S. antibrachium*, *S. raybouldi* and *S. perforatum* **6**

2. Filaments with thick walls strongly sclerotized and dark brown or blackish. Dorsal surface of pupal thorax and head covered with spine-like projections **3**

   - Filaments with walls not abnormally thick or sclerotized, paler. Dorsal surface of pupal thorax and head mainly or exclusively covered with disc-like tubercles **4**

3. The four filaments are very thick and relatively short. They arise directly from the base and are strongly divergent. Total length of pupal gills 2 mm. Cocoon finely woven without antero-median process (see main key couplet 32) ..... **duboisi** Fain

   - The four filaments are thinner and longer. They arise from 2 basal rather thick stems 300 and 500 µ long respectively. Total length of pupal gills 3 mm. Cocoon loosely woven with an antero-median process. Kenya ..... **aspericorne** Fain, Bafort & Silberstein

4. The four filaments arise directly from the base and lie closely together **5**

   - The four filaments arise from 2 generally unequal secondary stems (see Plate V). Cocoon finely woven without fenestrations, there is no median process ..... **aureosimile** Pomeroy

5. Basal vesicle very voluminous and longer than the basal trunk. Hairs of pilous plate very long and inflated apically. Cocoon with fenestrations laterally ..... **bulbiferum** Fain & Dujardin

   - Basal vesicle small and shorter than the basal trunk. Hairs of pilous plate shorter not inflated apically. Cocoon finely woven without fenestrations. Rwanda, Democratic Republic of the Congo ..... **rubescens** Fain & Dujardin

6. Filament walls with very short raised piliform structures disposed either on transverse lines (type 2) or on transverse and oblique lines forming occasionally an incomplete and slightly raised reticulum (intermediate between types 1 and 2) **7**

   - Filaments walls with very short slightly raised structures not forming a distinct pattern **11**

7. Walls of filaments with a pattern of raised transverse lines (type 2). The four filaments arise directly from the base and remain parallel. Cocoon with a rim but lacking a median process. Basal trunk very short **8**

   - Walls of filaments with transverse and oblique lines forming occasionally an incomplete, slightly raised reticulum (intermediate between types 1 and 2) **9**

8. Basal trunk bright orange, compressed laterally and enlarged dorso-ventrally, bearing small rounded and raised tubercles. Basal vesicle smaller. Cocoon finely woven, not reinforced by thick threads. Rwanda, United Republic of Tanzania ..... **flavinotatum** Fain & Dujardin

- Basal trunk brownish, flattened dorso-ventrally and enlarged laterally bearing more numerous not raised rounded structures. Basal vesicle larger. Cocoon strongly reinforced with numerous and irregular either thick or thin threads ..... **simplex** Gibbins

9. Base of pupal gills about 600 µ long. Cocoon with a broad antero-median process. Pilous plate with all the hairs branched. Tanzania ..... **raybouldi** Fain & Dujardin

   - Base of pupal gills less than 400 µ long. Cocoon without antero-median process. All the hairs of pilous plate simple..... **10**

10. Cocoon loosely woven with fenestrations in lateral and anterior parts. The uppermost filament describes a distinct slightly angulate dorsal curve ..... **perforatum** Fain & Dujardin

    - Cocoon finely woven without fenestrations:

      (1) The lowermost filament describes a wide ventral curve. Basal trunk short. Cameroon, Kenya ..... **antibrachium** Fain & Dujardin

      (2) Uppermost filament pairs with next one in distinct slightly angulate dorsal curve. Basal trunk short. Uganda..... **bwambanum** Krueger

      (3) All four filaments arise directly from the basal trunk and describe no angulation or spreading. Basal trunk 300–390 µ long. Uganda..... **itwariense** Krueger

11. Walls of filaments with very short irregularly rounded structures (type 4). The four filaments arise directly from the base. Basal vesicle strongly developed, sacciform, longer than the basal trunk. Hairs of pilous plate very long and drumstick-like. Cocoon finely woven. Rwanda, Burundi..... **sacculiferum** Fain & Dujardin

    - Walls of filaments with very short piliform structures (type 3). The four filaments arise either directly from the base or from secondary trunks. Basal vesicle less developed, not sacciform.

    Hairs of pilous plate much shorter and not inflated apically..... **12**

12. Base of pupal gills from 270 to 400 µ long and more than 27 times as long as its width in the middle of the trunk..... **13**

    - Base of pupal gills not longer than 200 µ and not more than twice as long as wide..... **15**

13. Cocoon very wide, transparent, with a short rounded antero-median process and reinforced laterally by thick obvious transverse threads. Kenya ..... **alatum** Fain & Dujardin

    - Cocoon narrower, not reinforced by thick transverse threads, with the anterior margin either slightly produced or not **14**

14. Basal trunk more than three times as long (380–400 µ) as wide (120 µ). Anterior margin of the cocoon slightly produced. Kenya ..... **nyanzense** Fain & Dujardin

    - Basal trunk about 2.7 times as long (270 µ) as wide (100 µ). Anterior margin of the cocoon not produced. Kenya ..... **baforti** Fain & Dujardin

15. The four filaments arise from two secondary stems and they are strongly unequal: one is relatively very short, one is distinctly longer than the others, and two are unequal and intermediate in length. All the hairs of the pilous plate are bifid or trifid. Cocoon finely woven..... **nigritarse** Coquillett

    - The four filaments arise either directly from the base or two arise from a short stem and the others directly from the base. The four filaments are subequal in length. Hairs of pilous plate simple, except in *S. sirimonense* where one hair is bifid..... **16**

16. The two dorsal filaments arise from a common stem distinctly diverging from the others. The uppermost filament describes an angulated curve. Cocoon finely woven ..... **arabicum** Crosskey

    - The four filaments arise directly from the base, they are spreading away at their base but the uppermost filament is never angulate. **17**

17. Only the uppermost filament describes a wide regular curve. Cocoon loosely woven with fenestrations ..... **brachium** Gibbins

    - Both the uppermost and the lowermost filaments describe a regular curve **18**

18. Uppermost and lowermost filaments almost opposite in direction. Cocoon finely woven. Tanzania ..... **candelabrum** Fain & Dujardin

    - Uppermost and lowermost filaments much less divergent. Cocoon very loosely woven with large fenestrations. Kenya, Uganda ..... **sirimonense** Fain & Dujardin

A1.3 References

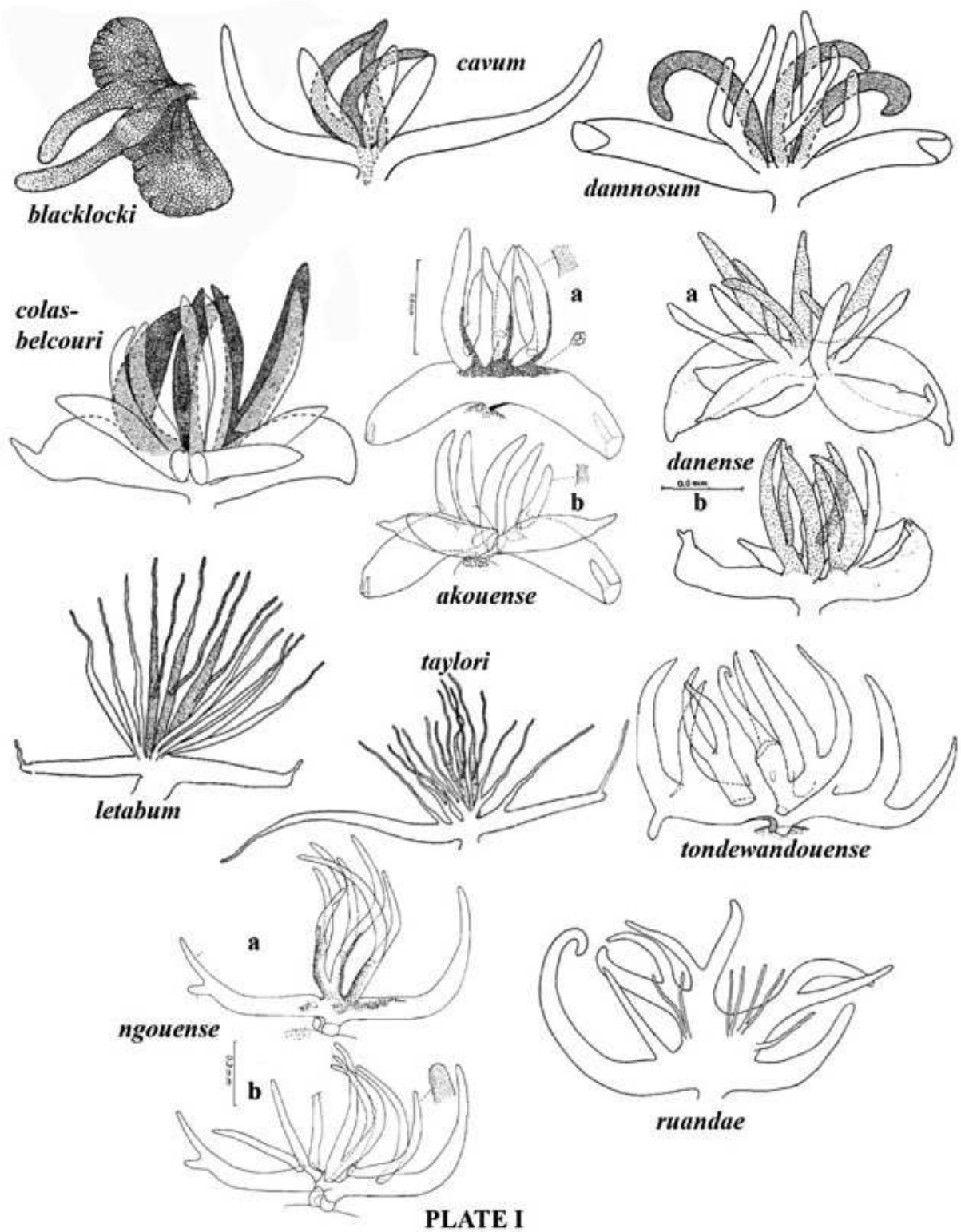
1. Freeman P, de Meillon B. Simuliidae of the Ethiopian region. London: British Museum (Natural History);1953.

2. Adler P. World blackflies (Diptera: Simuliidae): A comprehensive revision of the taxonomic and geographical inventory. Clemson (SC): Clemson University; 2021 (https://biomia.sites.clemson.edu/pdfs/blackflyinventory.pdf).

3. Fain A, Dujardin JP. The *Simulium nigritarse* complex (Diptera: Simuliidae). Rev Zool Afri. 1983:97(2):379–452.

4. Krueger A. The *Simulium nigritarse* subgroup (Diptera: Simuliidae) in Uganda: New species and country records. Zootaxa. 2016;4121(1):59–67.

Annex 2. Illustrations of African simuliid pupal respiratory filaments (gills)



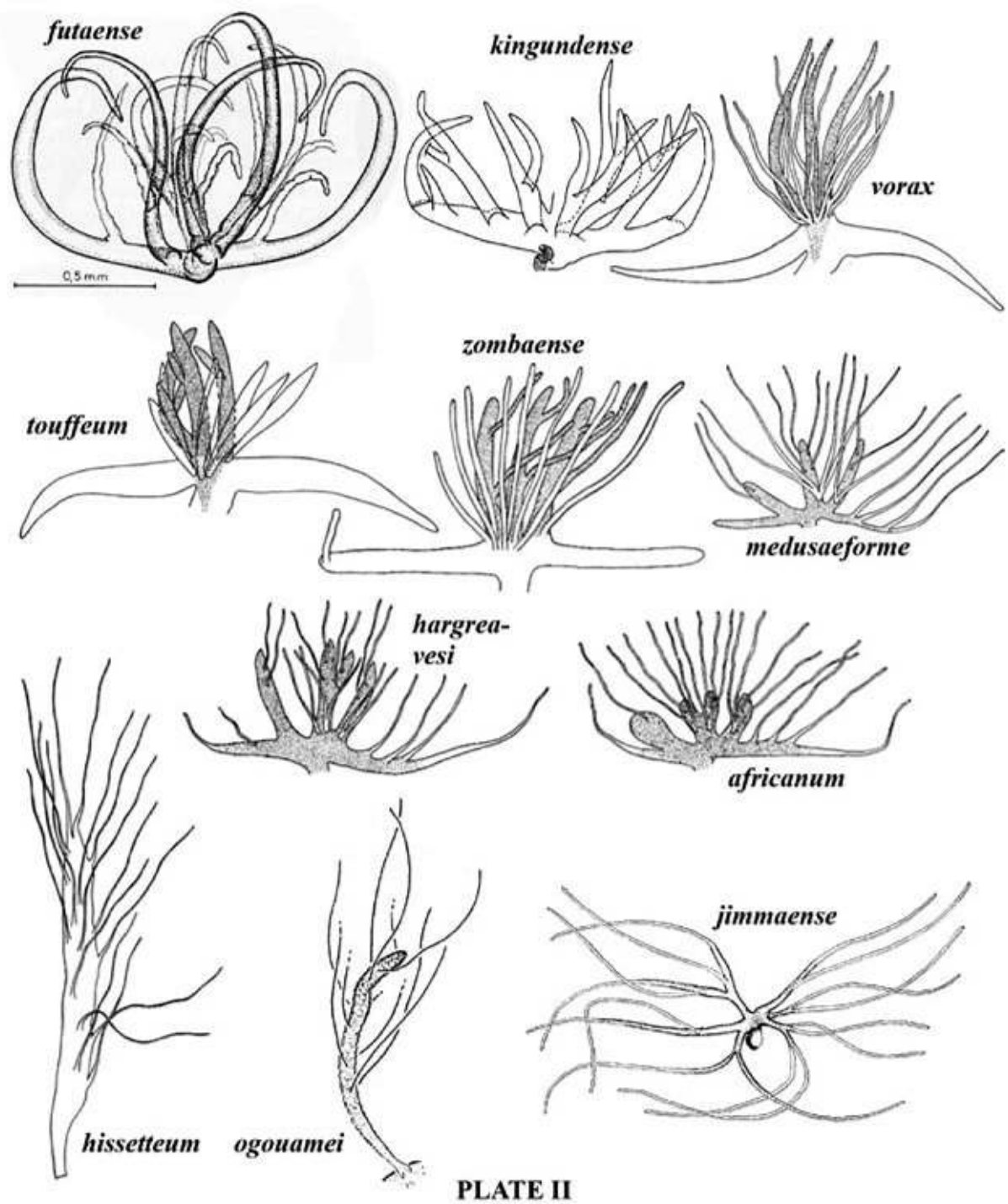

futaense
0,5 mm
kingundense
vorax
zombaense
touffeum
medusaeforme
hargrea-
vesi
africanum
jimmaense
hissetteum
ogouamei

PLATE II

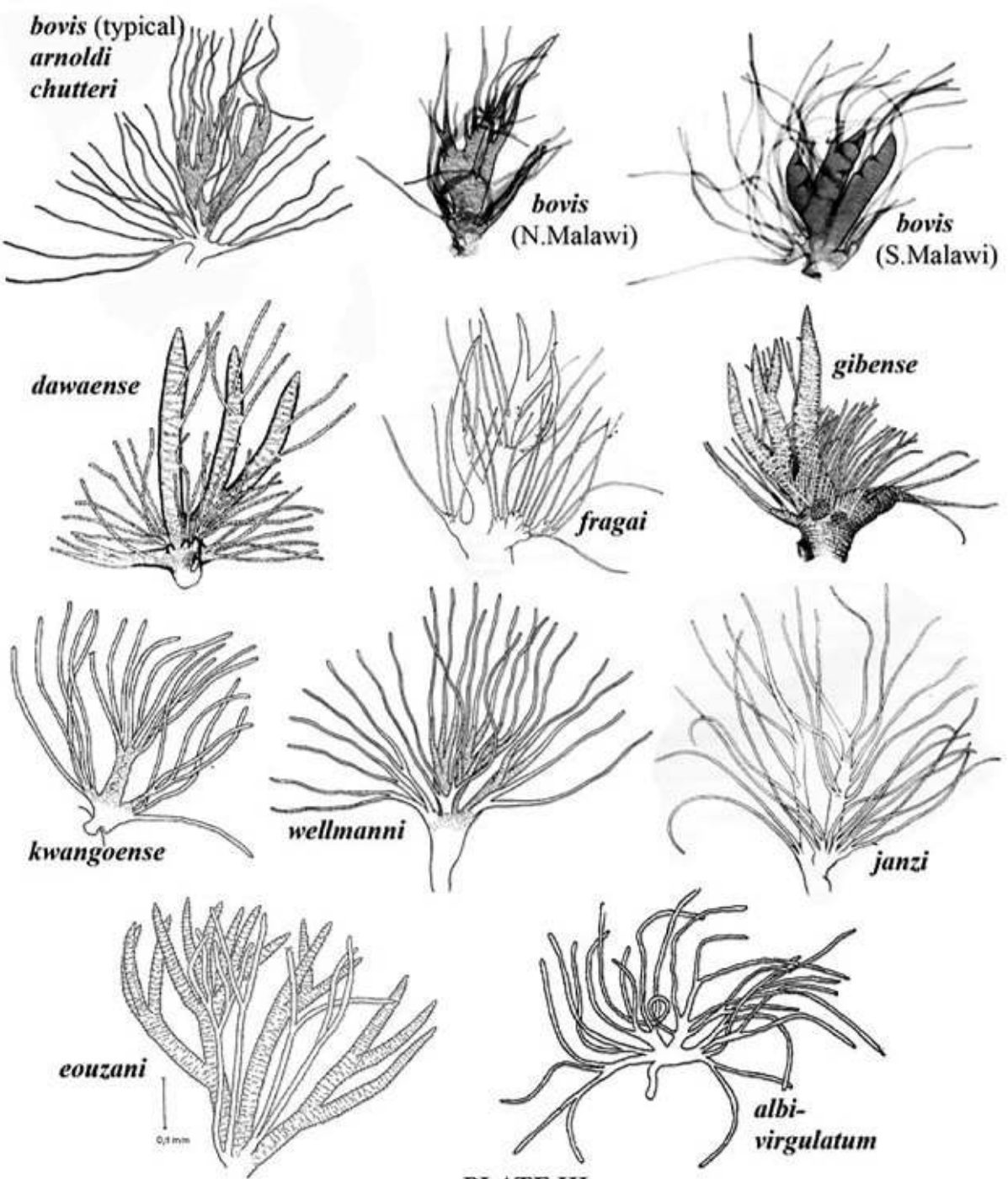

bovis (typical)
arnoldi
chutteri
bovis
(N.Malawi)
bovis
(S.Malawi)
dawaense
fragai
gibense
kwangoense
wellmanni
janzi
eouzani
0,1 mm
albi-
virgulatum

PLATE III

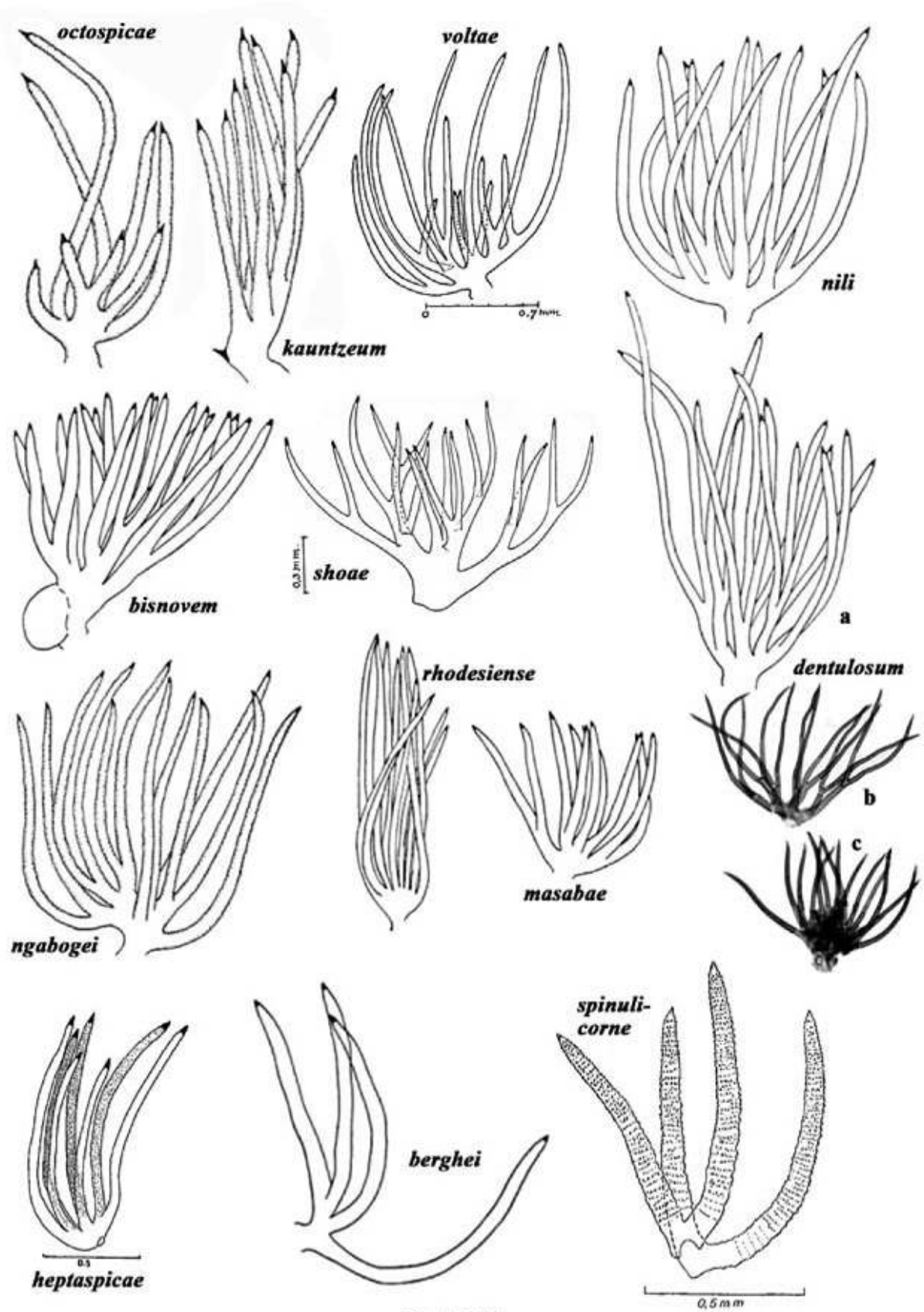

octospicae
kauntzeum
voltae
0.7 mm
nili
bisnovem
0.3 mm
shoae
a
dentulosum
b
c
rhodesiense
masabae
ngabogei
spinuli-
corne
0.5 mm
berghei
0.5
heptaspicae

PLATE IV

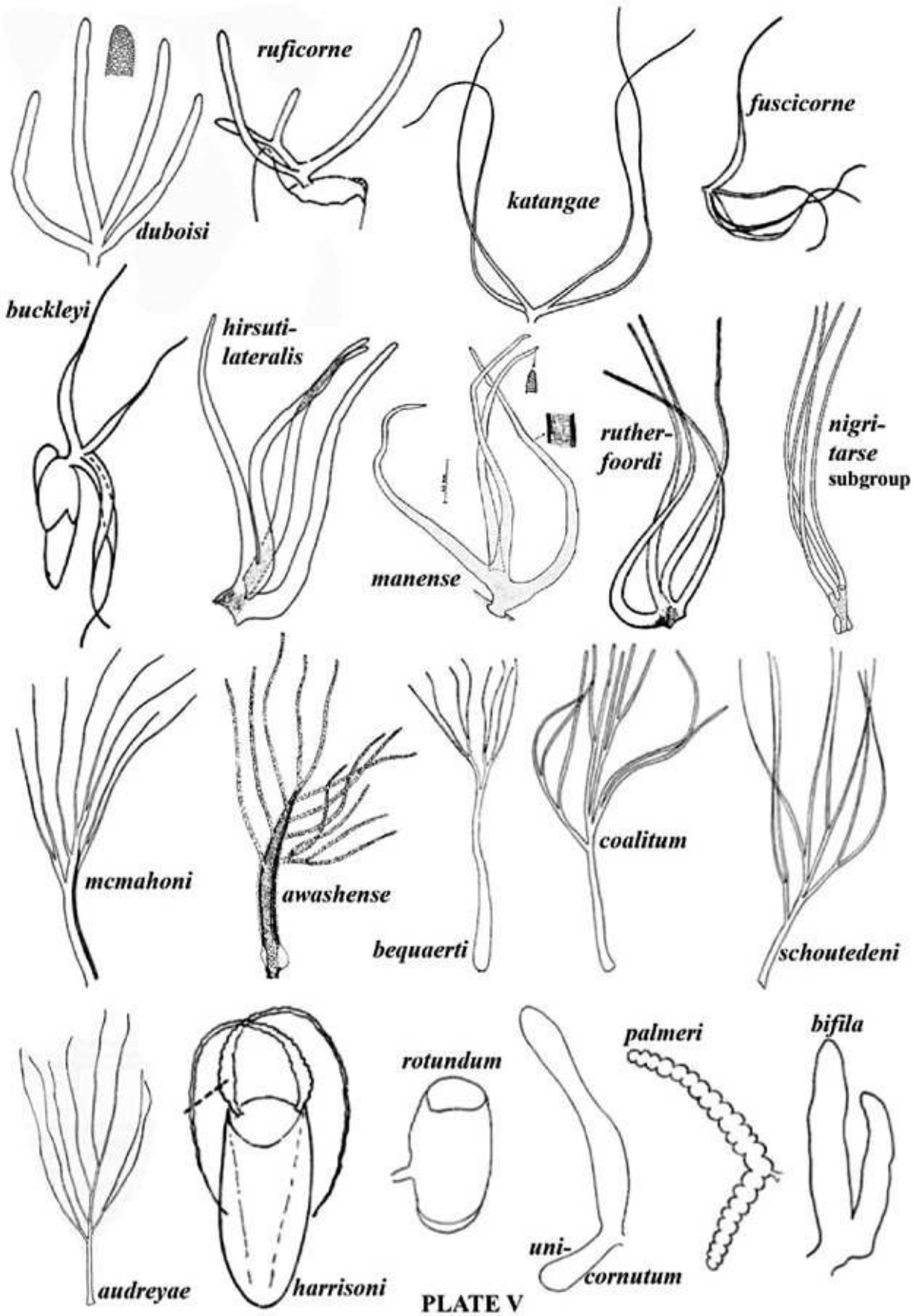

duboisi
ruficorne
katangae
fuscicorne
buckleyi
hirsuti-
lateralis
manense
ruther-
foordi
nigri-
tarse
subgroup
mcmahoni
awashense
bequaerti
coalitum
schoutedeni
audreyae
harrisoni
rotundum
uni-
cornutum
palmeri
bifila

PLATE V

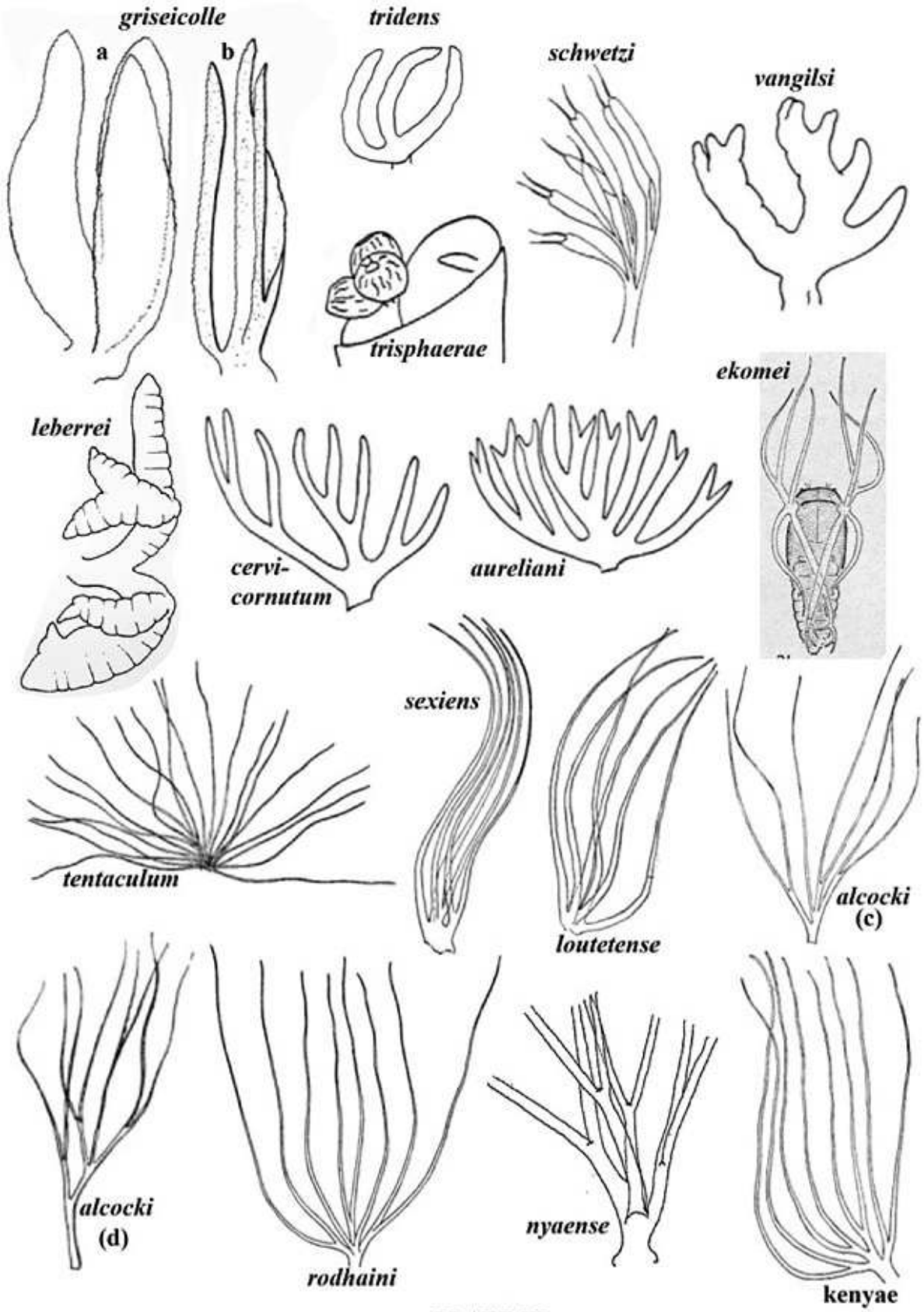

griseicolle
a
b
tridens
schwetzi
vangilsi
trisphaerae
ekomei
leberrei
cervi-
cornutum
aureliani
sexiens
tentaculum
loutetense
alcocki
(c)
alcocki
(d)
rodhaini
nyaense
kenyae

PLATE VI

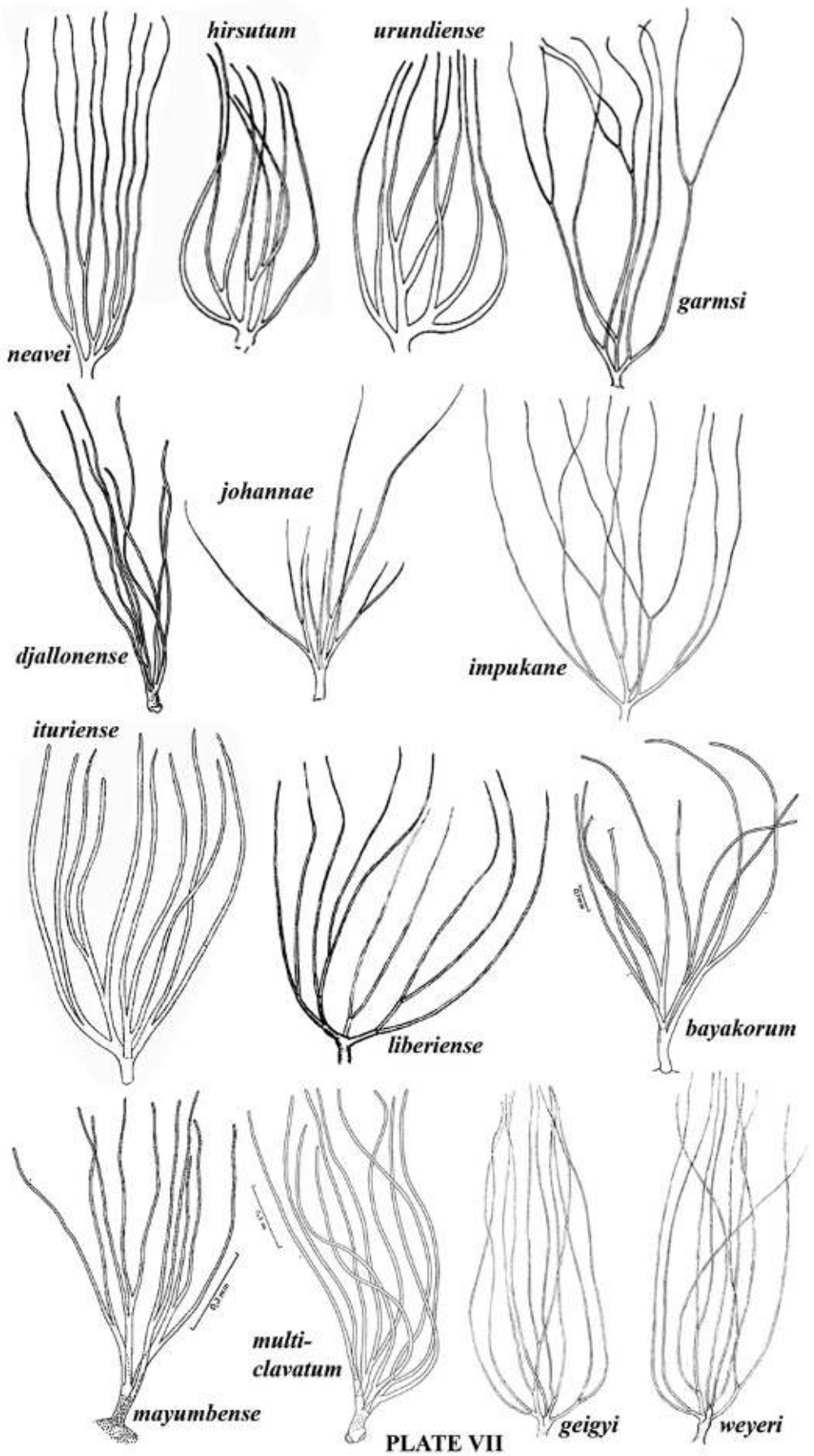
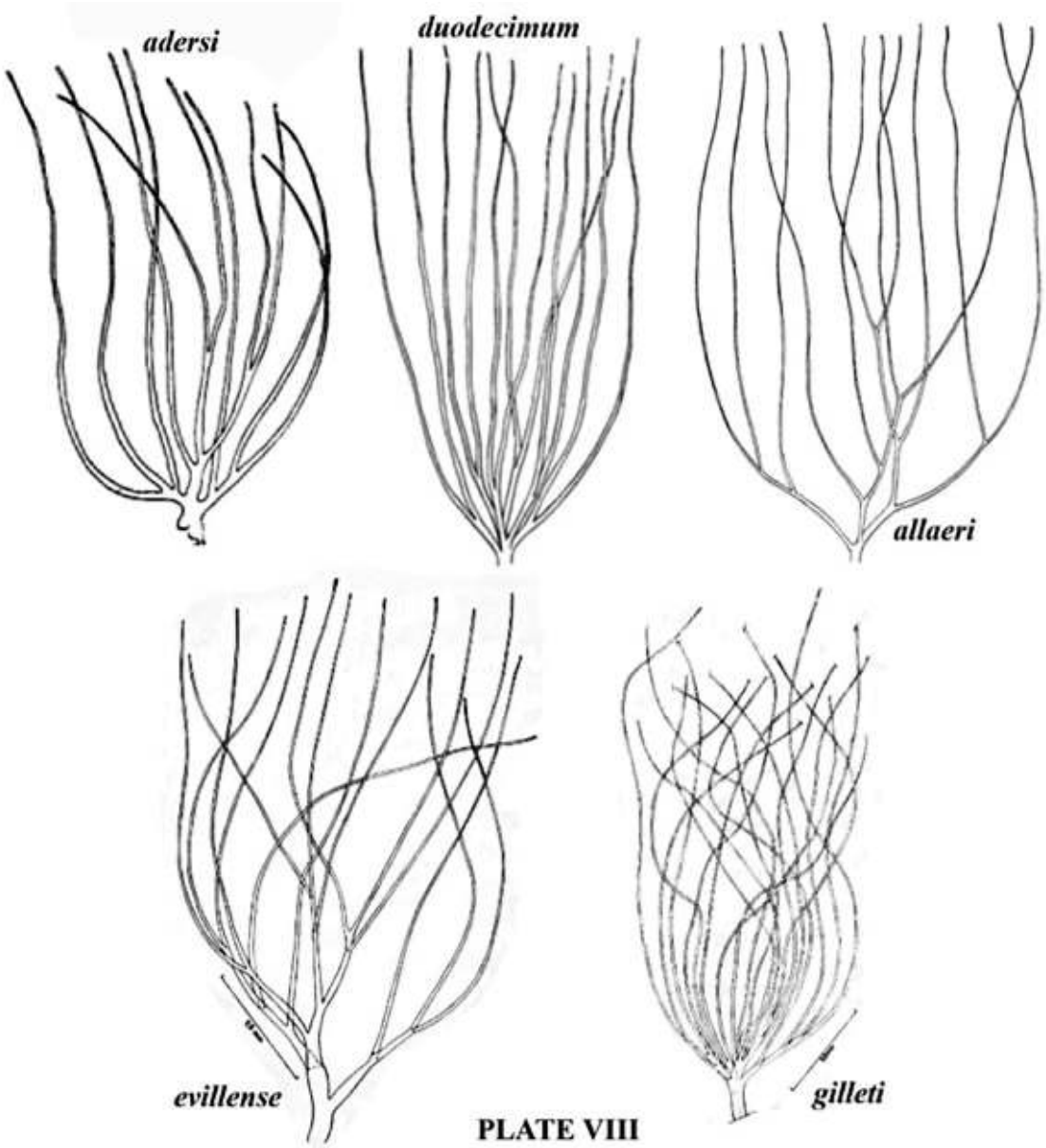

hirsutum
urundiense
neavei
garmsi
johannae
djallonense
impukane
ituriense
liberiense
bayakorum
multi-
clavatum
mayumbense
geigyi
weyeri

PLATE VII


adersi
duodecimum
allaeri
evillense
gilleti
PLATE VIII

For further information, contact:
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Tropical Diseases
World Health Organization
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1211 Geneva 27
Switzerland

Website: https://www.who.int

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