Diptera, Nematocera

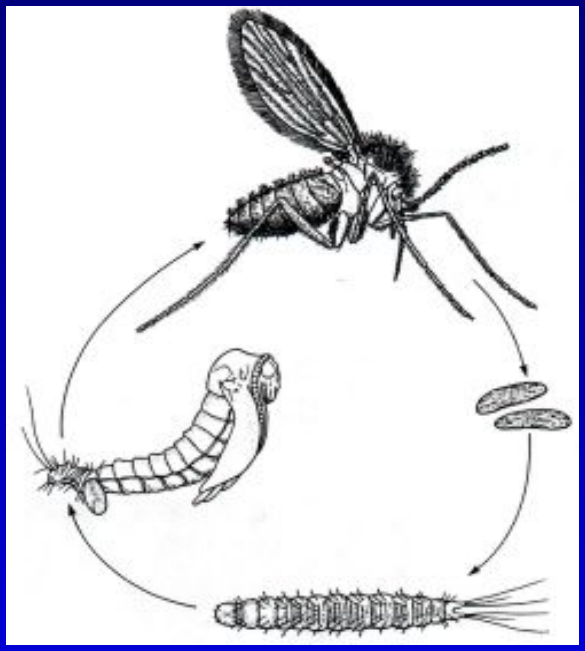
Psychodidae, Ceratopogonidae, Simuliidae, Culicidae
(= sand flies, biting midges, black flies, mosquitoes)

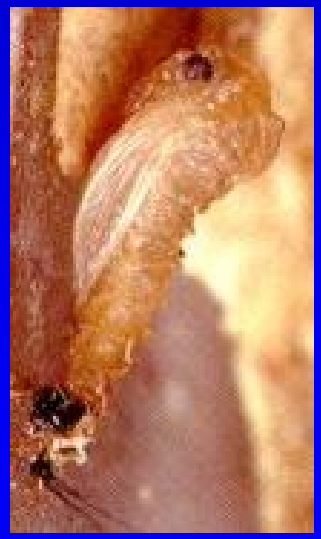


Mostly long legs and antennas, but black flies different.
Both sexes feed on sugar meal (nectar, honeydew, phloem).
Females: blood meal, gonotrofic cycle, concordant x discordant
Sugar meal directed to diverticulum (crop) x blood meal to midgut
Autogeny

Phlebotominae (Psychodidae)

Sand flies. About 800 species, tiny and hairy.
Traditionally two important genera: *Lutzomyia* (New World), *Phlebotomus* (Old World)
Sergentomyia: mainly on reptiles, few bite mammals.
Distribution: from tropics to temperate zone.
Exo and endophagic sand fly species.
Crepuscular and nocturnal, evening peak of activity, mating on or around the hosts, pheromones.
Breeding places terrestrial (rodent burrows etc.), four larval instars, feed on organic debris.





Medical importance of sand flies

1. Hypersensitive reactions, harara

In humans as well domestic animals, high IgE

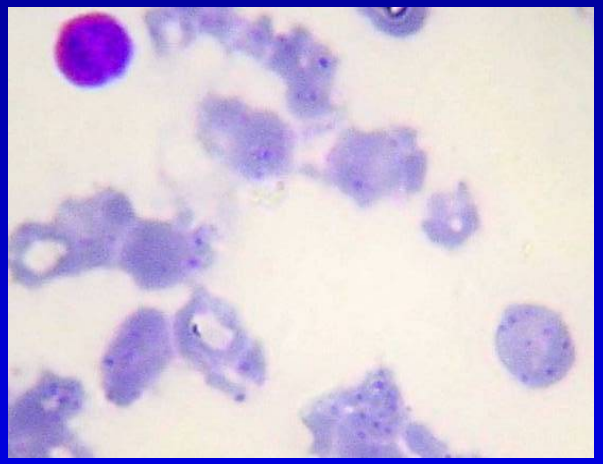
2. Transmision of viruses:

Phlebovirus (Bunyaviridae): Mediterranean

Sicilian virus, Naples virus: mostly just a fever (papataci f.)

but Toscana virus affect CNS. Reservoirs?

In sand flies transovarial and horizontal transmission.



3. Transmision of *Bartonella bacilliformis*:

Intracellular bacteria in endothelium and erythrocytes

Acute phase: anemia Oroya fever (1870 Lima-Oroya), up to 40% mortality.

Chronic form: Verruca peruviana (peruvian dwarf, see next slide).

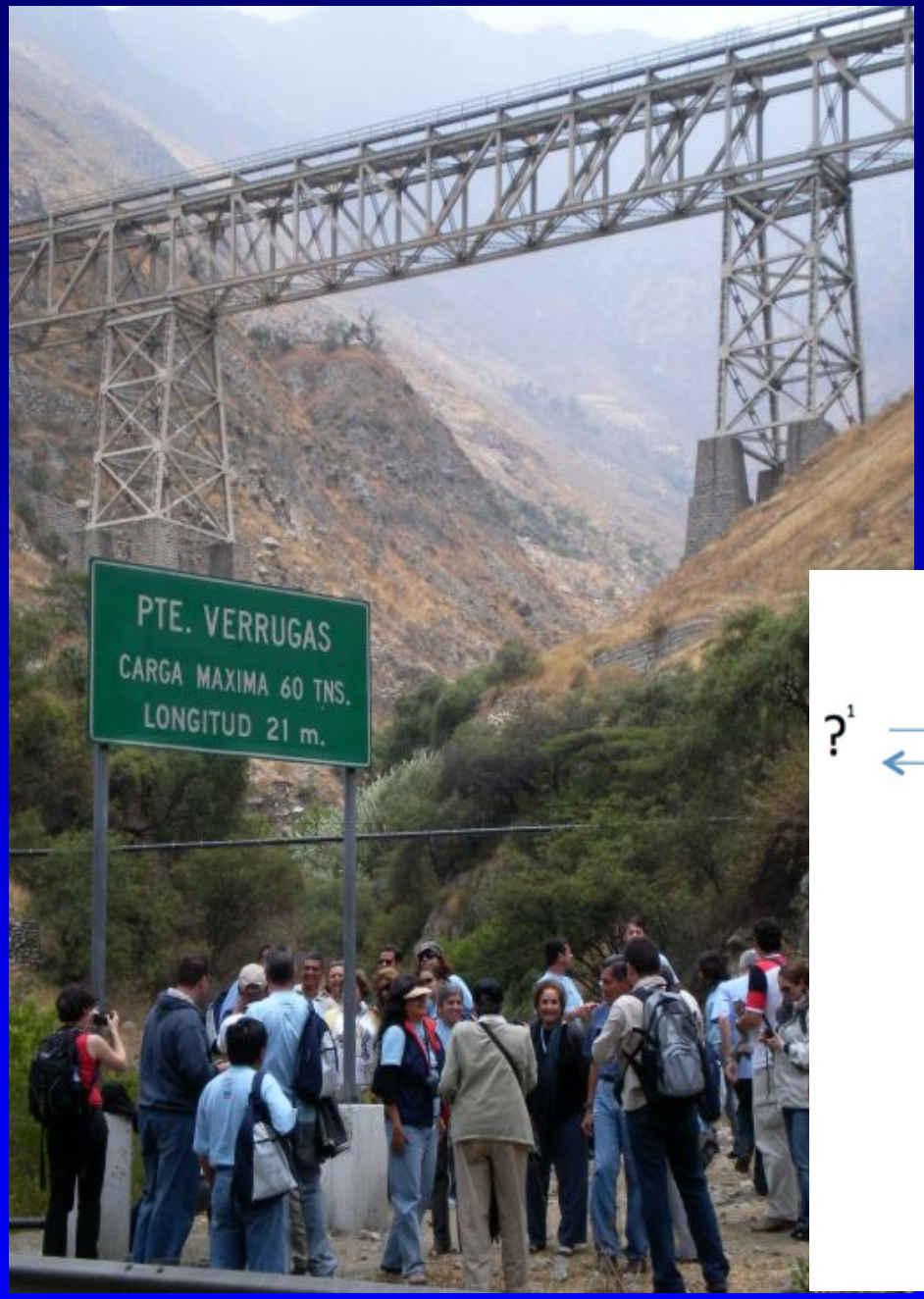
Carrions disease (young MD who died due to B. bacilliformis).

Agricultural communities in Andes: Peru, Ecuador, Columbia.

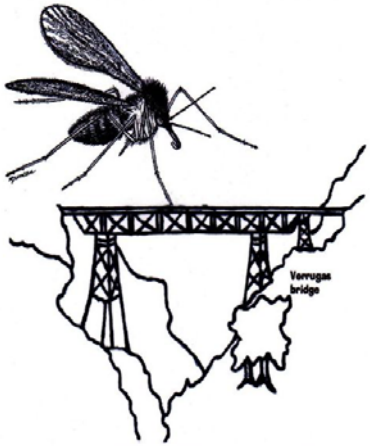
Lutzomyia species (*L. verrucarum*), transmission mechanism poorly known,

in humans vertical transmission, blood donors etc.

Diagnostic detection: various *Bartonella* species, DNA techniques. Antibiotics.

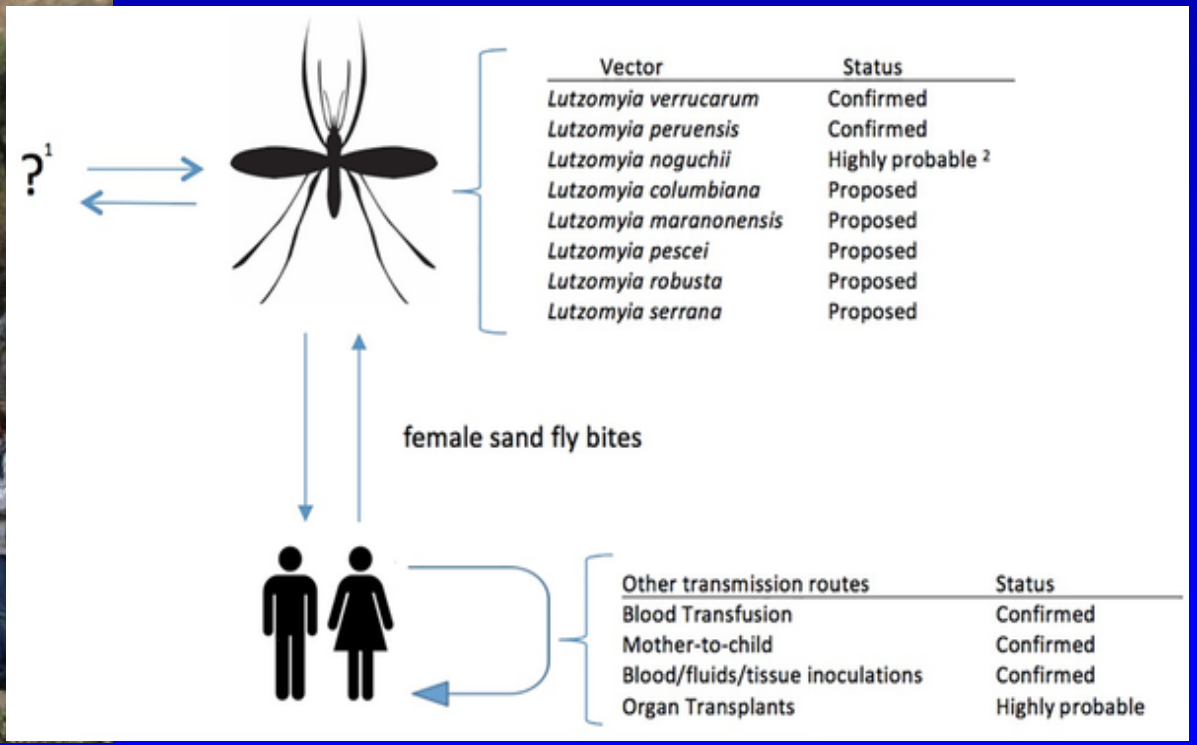


6th INTERNATIONAL SYMPOSIUM
on PHLEBOTOMINE SANDFLIES

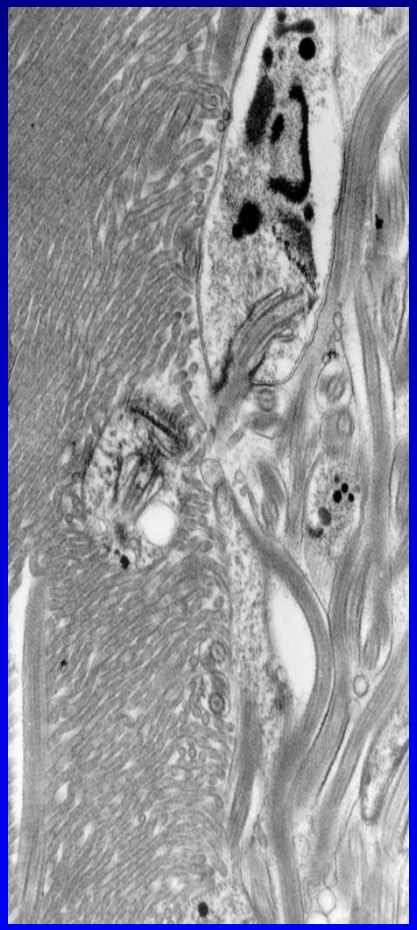


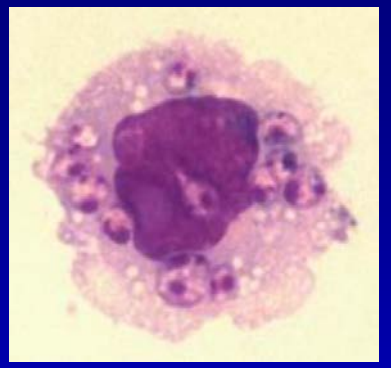
October 27-31, 2008, Lima, PERU

verruca peruviana

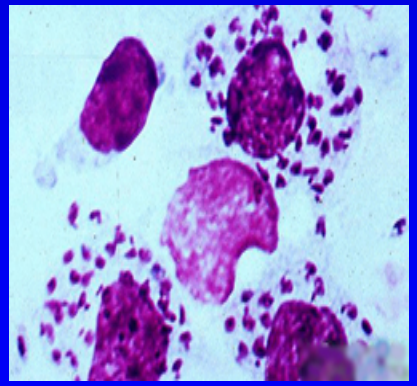


Leishmania life cycle





Promastigotes in sand fly midgut
Amastigotes in host phagocytes:
macrophages, temporarily neutrophils,
(Trojan horse model)



V mojí noze žije PARAZIT

Jana si ze svého výletu do Brazílie nepřivezla jen hromadu zážitků, ale i štípanec, který se změnil v krvavý kráter...

Přesně si vzpomínám, jak jsme s naší expedicí (v čele s mým přítelem Patrikem, obloženi batožinou) čekali na letišti. Konečně! Od šestnácti si plánuju vidět na vlastní oči deštné pralesy, nejmohutnější vodopády a krásnou Amazonku. Moje mamča už tak šťastná nebyla. Poprvé jsem cestovala tak daleko. Naposledy ke mně dolehlo její „dávej na sebe pozor", ale já už byla duchem v džungli. Jak jsem mohla tušit, že se po měsíci vrátím zoufalá - s odpornými parazity, kteří žerou lidské maso.

„PŘED TÚROU V PRALESE JSME SI DALI RELAX V THAJSKU..."

První zastávka byla hodně odpočinková. Většinu času jsme se váleli na thajských plážích a poté se vydali na menší výlet do tropické džungle. Příroda kolem mě naprosto uchvátila, a dokonce ani číhající hadi, protivný hmyz a všudypřítomné pijavice mi nemohli zkazit náladu. Po téhle předehře jsem byla tak akorát naladěná na cestu do Jižní Ameriky. Když jsme dorazili do Brazílie, naplánovali jsme několikadenní výpravu po deštných pralesech. Moji nezdolnou euforii nenarušilo ani nejmenší tušení, že tenhle trip se mi stane osudným.

„CO MĚ TO, PROBOHA, KOUSLO ZA MRCHU?"

Čekal nás pořádný záběr, každý den kolem osmi hodin stoupání do svahu. „Přece něco vydržím!" chvástala jsem se před Patrikem a byla o tom přesvědčená. Vlhkost v takové krajině dosahuje téměř

CO JE TO LEISHMANIASIS?

• Maso požírající parazit, přenášený muchničkami (sandflies), které se nakazí kousnutím infikovaného savce. • Příznaky jsou malé zarudlé otoky obklopené vyrážkou, které se mohou změnit v mokvající ránu. Ta se zvětšuje úměrně s rostoucím množstvím parazitů. • Pokud se choroba neléčí antibiotiky, může trvat roky, a i když odezní, může se znovu obnovit. • Infekce je schopná rozšířit se k nosu a ústům a „nechat zmizet" okolní tkáň (slizniční Leishmaniasis). • Leishmaniasis se vyskytuje v 88 zemích na území deštných pralesů ve Střední a Jižní Americe až k pouštím v západní Asii. • V průběhu jednoho roku se objeví kolem 1,5 milionu případů nemoci. • Zatím neexistuje žádná účinná vakcína proti onemocnění.

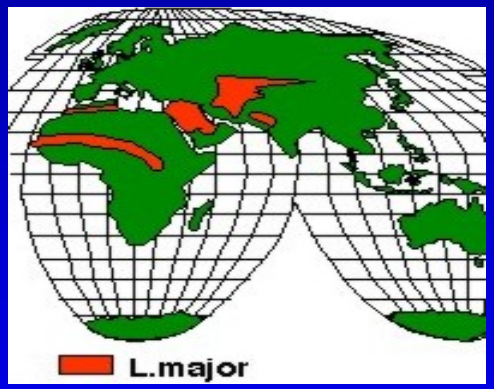
Leishmania transmission and epidemiology of leishmaniases

Mostly zoonoses, mammalian reservoirs, role of asymptomatic hosts

Spread: climate change, human migration (seasonal workers, refugees),
lack of anti-malaria or anti-rabbies campaigns

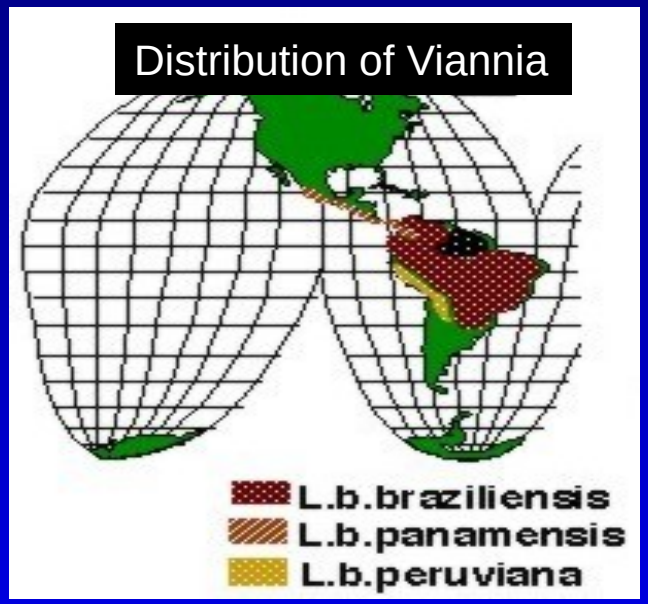
Cutaneous leishmaniases in the Old World:

1. *Leishmania major*: zoonosis, gerbils, *Phlebotomus papatasi (P. duboscqi)*
2. *L. tropica*: anthroponosis in old cities x zoonotic foci with hyraxes



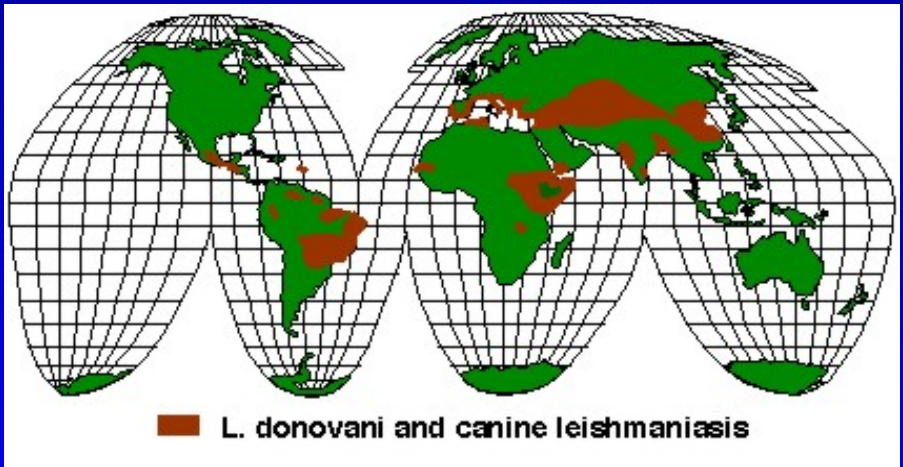
Cutaneous and mucocutaneous leishmaniases of Latin America

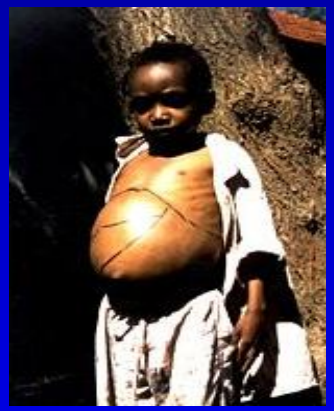
subgenera *Leishmania* and *Viannia*, zoonoses.
Reservoirs are rodents or other forest animals, vectors various *Lutzomyia* species



Visceral leishmaniases: *L. donovani* complex

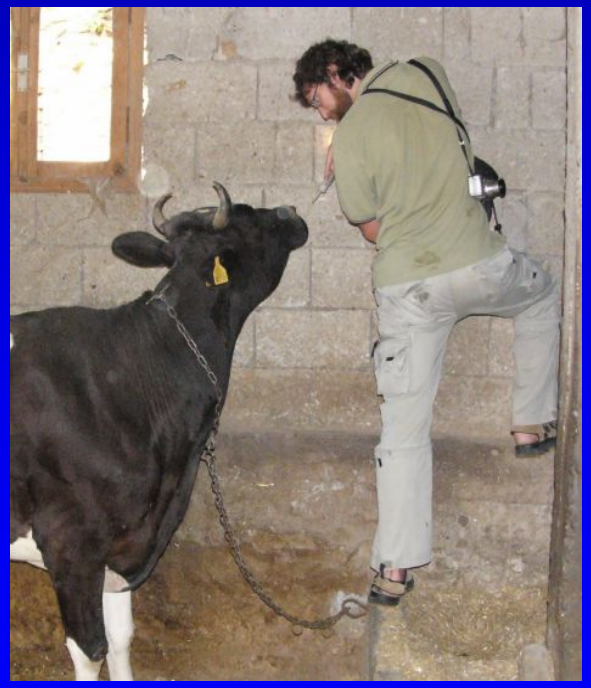
L. donovani: kala azar, high mortality (20-90%), affected by nutrition and immune response. India, Bangladesh and Nepal: antroponosis, East Afrika: Ethiopia, Sudan: zoonosis?
L. infantum (=L. chagasi): zoonosis, reservoirs canids (dogs).
Transmited by *Phlebotomus* in Mediterranean but *Lutzomyia* (mainly *L. longipalpis*) in Latin America, most cases in Brazil.





Control of sand flies

Destruction of hosts (dogs, gerbils) but breeding places mostly unknown.
Resting places in stables and chicken houses. Residual spraying.
Impregnated bed nets (Olyset): lower mesh size than for mosquitoes.
Nets in windows and around houses.
Dog collars and spot-on. Repellents on humans.
Oral insecticides isoxazolines (afoxolaner, fluralaner)
licensed in domestic animals.



Ceratopogonidae

Biting midges, about 5000 species and 60 genera
Only 3 genera parasitic: *Culicoides*, *Leptoconops*, *Lasiohelea*
Difficult identification, changing taxonomy, new species.
Four larval instars, both males and females feed on nectar, females feed on blood of vertebrates.
Exophilic, frequent autogeny.

Leptoconops
About 150 species, subtropics and tropics, mainly America (Florida, California). Breeding places in brackish wetlands, near beaches. Diurnal (active during day)
New biotopes (golf courses), problems for tourists.

Lasiohelea (formerly *Forcipomyia*): all over the World, important for fertilization of plants (cocoa etc.)

***Culicoides*: about 1000 species.**

Whole world up to arctic circle.

Breeding in humid soil with organic substrate, swamps,

4 larval instars feed on microorganisms or invertebrates,

in temperate climate overwinter.

Medical importance and control

Dermatitis, skin hypersensitivity.

Bite even during day, depends on wind speed and light intensity. Almost exclusively exophagic.

Problems with individual protection in countryside: repellents, hiding indoor. Affect forestry and tourists.

Seasonality, Scottish highlands, Lapland.

Chemical control difficult, extensive breeding places, barrier spraying of vegetation.

Mechanical control: regulation of the groundwater

Biting midges as vectors

Relatively low medical importance
filarioses: non-pathogenic Mansonella in tropics of the New World
Bunyaviridae: Oropouche (South America), Rift Valley (*Phlebovirus*, Africa)
Leishmania enrietti complex (subgenus *Mundinia*).

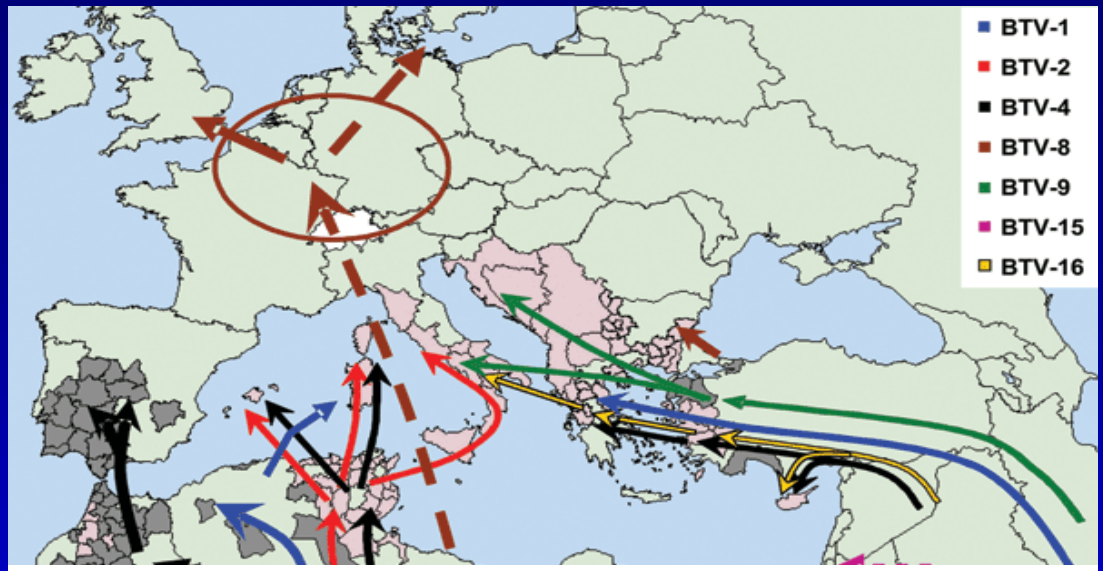
High veterinary importance
Filarioses: onchocerciases in cattle
Blood protozoa: *Haemoproteus*, *Leukocytozoon*

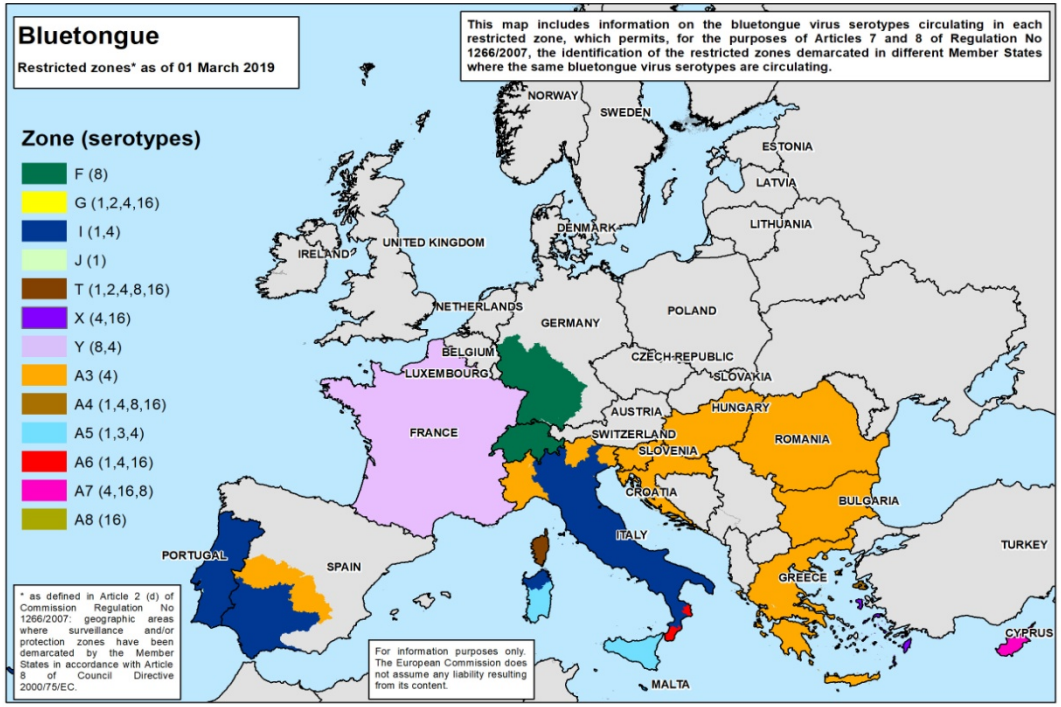
Viruses: **Bluetongue** (BTV), *Orbivirus*.
All around the World (America, Australia etc.), Europe.
High fever, excessive salivation, cyanosis of the tongue
High mortality of sheep. Cattle, goats and wild ruminants asymptomatic but with high virus levels in blood.
Vectors: *Culicoides* (*C. variipennis* complex in USA)
Prevention: quarantine, vaccine, vector control.

Schmallenberg virus (SCV) (*Orthobunyavirus*):
Fever, aborts in sheep, goats and cattle. Epidemy in 2012.

Blue tongue virus in Europe

Till 1998 only Cyprus andTurkey, then south of France and Spain. *C. imicola*.
2006: Netherlands, Belgium, Germany. Source??
Transmition by local midges.
August 2007: England
November 2007: Czech Republic
Local midges susceptible.
2008: Overwintering in leucocytes, Transplacental transmission, Veneral transmission in cattle.
Vaccine with attenuated virus unreliable.
2009-11: reliable vaccine
Czech Rep. free of BTV since 2013.
2019: mainly France, Italy, Spain/Portugal, Greece and Cyprus

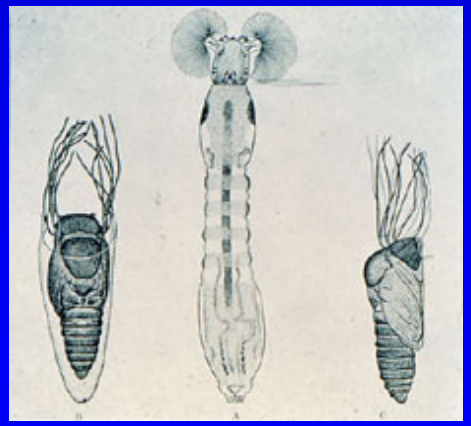


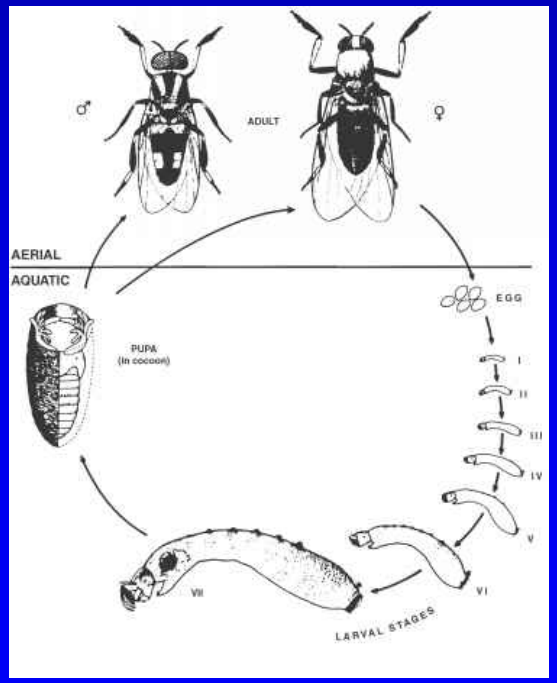


Simuliidae

Eggs laid on stones and vegetation in running water, in some species aggregations of females, egg and larval masses.

In tropics up to 20 generations per year, 2-3 week life cycle (eggs 2 days, larvae one week).
In temperate regions
usually a single generation,
eggs or larvae overwinter.



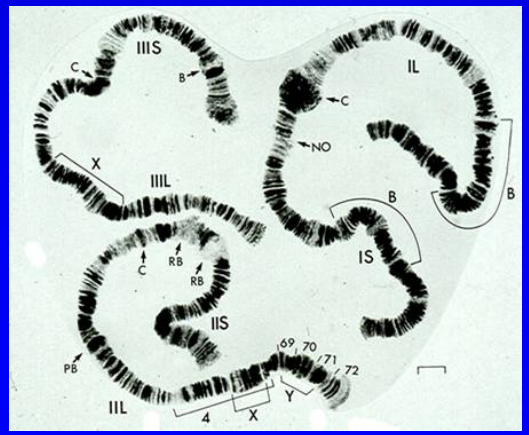


Larva: in running water, unstable number of instars, filtrators.
Attachment versus migration of larvae,
silk fiber, phoresis by aquatic invertebrates.

Pupa: attached, coccon, „gils“ (tracheal tubes)

Adult: waiting in pupa, with a buble to surface.
Mating, sometimes in swarms. Holoptic eyes of males, visual search for females, various omatidia and eye pigment.
Diurnal, exophagic. Aggressive.
Fly for several km from the breeding sites.
Long migration (hundreds of km) of nuliparous females in Canada or Sahel (Africa, savannah).

Species complexes (sibling species)
Sympatric, do not crossbreed,
Various susceptibility to pathogens,
Polytenne chromosomes in
larval salivary glands, isoenzymes,
DNA barcoding.

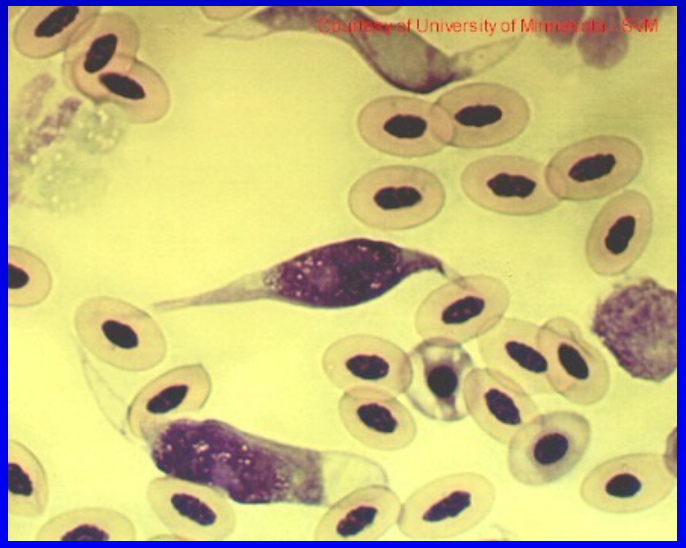


Medical and veterinary importance

Bite only some species/populations (mainly Siberia, Scandinavia, Canada, tropical Africa, Amazonia). Other populations autogenous.
Various host specificity: ornithophilic (*Eusimulium*), mammaliophilic (*Odagmyia*).
Painful is resulting hypersensitivity reaction in sensitized individuals.
Numerous bites: oedema (face, limbs), astma-like problems, headache, swelling of lymphatic nodes, chronic skin problems.
Restrictions for the field work (Siberia, *Gnus gnus*) or cattle farming (formerly Iron gates on Danube river, *Simulium golubatchense*)

Transmission:

Veterinary important:
Leukocytozoon
(poultry, but also wild birds, owls, birds of prey)



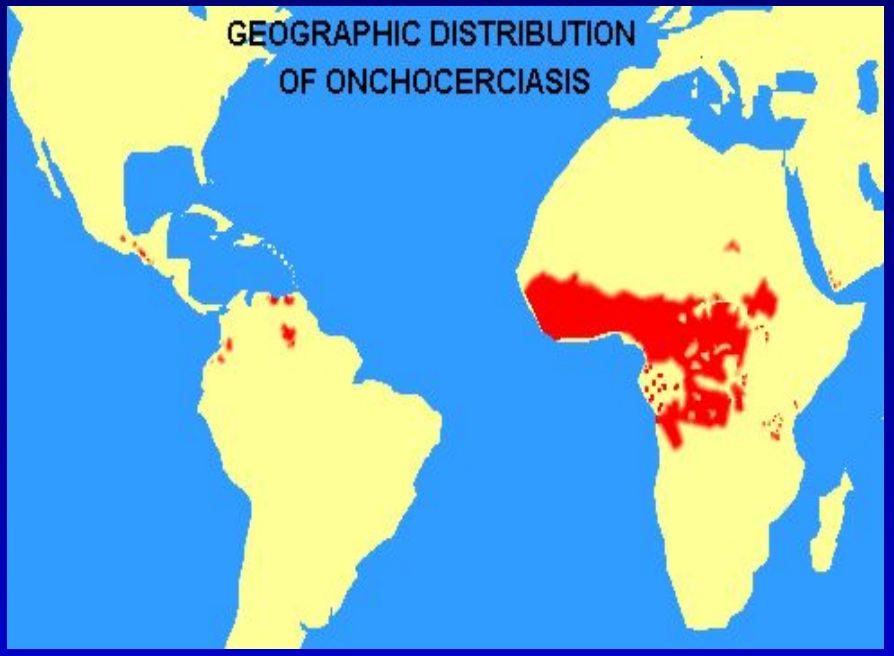
Onchocercosis:

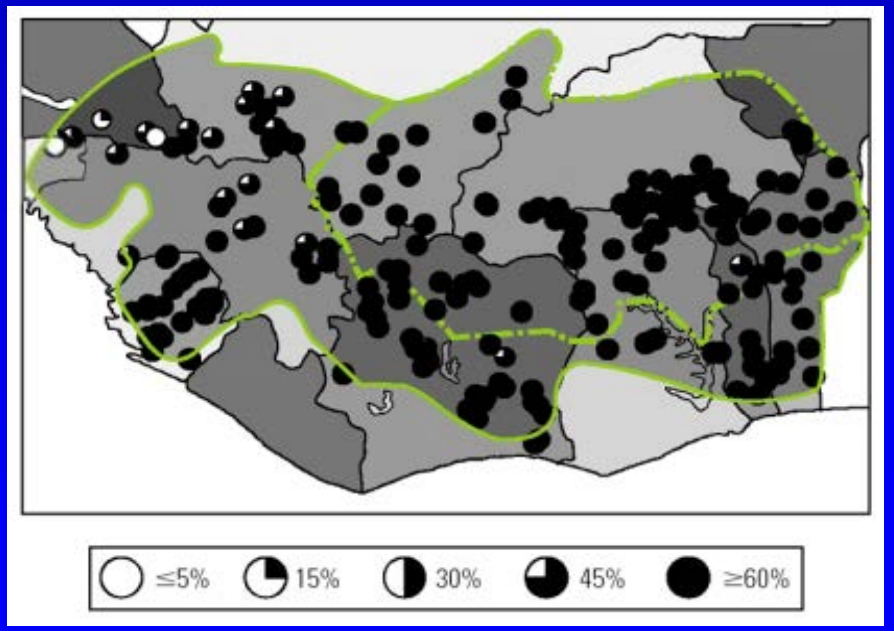
Chronic, antroponosis, nodules with adults, skin full of microfilaria, eye lesions, „river blindness.

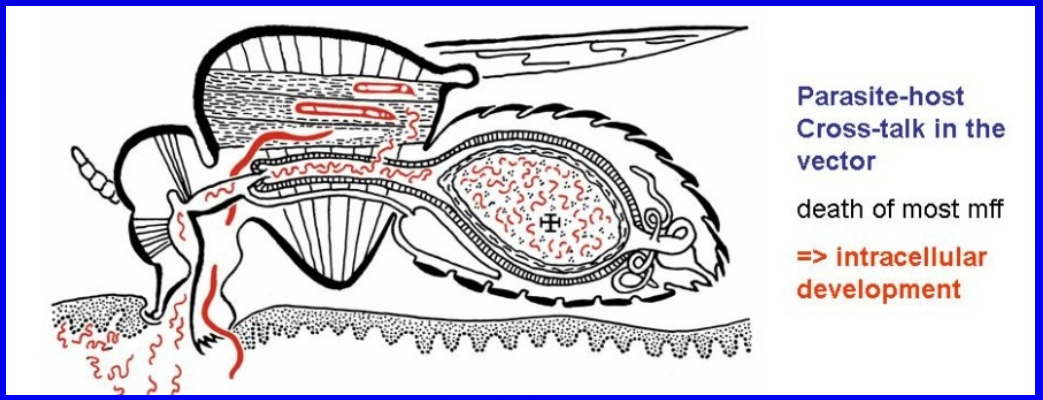
Tropical Africa, imported to Latin America.

In 1990-2000 around 20 millions infected, in some villages 100% infected and 10% blind. In 2017 more than 1 million blind.

Life cycle: two hypotheses about escape from midgut, intracellular development in fly mussels, L3 to labium.







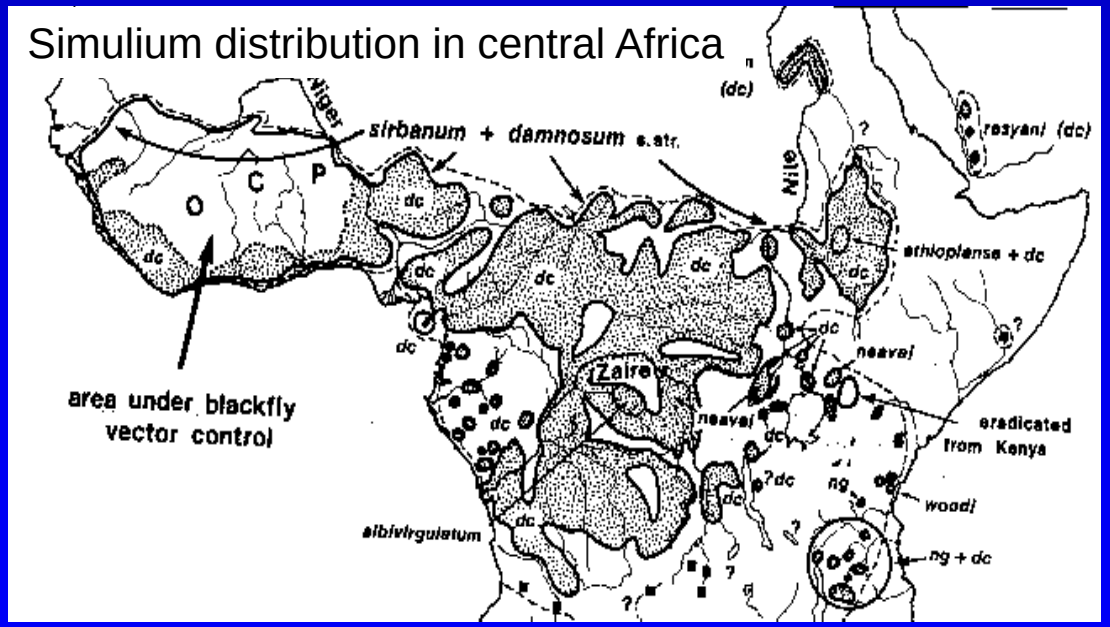
River blindness

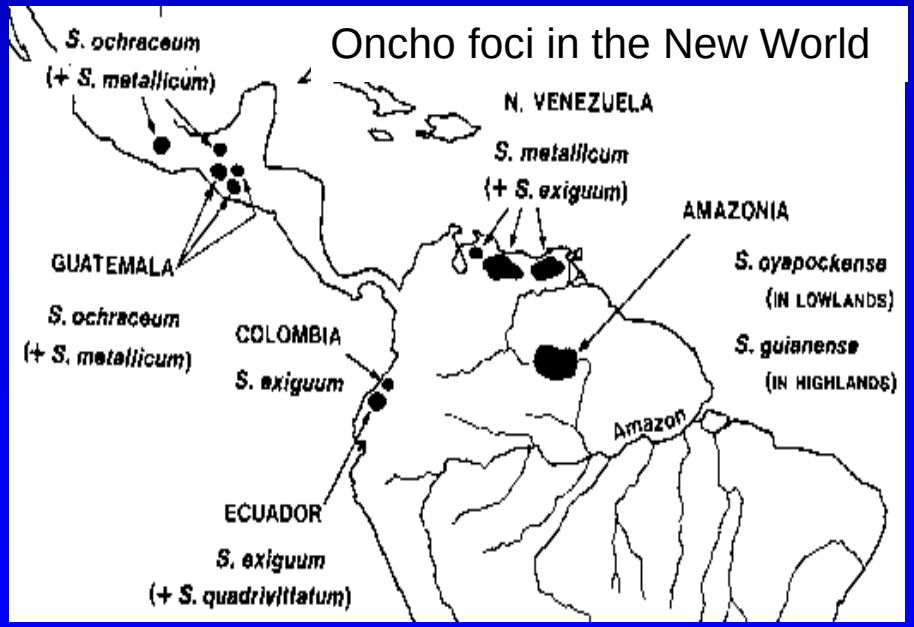
Vectors of *Oncocerca volvulus*

***Simulium damnosum* complex**:
„Ideal vector“: competent, high capacity to transmit (antropophilic, several gonotrophic cycles). **A**bout 20 species, *S. damnosum* s.s., *S. sirbanum* (95% of onchocerciases). Long migrations of nulliparous females across savannah. Breeding sites in large rivers, parous females stay close to breeding sites.

Other *Simulium* sp. in mountains and forests: competent but less important.

Different *Simulium* species transmit *O. volvulus* in Yemen and Latin America





History of chemical and biological control

directed against breeding sites

1. pre DDT era: migration of human population.
Side effect of changes in water regime
(Danube and *S. colombaschense*).

2. DDT (1946-1970) larvicidal effect.

3. Post DDT era: temephos, Bacillus thuringiensis H14
(destruction of larval midgut)

OCP (Onchocerciasis Control Programme 1974-1995):
West Africa, breeding sites: 1974 temephos,
1991: BT H14 plus chemical insecticides.
1987: Ivermectin (Mectizan, Merck), mass drug administration twice a year, kills microfilaria. Big success.

APOC (African Programme for Onchocerciasis Control)
1995-2015: 20 countries, incl. Central and East Africa
Mainly Mectisan. Funded by WHO and BMGF. Partial success.

Onchocerciasis Vaccine for Africa: from 2015

Culicidae

About 4000 species, 3 subfamilies,
relative to nonparasitic Dixidae, Chaoboridae

1. Toxorhynchitinae: *Toxorhynchites*: tropical species, large, colorful, curved proboscis, diurnal, nonparasitic, predatory larvae used for biological control.

2. Anophelinae: 3 genera, important only *Anopheles*: various continents, transmission of *Plasmodium*, lymphatic filaria, viruses

3. Culicinae: about 30 genera, important 6-7

Aedes: varius continents, yellow fever, dengue, encephalitis, lymphatic filariases.

Culex* a *Mansonia: various continents, WNV and other viruses, filariases.

Culiseta: Old World: filariases

Haemagogus* a *Sabethes: Latin Am., breeding in plant cavities, Yellow fever.

Mosquito breeding places

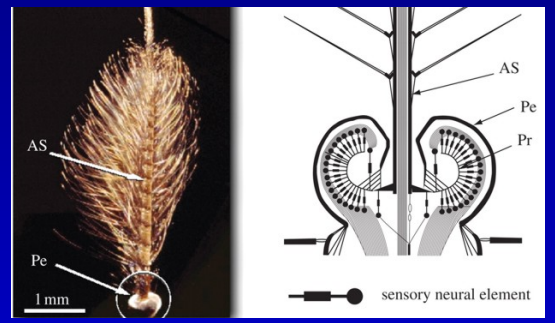
Eggs: 20-200, either on water surface (*Culex* and *Mansonia* in rafts) or to humid substrate (*Aedes* and *Haemagogus*), hatch after submersion to water.
Local *Aedes* need winter diapauze to hatch. Other diapauze as adults (most *Anopheles*, *Culex*, *Culiseta*).
Haemagogus and some *Aedes* breed in tree holes.
Some prefer salt water (or mixed) habitats (*Anopheles atroparvus, sacharovi*, *melas, merus*).

Larvae: 4 instars, eucephalic, filtration apparatus.
Sipho in Culicinae, plate in Anopheles. *Mansonia* from aerenchym of water plants. *Anopheles* larvae filtrate mainly from water surface.
Different requirements for clean water
(*Culex pipiens* in dirty water).

Pupae: mobile, breath from surface but *Mansonia* from aerenchym.

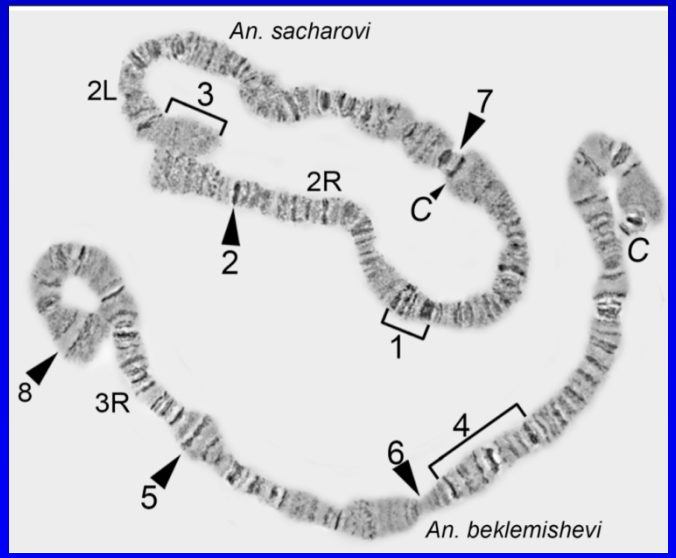
Biology of adult mosquitoes

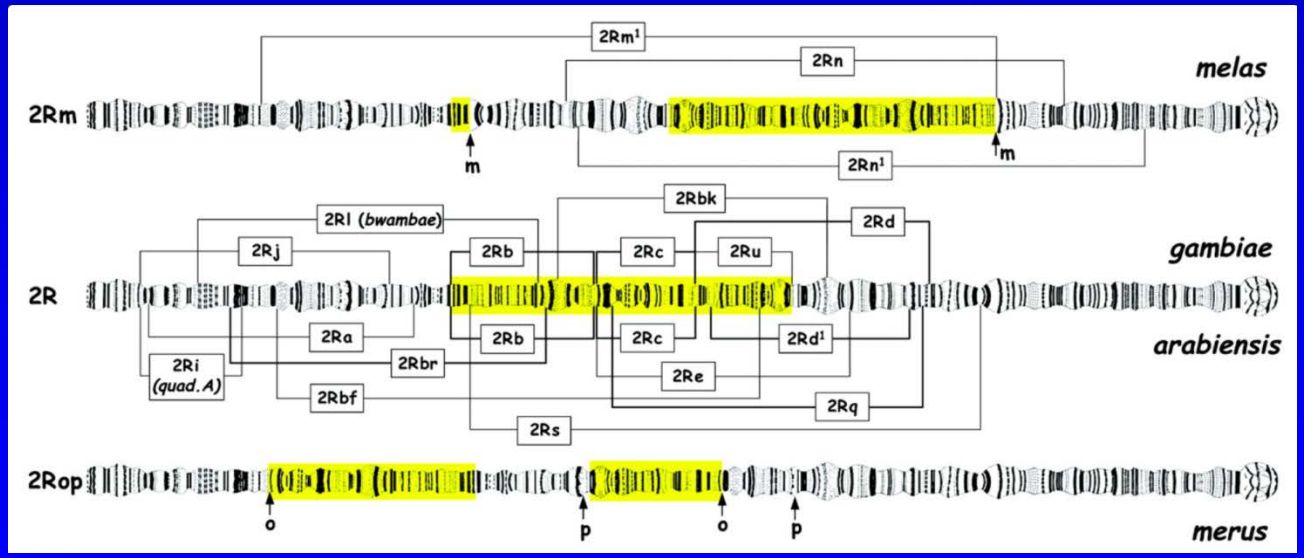
Males emerge earlier (protandry), rotation of genitalia.
Mating usually in flight, some species swarm, male hears female by Johnston organ in pedicel = 2nd segment, contact pheromones important for short distance.
Active at dust but *Mansonia* and *Aedes* also during day
Biting: anthropophilic, zoophilic, ornithophilic, exophagic versus endophagic.
Bloodmeal digestion and resting: endophilic, exophilic. Importance for transmission and vector control
Aedes aegypti: anthropophilic, exophagic, exophilic. Risk: outdoor activities, sleeping outdoor.
Anopheles gambiae: anthropophilic, endophagic, endophilic. Risk: even infants inside the house.
Gonotrophic cycles, discordant species (*A. aegypti*).
Autogeny: depends of species, larval food, adaptation of *Culex molestus* laboratory colonies.
Adults live for about month, females longer than males, *Anopheles* and *Culiseta* adults overwintering.



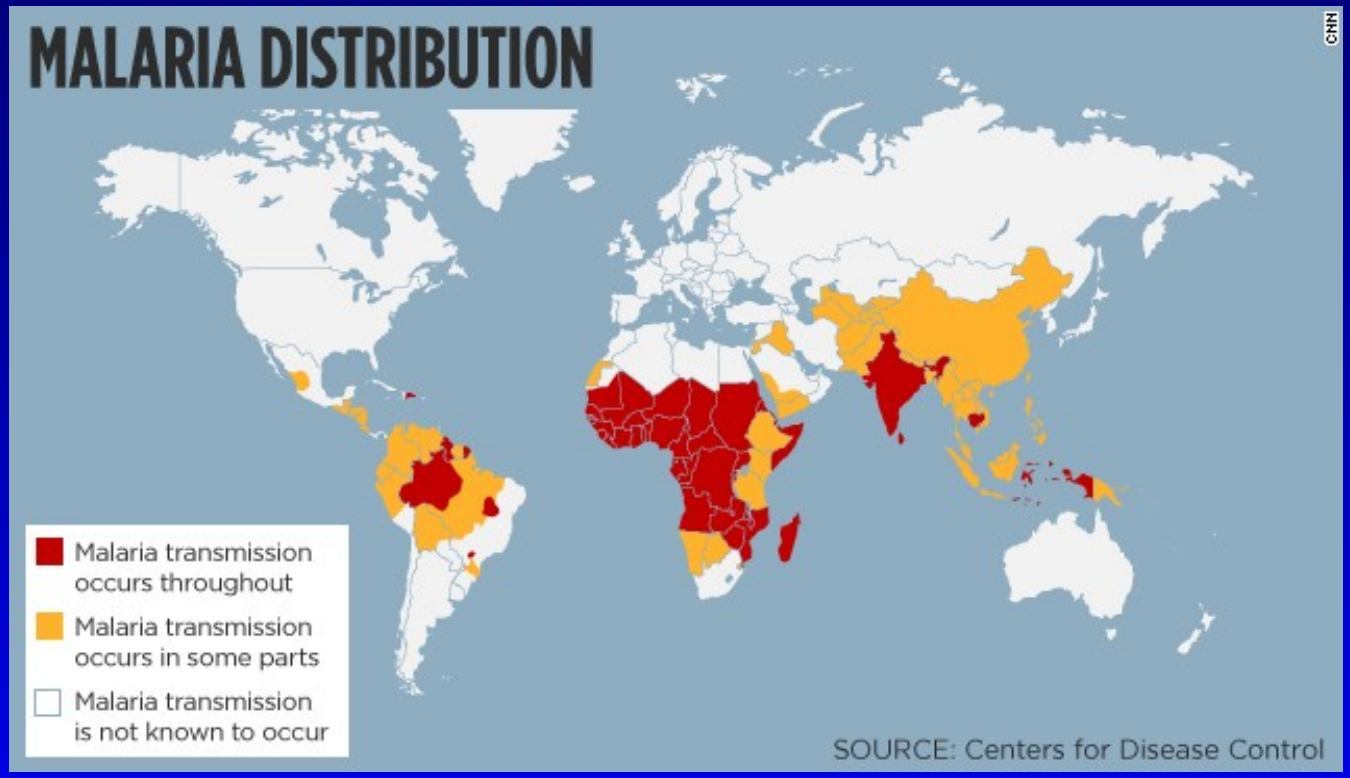
Species complexes

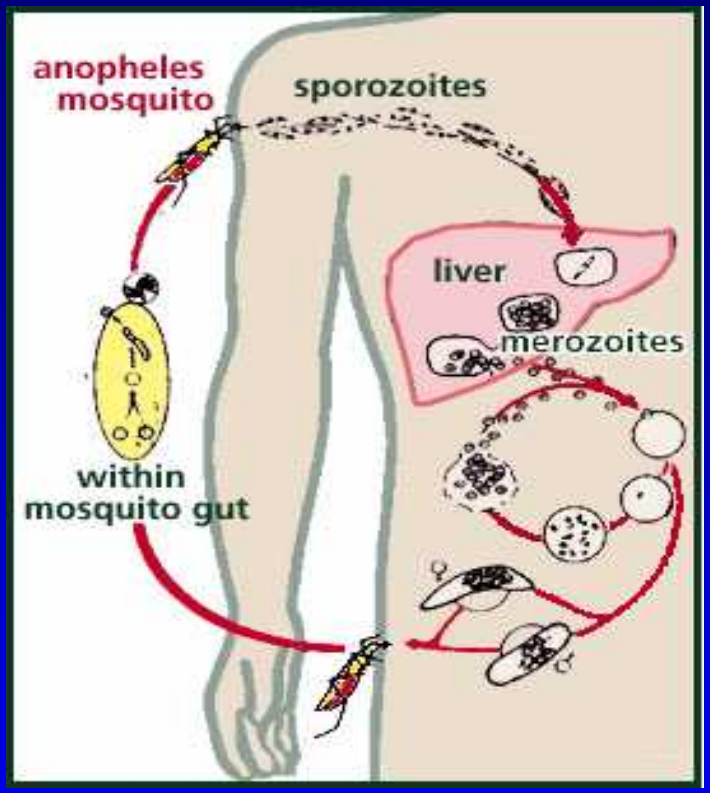
Hybrids sterile or with reduced fitness/fecundity. Vary in biology, vector competence.
History: isoenzymes, polytene chromozomes: large with thousands of DNA strands, various tissue like larval salivary gland, bands of euchromatin and heterochromatin,
Mosquitoes: 2n = 6
Sub-Saharan Africa: *A. gambiae* complex, Europe: *A. maculipennis* complex
Worldwide: *Culex pipiens* complex
C. p. pipiens: ornithophilic, eurygamous, adults diapauzing, not autogenous
C. p. molestus: oportunistic, stenogamous, no diapauze, autogenous strains.
C. p. quinquefasciatus: tropical, oportunistic, stenogamous, no diapauze





Malaria: *P. falciparum, P. vivax, P. ovale, P. malariae, P. knowlesi*





Vector part of the life cycle: gametocysts, ookinete, oocysts, sporozoites History: Alexander the Great, Ephesus, Rome
300-350 milions infected, 1.5 milions of deaths per year, mainly *P. falciparum*
Africa (80% cases), India, Brazil, Sri-Lanka, Thailand, Indonesia, China
About 50 *Anopheles* species of subgenera *Celia, Nyssorhynchus, Anopheles*

Endemic areas and most important vectors of malaria

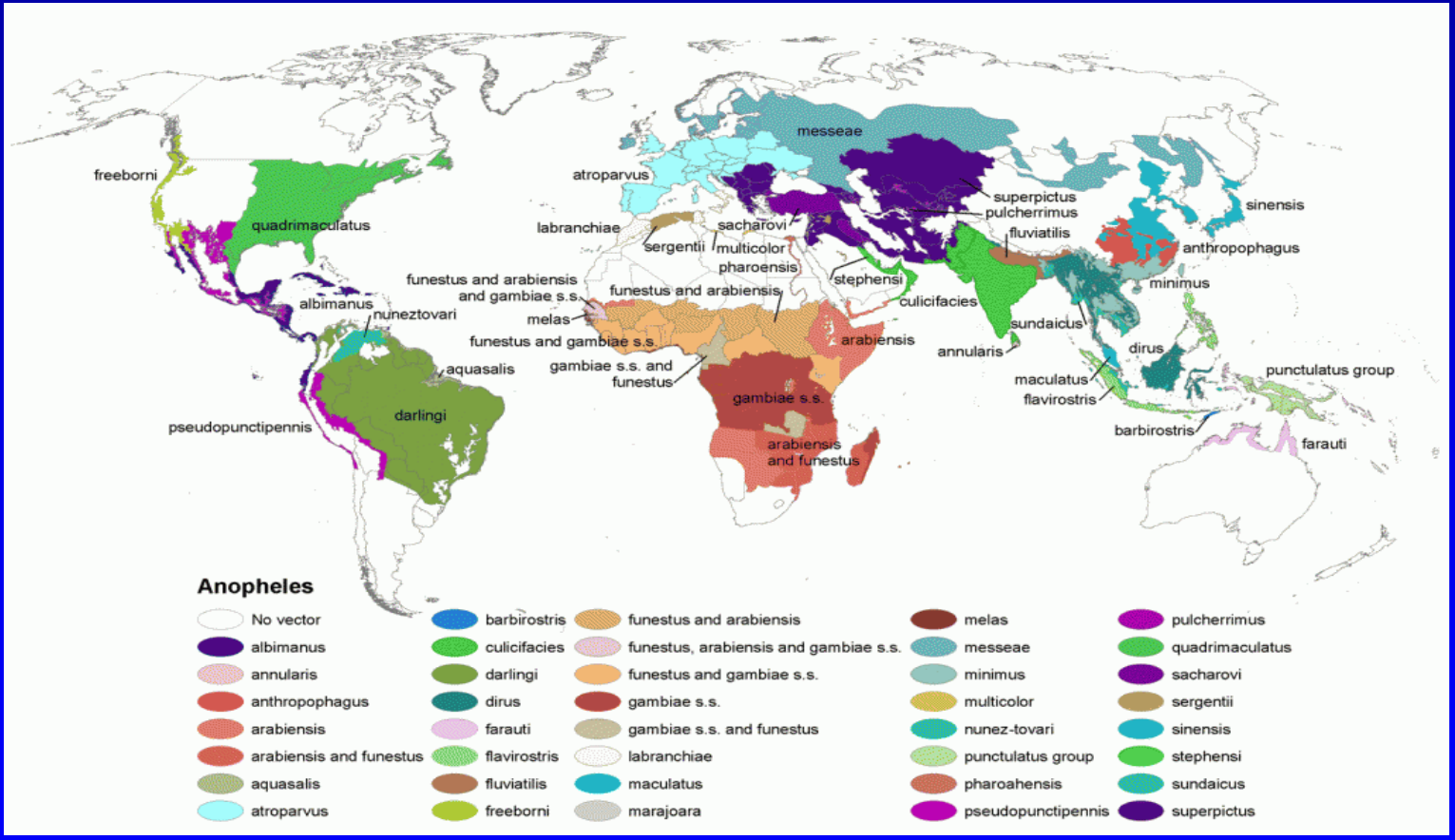
A. (Celia) gambiae complex: Sub-Saharan Africa, at least 7 species, different bionomy

- *gambiae* (molecular S form): anthropophilic, endophagous (children!), hundreds of bites every night (infection rate 1-5%), periodical water, tropical Africa.
- *coluzzi* (M form): similar but only West Africa, permanent waters and rice fields.
- *arabiensis*: more often zoophilic and exophilic (advantages versus complications)
- Two unimportant (zoofilic or limited distribution, two (*A. melas, merus*) only brackish.

A.(C.) stephensi:
Middle East,
even dirty water

A.(N.) albimanus:
Central America,
even brakish water

A. (A.) maculipennis
Europe and Asia,
complex of 15 species



Anopheles and malaria in Central Europe

Mainly *A. maculipennis* complex
less important: *A. maculipennis* and *A. messae*: our populations zoophilic
more important: *A. labranchiae* (Mediterranean), *A. sacharovi*
(Mediterranean, Middle east, Russia), *A. atroparvus* (most of Europe)

Other Anopheles species in Central Europe:
A. claviger (vector in Middle East),
A. plumbeus (vector in England, 16-19th century)

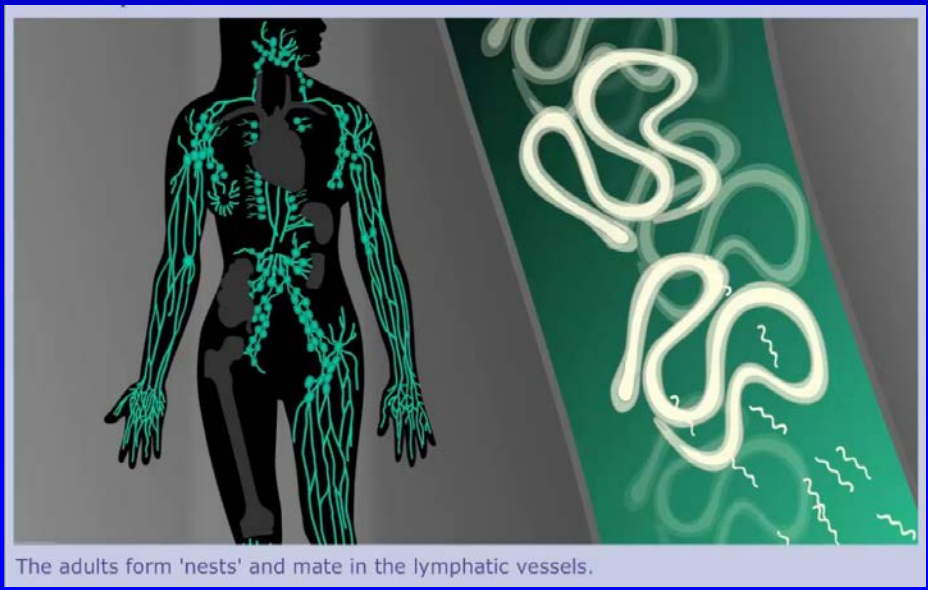
P. vivax: *A. maculipennis* c., 19th century, 1945-1955

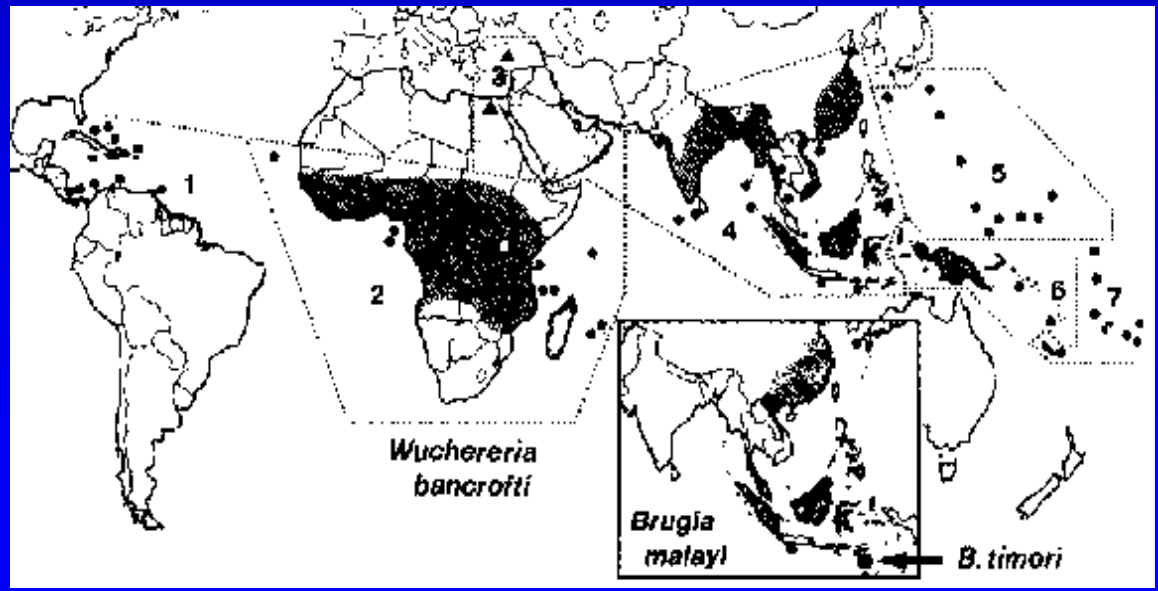
P. falciparum: *A. maculipennis* c. is not susceptible.
Autochtonous infections: blood transfusion, airport malaria,
A. plumbeus: tree holes, highly susceptible,
two cases in Germany (Kruger et al., 2001)

Lymphatic filarioses

Wuchereria bancrofti: tropics in different continents, often urban anthroponosis.
Brugia malayi: anthroponosis or zoonosis, monkeys, dogs, cats, typically rural.
Less than 1% infected mosquitoes, low numbers of L3, thoracic muscles
Both filaria species occur in periodic and subperiodic form,

W.B. periodic (nocturnal, MF in peripheral blood during night): most regions, during day hidden in capillaries of internal organs. Vectors: *Anopheles* and *Culex p. quinquefasciatus.*
W.B. subperiodic (diurnal): Polynesia, islands of Indian ocean, MF in peripheral blood mainly in the evening. Vectors: *Aedes* active during day, exophilic.


The adults form 'nests' and mate in the lymphatic vessels.

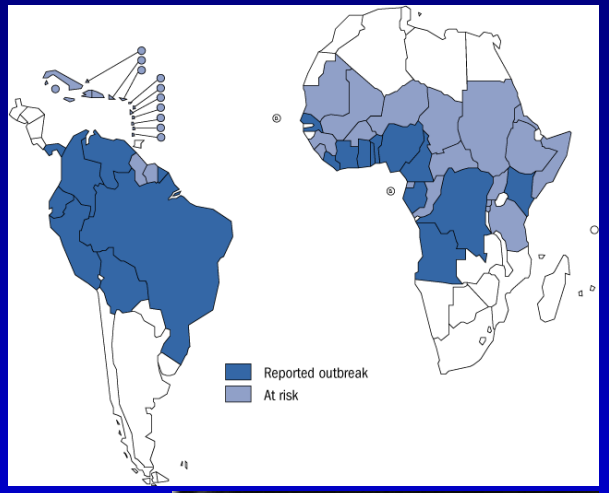


Important human arboviruses transmitted by mosquitoes

Togaviridae (Alfavirus)		mortality (%)	main vectors	reservoirs
Eastern equine e.	encephalitis	50-75	*Culiseta (Coquilletidia)*	birds
Venezuelan eq. e.	encephalitis	10-20	*Culex pipiens complex*	rodents
Western eq. e.	encephalitis	5-10	*Culex tarsalis*	birds
Sindbis	fever	0-1	*Culex*	birds
Chikungunya	fever, joints	0-1	*Ae. aegypti, albopictus*	monkeys, man
Flaviviridae				
Yellow fever	hemorh. fever	20-50	*Aedes, Haemagogus*	monkeys, man
Dengue 1-4	(hemorh) fever	0-20	*Ae. aegypti, albopictus*	man
Zika	fever (neuropat)	0-?	*Ae. aegypti, albopictus*	man
West Nile	fever (neuropat)	1-5	*Culex pipiens, tarsalis*	birds
Murray Valley	encephalitis	30-70	*Culex*	birds
Japanese ence.	encephalitis	30-40	*Culex*	birds
Bunyaviridae				
La Crosse	encephalitis	1-2	*Aedes*	rodents
Rift Valley	fever	0	*Aedes, Culex*	ruminants!
Ťahyňa, Čalovo ap.	fever	0	*Aedes*	Lagomorpha

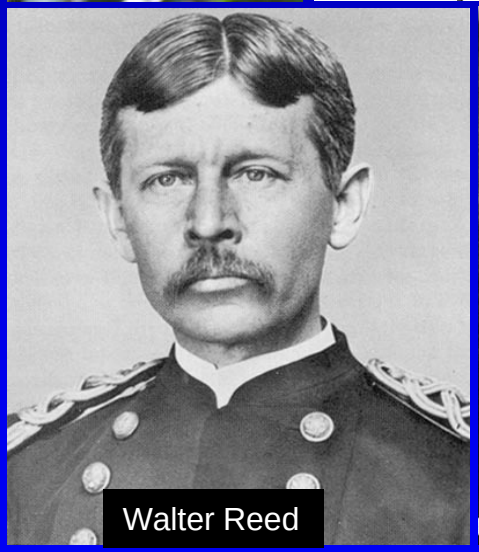
Yellow fever

Zoonosis of monkeys
Aedes (Stegomyia): not transovarially (x ticks)
Incubation period 5 days, two phases:
1. Fever (viremia, infectivity to mosquitoes)
2. Viscera (jaundice, vomito negro), hemorrhagic syndrome, up to 50% lethality.
Vaccine: attenuated virus, life-lasting protection.



Circulation in Africa:
A. africanus in tree canopy
A. simpsoni on plantations (during day)
A. aegypti in urban areas

Circulation in America:
Haemagogus, Aedes in tree canopy:
Forest workers
A. aegypti in urban areas


Walter Reed


William Craford Gorgas

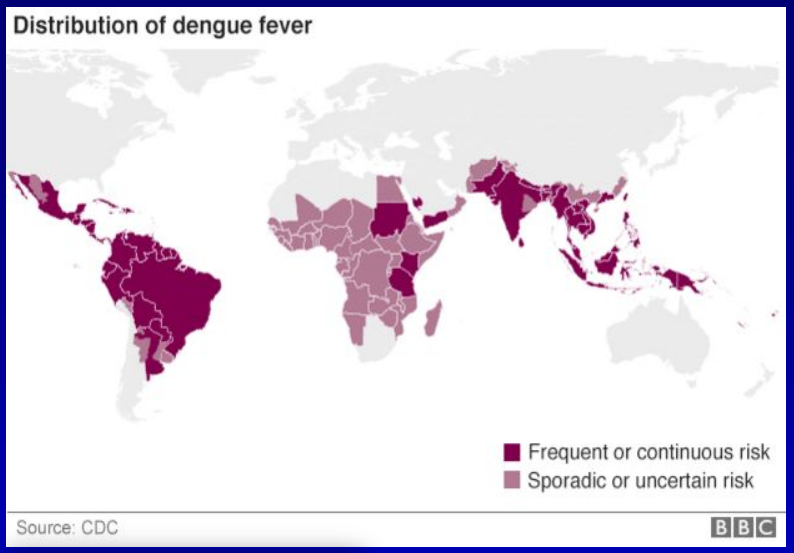
Epidemies in Cuba and construction of Panama canal (1880-1889, 1904-1905-1914)

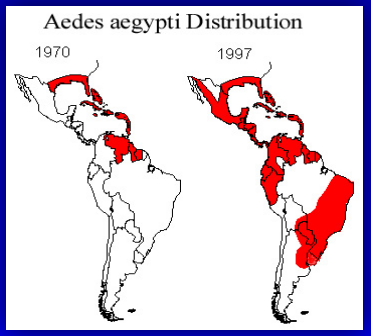
Dengue

Humans and monkeys, *Aedes* (*Stegomyia*)
A. albopictus (Asian Tiger mosquito)
A. aegypti (Yellow fever mosquito)
Four viruses, tropics and subtropics, urban!
Dengue hemorrhagic fever (DHF) lethal in 20%
50 milion cases per year, 1 million DHF.
2500 millions in 100 countries at risk.
In SE Asia and Pacific region: mortality in children.
Madeira (epidemy 2012-13), Croatia (cases 2010)

Spread of dengue: spread of the vectors due to climate changes, urbanization, air transport, resistence to insecticides. No reliable vaccine!!

Spread of *A. albopictus* by boats and used tires: Hawaii ~1890, USA 1983, Brazil 1986, Albania 1979 (from China), Italy 1990 (from USA), now everywhere in Mediterranean, transport by trucks with animals to Central Europe.



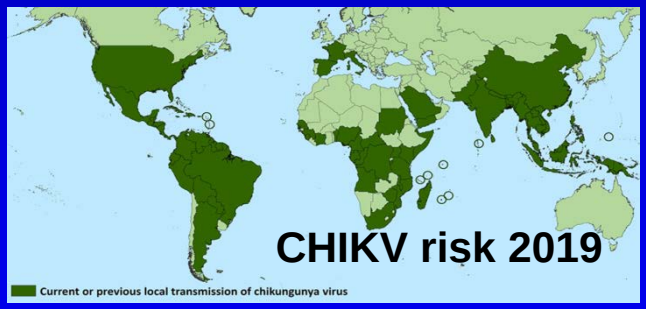


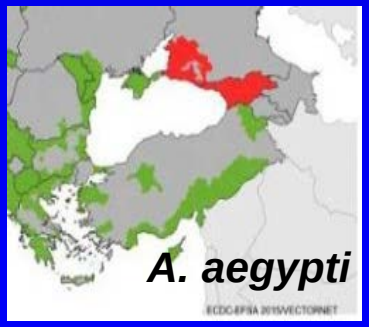
Zika virus (ZIKV, Flaviviridae)

Polynesia, South America,
Brazil 2015: more than one million cases, peaked 2016,
Risk for pregnant: microcephaly, brain defects
Reservoirs: monkeys and humans, sexually transmitted
Vectors: *A. albopictus* and *A. aegypti*
Europe: tourists, small potential risk

Chikungunya virus (CHIKV, Togaviridae)

Acute: High fever and joint pain, rash
Chronic: painful joints (for years)
Epidemics in SE Asia, Africa, S America
Reunion 2005 (266 thousands)
India 2006: more than 1 million
Reservoirs: primates and small mammals
Italy and France (autochtonous in 2006, 2007, 2014), introduced by travelers from India and Reunion, resp.
Vectors: *A. albopictus* and *A. aegypti*

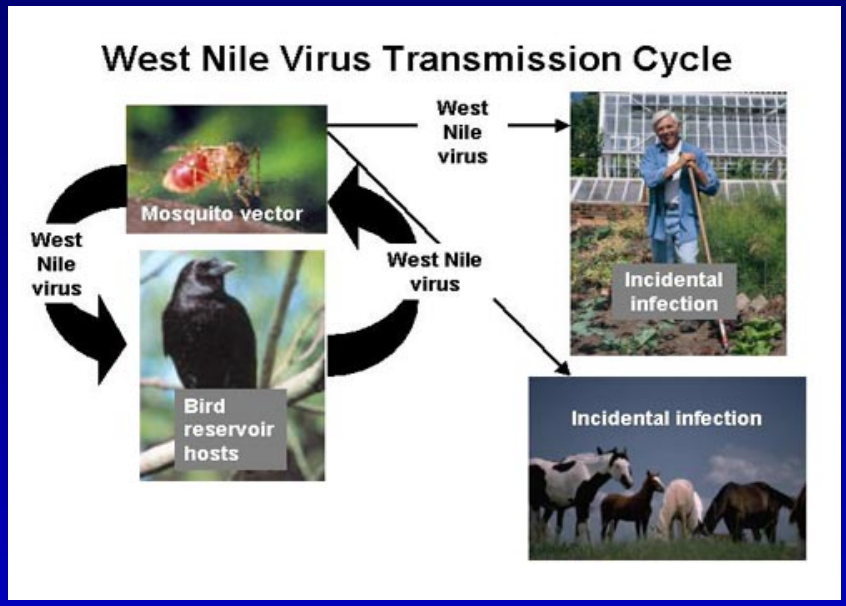


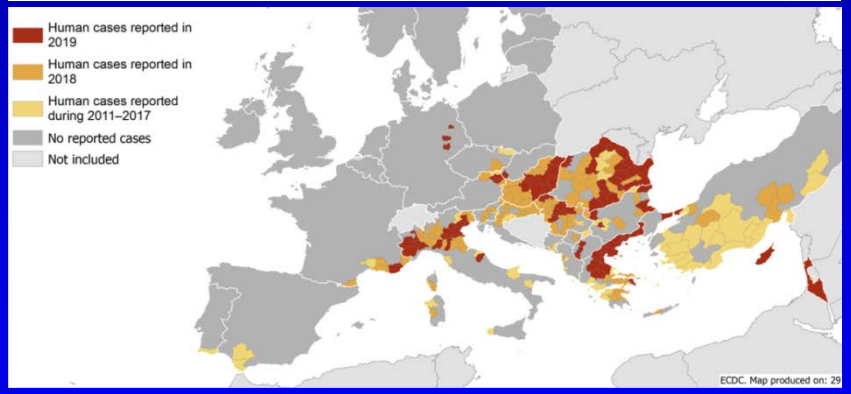


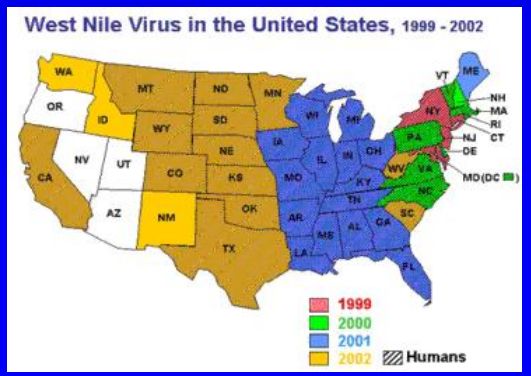
West Nile Virus

The most spread flavivirus (even in Australia)
Uganda 1937, Nile delta
Europe: ~1000 cases in Romania (1996-97) and Ukraine (1999). EU 2019: 470 reported cases.
Reservoirs: migratory birds, Corvidae !
Hosts: broad spectrum of mammals, horses!
Humans: 20% infected get fever,
1-5% serious symptoms (then 10-15% fatal).
Risk factor: age over 60
In Europe mainly *Culex pipiens*, *C.modestus*

New World, USA: introduction 1999
Human cases peaked 2002 (4161).
Culex tarsalis, mortality of American crows
Czech Republic: South Moravia, Břeclav
Seropositivity: domestic animals 2%,
game animals: 5-15%, humans 0-2%.
Few serious cases (two reported in 2018)
Anthropophily of *Culex pipiens*
Spread of *C. modestus*







Protection and control

Personal protection

1. Impregnated bednets: permethrin, deltamethrin
2. Screening of windows and doors
3. Mosquito coils, electrically heated pads with pyrethroids
4. Wearing protective clothing (long sleeves and trousers)
5. Topical repellents: diethyltoluamid (DEET), picaridin
6. Prevent the contact by behaviour

Control of adult mosquitoes

1. Indoor spraying

DDT since 1957. Pros and cons:
cheap but resistance, exophagy.
Recently: synthetic pyrethroids.

2. Barrier protection (spray vegetation) and aerial spraying by residual insecticides (epidemics like dengue or EEE)

3. Spraying and fogging in cities (ZIKV)

Control of breeding places

1. Environmental management (physical methods)

Drainage of marshes, filling trenches (Italy, *Anopheles maculipennis*), cutting of water plants (*Mansonia*), collecting garbage (*Aedes, An. gambiae*).
Natural reserves and new habitats: irrigation projects, dams, rice fields.
Rice fields: intermittent irrigation (1-2 days twice a week, limit: water supply)
Marshes and floodplain forest: water management.
Sewer systems: *Culex* and *An. plumbeus*

2. Insecticides (chemical control):

Historically: Copper sulphate, diesel oil, other oils.
DDT: after WW2, resistance, residues.
Recently: usually combinations of organophosphates (metathion), growth regulators (methoprene) or chitin inhibitors (diflubenzurol, Dimilin).
Sprays or granules to penetrate the vegetation.

Control of breeding places

3. Biological control

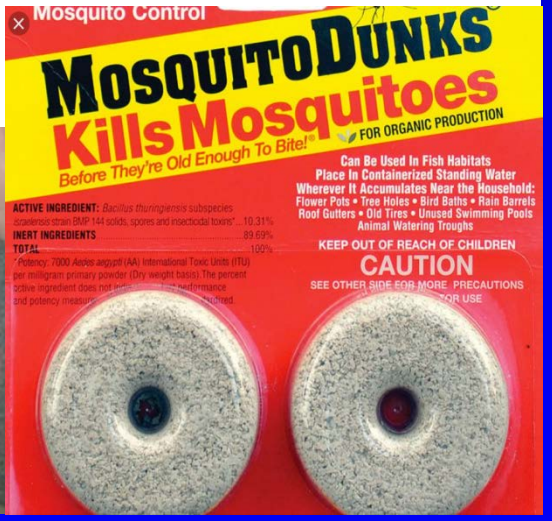
Poecilia, Gambusia, Cynolebias

Toxorhynchites

Bacillus thuringiensis var. israelensis = BTI
Endotoxin (crystals, Cry toxin)
Formulations: aqueous emulsions (AS), water-dispersible granules (VectoBac G, WGD for aqueous spray mixes or direct application), tablets, BTI donuts.

Bacillus sphericus: two toxins, differ from BTI

Genetic manipulations.
Problems with introduction and public relations.



Brachycera

Tabanidae: horse flies, deer flies and kegs

Eggs in masses on leaves, wet ground etc.

Larvae predatory, cannibalistic.
Retractible head capsule
(hemicephalic larvae),
3-4 pairs of pseudopods,
short respiratory siphon, abdominal
segments with fringes or spines.

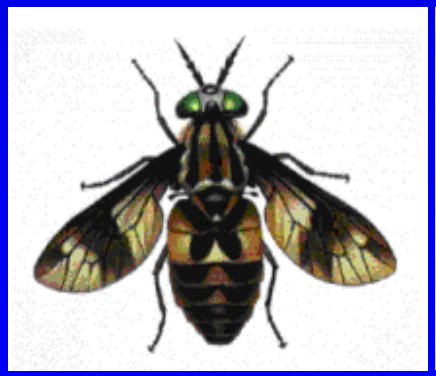
Marshes, wet meadows, mud, river banks,
edge of aquatic habitats.
Long life cycle, 6-12 larval instars, most
temperate species are univoltine,
in tropics ~ 3 generations per year.
Pupa: 2-4 weeks.

Tabanidae

Strong fliers for long distances (but not regular migrations).
Only females parasitic and bite (some species autogenous for first egg batch)
Active diurnally, hosts located by sight (color and movement) and CO2 emission.
Attracted to large moving objects. Both sexes feed on sugars at flowers, sap from wounded trees, drink water in flight.

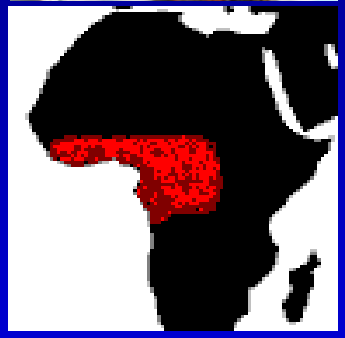
Control: difficult in breeding sites
(water management, pesticides, clearing vegetation),
Limited effect of repellents. Traps, e.g Manitoba trap.

~ 4000 described species, in Europe two subfamilies with four most frequent genera
Chrysopinae: *Chrysops* (deer-flies, worldwide),
Tabaninae: *Tabanus*, *Hybomitra* (horse flies, worldwide), *Haematopota* (clegs).

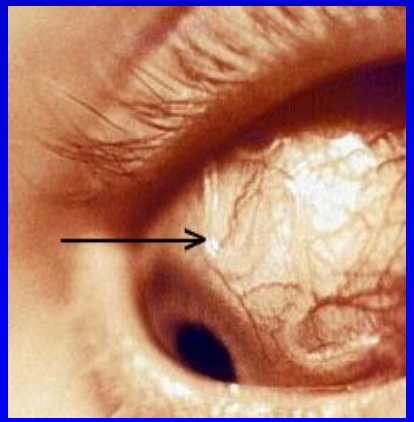


Medical and veterinary importance of tabanids

Aggressive and persistent biters, skin hypersensitivity
(antihistamics in creams). Protection: clothing better than repellents
Painful bites, grooming and defensive behavior of hosts,
Interruption of feeding, large mouthparts: ideal mechanical vectors
: Viruses of domestic animals (Equine infectious anemia virus, EIAV)
: Bacteria: *Bacillus anthracis, Francisella tularensis* (Canada, Russia)
Anaplasma marginale (cattle), *Borrelia burgdorferi???*
: *Trypanosoma evansi*, *T. vivax*, nonpathogenic *T. theileri*



Loa loa: the only human disease transmitted by tabanids
biologically to humans, ~ million of infected.
Zoonosis of monkeys (*Mandrillus*).
Rainforest of tropical Africa (Congo).
Long-lived adult worms (~10 years),
migration under skin and conjuctiva
Temporary subcutaneous swellings
(Calabar swelling), mainly on limbs.



Microfilariae in perif. blood during day, L3 in fat body of *Chrysops.*
Vectors: several *Chrysops* species (*C. dimidiata* and *C. silacea).*

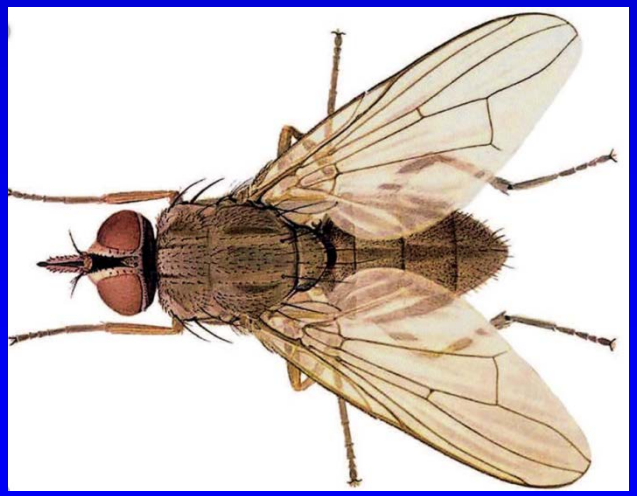
Stomoxyinae (bloodsucking Muscidae)
Stable flies and horn flies

Stomoxys calcitrans
(stable fly, bodalka stájová)
Word-wide (introduced to America in 17th cent.)
Sclerotized and projecting proboscis,
abdomen shorter and broader than in Musca.
Both sexes bite, more than once a day, even under strong sunlight, short but painful bite, opportunistic, in and around farms, in animals endophilic, in humans mainly exophilic.
Veterinary pest: reduce milk yield and weight gain,
Possible mechanical vectors of *T. evansi* etc.
Breeding sites in manure, animal bedding, rotting vegetation, three larval instars (maggots) saprophagous, pupation in drier parts.
Control: improved sanitation, reducing breeding places, residual insecticides in stables, insecticide-impregnated strips, ear tags in cattle, sprays etc.

Haematobia (horn flies)

several species, two common in temperate habitats
H. irritans (northern hemisphere and Australia), small: 3-4 mm
H. stimulans (Europe only), bigger (6 mm)
98% of time on the host, leave only briefly to lay eggs in fresh dung, batches of 20-30 eggs, short life cycle (2 weeks),
~ 5 generations during summer, pupae overwinter in the soil below dung.
Cattle (occasionally other animals)
Irritation, losses of weight and milk yield, dermatitis.

Control: topical insecticides directly to animals (spray, pour-on, ear tags).



Hippoboscoidae (Pupipara)

Unique development of larva: within modified common oviduct, fed internally by „milk glands“ , viviparous, immobile final (3rd) stage larva pupates *in situ*.

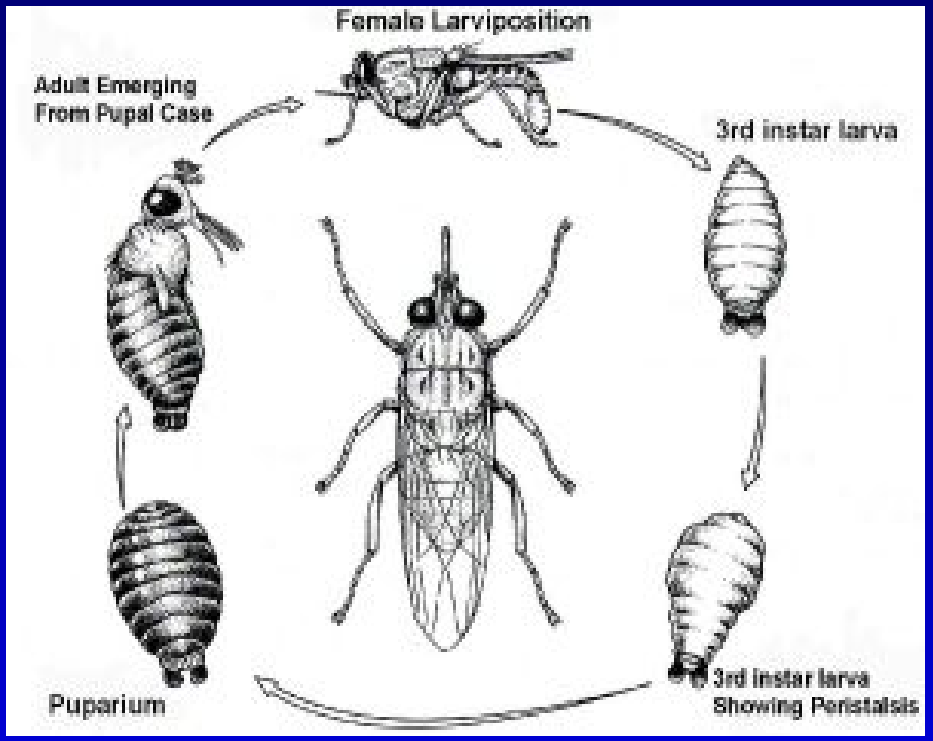
Nycteribiidae, Streblidae

Bat flies, specific parasites, mostly wingless, possible transmission of rabies

Hippoboscidae: keds and louse flies

Wingless or temporary or permanent wings.
Melophagus ovinus (sheep ked), veterinary important, neck region, dermatitis, more in winter. Vector of benign *Trypanosoma melophagium.*
Lipoptena cervi (deer ked), also attacks humans.
Hippobosca equina (and others) in horses, cattle.
Louse flies: many species in birds (*Ornitomyia*).

Glossina (moucha tsetse)



Adenotrophic viviparity
(adenotrofní viviparie):
larvae develop in the „uterus“, nourished by products of modified accessory glands, 3rd instar larvae laid just before pupation.

Two ovaria, each with two ovariols,
ovulate alternatively every ~ 7-10 days (same for larval development).
Laid larva 5 mm long, excretes metabolites and burrows several cm into ground.
Formation of puparium within few hours.
Larvae laid into relatively cold and humid places (under stones, near the base of trunk etc.). Pupa develops inside puparium (larval cuticle), for about one month.
Freshly emerged adults before first bloodmeal called "teneral" , relatively fragile and more susceptible to infection.

Glossina biology

Activity diurnal (daytime). Resting for 90% of time.
Resting sites in the shadow, in canopy on tree trunks.
Wait and watch the hosts, short and fast flights, speed up to 30 km/hour.
Only hungry ones search the hosts by flying (perpendicularly to air flow),
Chemical sensors, arista (special hair on the third segment of antenna)
Eyesight relatively good, recognizes shape and movement, cattle for 100-150 m.
Attractive colors blue and black, repellent yellow, see tsetse traps.

Males search for females around the host, or moving objects.
Adults live several moths, (1 larva weekly).
Females mate once in life, males repeatedly. For both sexes the blood is the only source of energy.
Blood-feed every second or third day.
Energy for flying by oxidation of proline (synthesized from other aminoacids during resting).

Glossina taxonomy

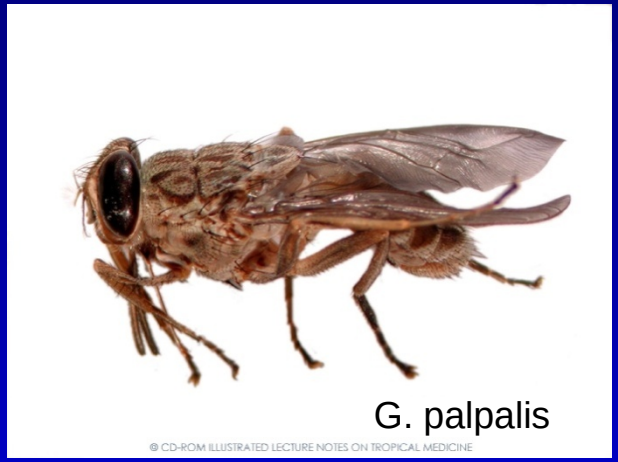
3 groups of related species, usually coherent with the biotope: groups „palpalis“, „morsitans“, „fusca“.

fusca: large, lowland rain forest, forest species. medically not important.

palpalis: palpalis, tachinoides, fuscipes (riverine sp.)
Along rivers, „gallery forest“, forest edge, villages.
West and Central Africa.
Opportunistic: pigs, reptiles, humans etc.

morsitans: morsitans, pallidipes (savanna sp.).
Savanna woodlands in East Africa. Specialized on wild game (warthogs, buffalos, antelopes) and cattle.

Importance of tsetse flies:
Low population densities, minor skin reactions, BUT: Transmission of trypanosomes to domestic animals and humans. Teneral flies more susceptible, infection lasts for the rest of life of the fly.



G. palpalis

G. morsitans

African trypanosomes

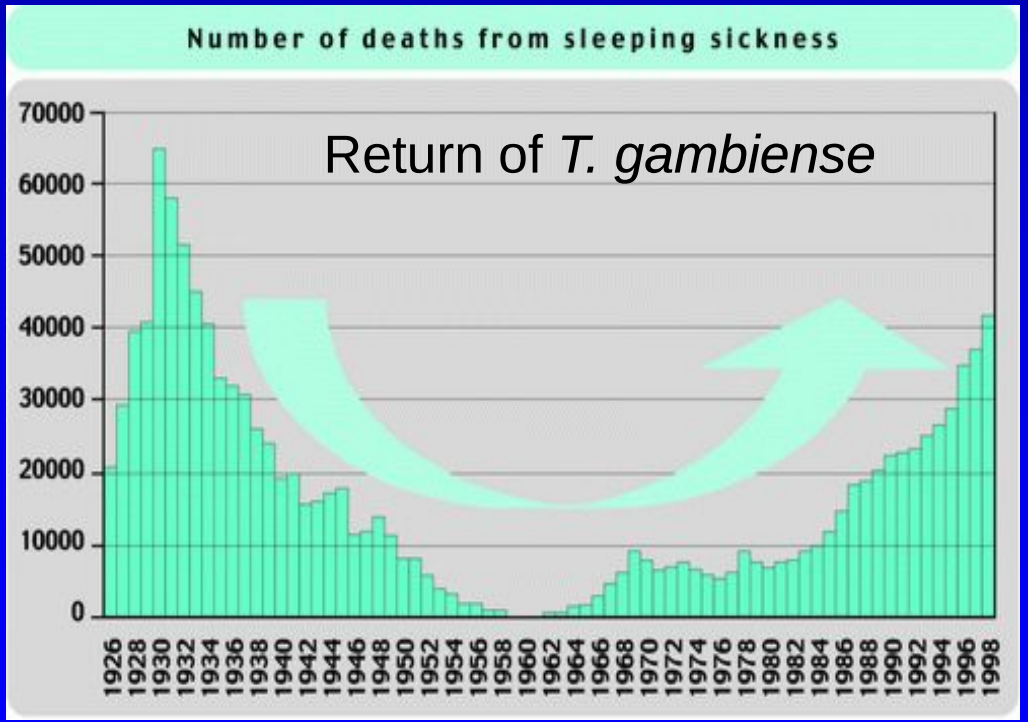
Nonpathogenic for wildlife, prevent animal farming (mainly cattle)

Thee important subgenera differing in the vector part of the life cycle:
T. vivax (*Duttonella*): only in proboscis, important pathogen for cattle
T. congolense (*Nannomonas*): mesenteron and proboscis, cattle pathogen
T. brucei complex (*Trypanozoon*): mesenteron, migration to salivary glands.
nonpathogenic for cattle, but important for human health.

Human trypanosomes
2000: 50.000 human cases, 2018: 1.000)

T. gambiense: *Glossina palpalis* group,
98% of human infections.
Reservoirs: domestic animals and
wildlife, humans involved in the cycle.

T. rhodesiense: *G. morsitans* group,
2% of human infections,
Humans are tangential (accidental) hosts.
Zoonosis of wild ungulates in savanna.
Cattle are asymptomatic reservoir.



Trypanosoma brucei complex

Glossina: low infection rates with human parasites (0.1%) higher with *T. b. brucei* and other cattle parasites (10%).
Difficult to distinguish morphologically: D. Bruce (1905).

Geographical distribution: map from 1990, now reduced.

Different pathogenicity (chronic versus acute disease)
Hypothesis about co-evolution with hominids,
T. b. gambiense in the West with apes, chronic.
T. b. rhodesiense in East and South, acute.
closely related to *T. brucei* (same isoenzymes).
Two *T. b. brucei* populations acquired gene for resistance against lysis by human serum (Trypanosome lytic factor 1 = Haptoglobin-Related Protein + High Density Lipoprotein, TLF2).


David Bruce

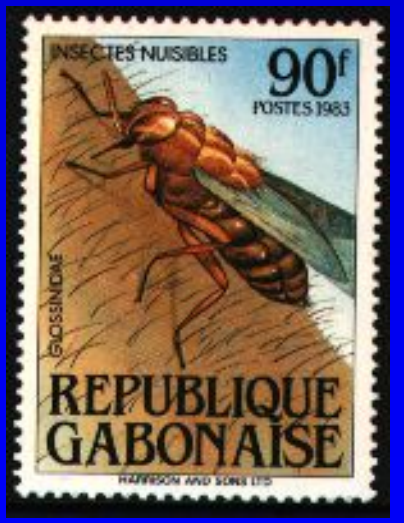
Prevention and control:
T. gambiense: early diagnosis, treatment.
T. rhodesiense: chemoprofylaxis of cattle.
WHO programme: treatment free of charge for endemic countries

Glossina control: history

Reduced reproductive potential, K strategy. Easy to remove but difficult to prevent reinfestation of areas. Eradication possible in isolated areas or if the biotope is changed. Important to consider the environmental cost.

Formerly used clearing of vegetation, killing of wildlife, persistent insecticide spraying on vegetation, all have proved inadequate.
Insecticides: From mid 1950s spraying DDT, lindane etc. (negative effect on insects, birds, reptiles), from mid 1970s pyrethroids (better for vertebrates, danger for insects and crustacians).
"Acceptable level of environmental damage": different opinions of farmers, politicians, medical doctors, biologists.
Example of reintroduction:
1991 Nigeria cleared 300 000 km2, 1999 glossina back.
Sustainability!!!

Today: combination various techniques, see next slide,
focus to *G. palpalis* group (vectors of *T. b. gambiense*)



Glossina control: present and plans

Combination of insecticides and traps

: Insecticides selectively to glossina resting sites.

: Cloth targets (wide blue and black strips), insecticide-impregnated

: Traps with odour attractants, biconical traps and other models,

Botswana, Zambia and Zimbabwe, more than million of traps.

Result: 90% reduction of *G. palpalis* numbers.

: Insecticides on cattle: pour on or dips, see the lecture on ticks.

Efficient, but negative effect on dung beetles.

Biological control: mainly Sterile insect technique (SIT):

Males sterilized by gama irradiation, relatively expensive. Used in Tanzania, Nigeria etc., success in Zanzibar and other islands (mainly *Glossina austeni*), useful in animal-biting species as males bite.

Transgenic glossina resistant to tryps:

Plan: genetically modified symbionts *Sodalis* and *Nocardia* produce peptides killing trypanosomes.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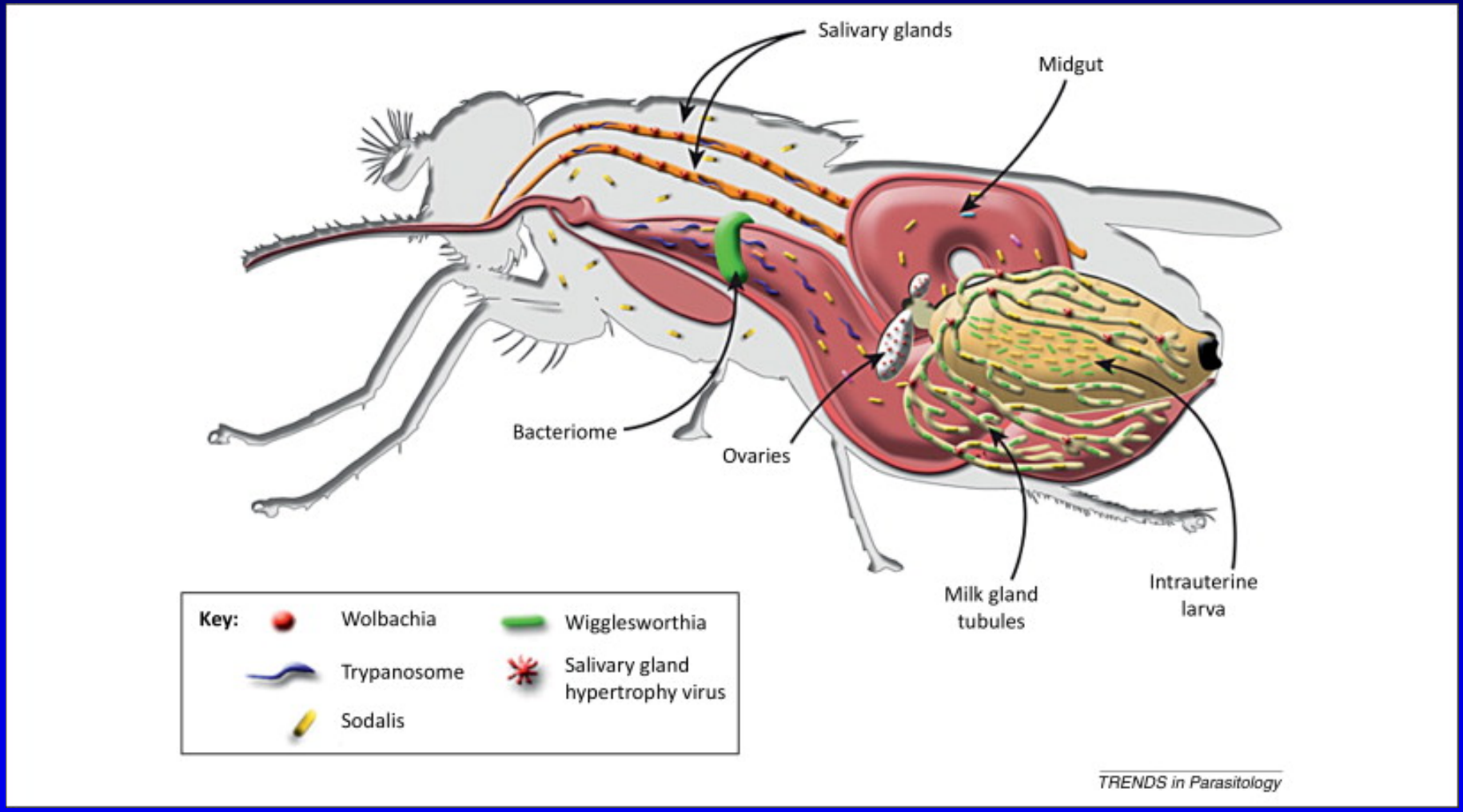

Salivary glands
Midgut
Bacteriome
Ovaries
Milk gland tubules
Intrauterine larva
Key:
Wolbachia
Wigglesworthia
Trypanosome
Salivary gland hypertrophy virus
Sodalis
TRENDS in Parasitology

Myases

Diseases caused by larvae of Diptera (Cyclorapha). Larvae of several fly families feed directly on the tissues of living human and animal hosts. 3 larval stages.
Specific (obligatory), semi specific (facultative), accidental (pseudomyases).
Various tissue: cutaneous, nasopharyngeal, ocular, intestinal, urogenital.

Sarcophagidae (flesh flies)

viviparous, two important genera

***Sarcophaga*:** most species on carrion or dung, pseudomyases **of gastrointestinal tract**, peroral infections, pain, vomiting (nausea).

Wohlfahrtia: similar appearance, several species of obligate parasites of mammals and eventually birds. Larvae deposited in groups, penetrate thin or broken skin, full grown in 6-7 days. Active during day, outdoor.
Various domestic animals, mainly sheep (rams and fatalities of newborns)
Humans: ears, nasal region, rapid destruction of tissue, attracted by exudates.
W. magnifica: Mediterranean, Eastern Europe, Middle East.
W. meigeni: Holarctic, other species in Africa and Americas.
Prevention of injuries, sanitation, screening prams of coverring of sleeping infants.

Calliphoridae (blow flies: bzučivkovití)

Most non-parasitic, few facultative or obligatory parasites.

Auchmeromyia senegalensis (Congo floor maggot). Sub-Saharan Africa. Eggs on ground, bloodsucking larvae search for hosts at night. Prevention: do not sleep on ground.
Protocalliphora: in bird nests, suck the blood of nestings.

Cordylobia anthropophaga (tumbu fly).
Sub-Saharan Africa, eggs on ground with urine or excreta, on used and wet clothing.
Cutaneous myasis in mammals, including dogs and humans (mainly children).
Larvae detect host by heat, odour and CO_2 penetrate skin, boil-like swelling for 7-9 days, maggots up to 15 mm.
Prevention: avoidance of contact with ground larvae (shoes), clean clothing, ironing.
Removal of larvae: occluding the breathing hole with cream.


Calliphora

Calliphoridae causing screw-worm myasis

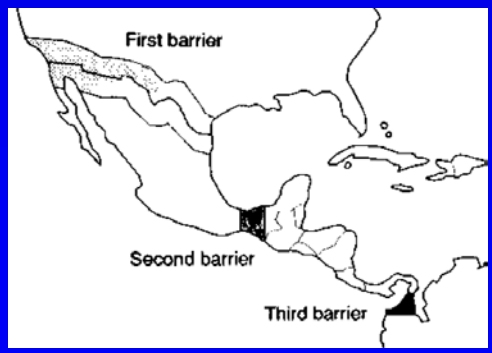
Two important species causing wound (traumatic) myasis metallic-blue body.

Cochliomyia hominivorax
(New World Screw-worm, formerly *Callitroga*)
Currently limited to Central and South America.

***Chrysomya* bezziana** (Old World Screw-worm, mainly India)

Batches of 100-300 eggs into and around wounds, orifices, larvae lacerate tissue and tunnel deeply (several cm).
Fast development (one week), many, deep destruction.
Control: treatment of livestock with insecticides, traps with chemicals attractant for adults.

Cochliomyia: SIT programe: USA (since 1962, 100-200 million males per week), then Mexico (since 1991), barrier by sterilized (irradiated) males.
Introduction to Lybia in 1991, SIT for 6 months.



Calliphoridae causing facultative myasis (bzučivky)

Breed mainly in carion, facultative agents of myases in domestic animals (sheep) and eventually humans.
Phormia (several New World species)
Calliphora (worldwide)
Lucilia sericata (worldwide, important in Europe)
L. cuprina *(*Africa, Asia, Australia), economic importance.

Sheep: anal and tail area contamined by feces, wounds, (toxaemia and fever if many, even death). Insecticides, development of vaccine.
Humans: discomfort, alarming. Infants, homeless, elderly or wounded people. Screens in hospitals, sanitation. Treatment: physical removal of larvae.

Use of *L. sericata* in human medicine: larvae **f**eed on exudates, bacteria and necrotic tissue. Deep or chronic wounds with resistant bacteria (diabetes patients).
Maggot therapy, certified in Israel, UK, Czech rep. etc.
Future: transgenic maggots producing human growth factor?

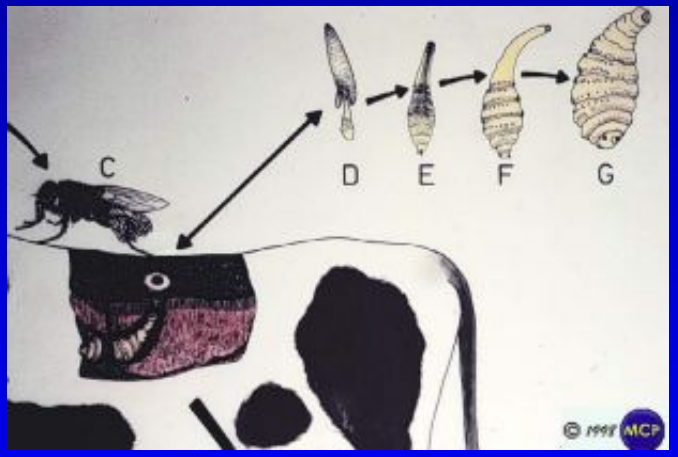
Oestridae

obligatory parasites of mammals and birds, rudimental mouthparts
usually 4 subfamilies: Cuterebrinae, Hypodermatinae, Oestrinae, Gasterophylinae

Cuterebrinae

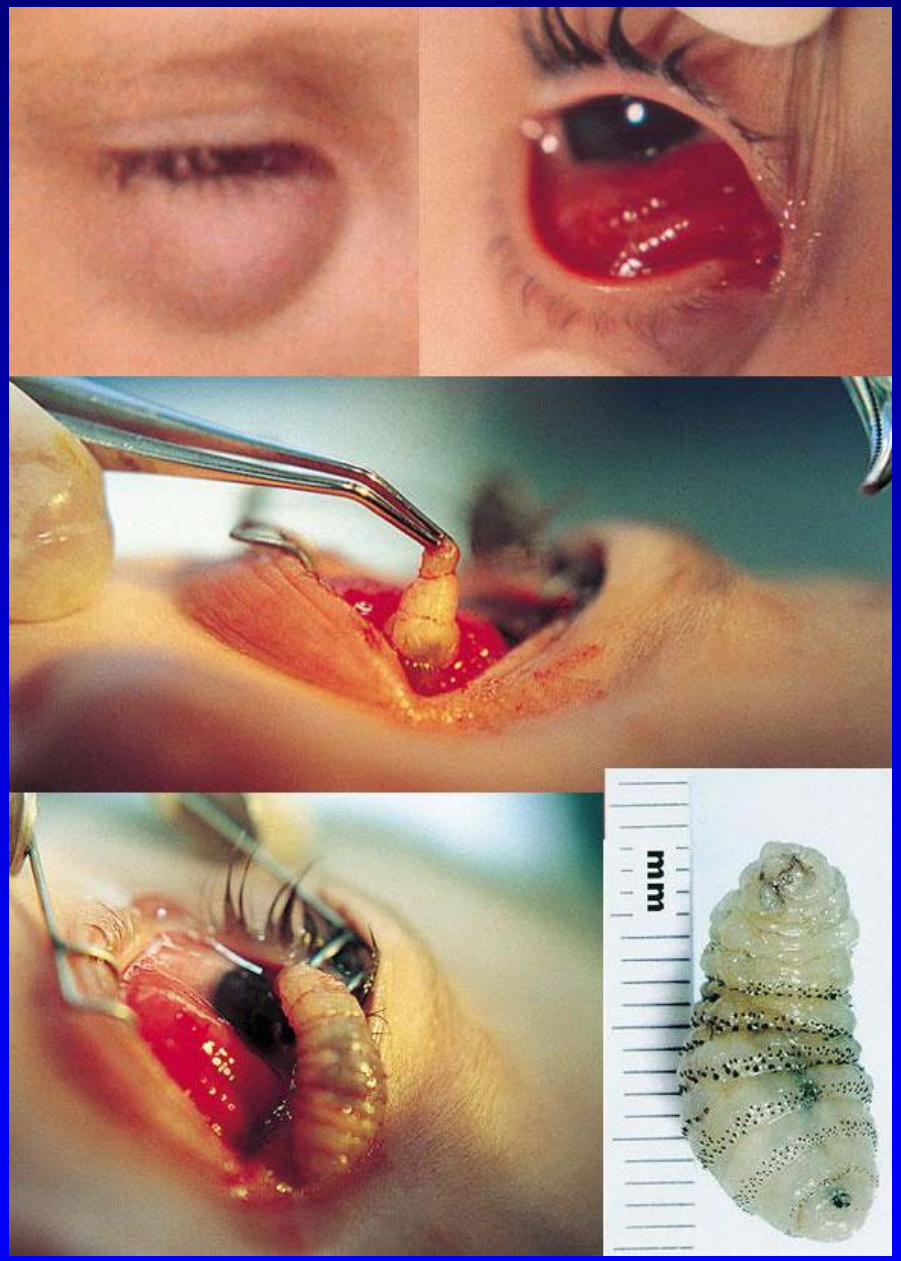
Cuterebra: rodents and lagomorphs in North America

Dermatobia hominis *(human bot fly, American bot fly)*
Latin America, forest margins, robust fly,
eggs attached in clusters to the abdomen of „carrier insects“ (muscids, mosquitoes), hatch delayed until a sudden increase of temperature.
3 morphologically different larval instars (1-25 mm), 2nd with typical shape.



Veterinary problem, economic loss in cattle.
Sheep, dogs and humans. Travelers.
Develops for 5-15 weeks, painful
Risk of contamination and other myases.
Surgical removal, avoid rupturing.

Dermatobia: nontypical localization, 3rd instar



Gasterophilinae (stomach bot flies)

Gasterophilus

World-wide, economically important species. Equines, mainly horses and donkeys.
Colorful, robust, hairy, dark wings, females with elongated abdominal segments.
Eggs laid on the host (around lips, on frontal legs) or vegetation (species-specific), L1 penetrate tissues of oral cavity, L2 and L3 attached to the intestinal tract for several months (stomach, duodenum, rectum). Pupae on/around dung.
One generation in temperate region (Mediterranean basin), several in tropics.
Stomatitis, chronic gastritis, ulceration, loss of condition and appetite.
Oral insecticides (ivermectin), single treatment during winter.

Other genera infest elephants and rhinoceros.



Hypodermatinae (warble flies)

Oestromyia leporina: in woles, nonmigrating larvae

Most others in Artiodactyla and larvae migrate
H. diana, actaeon etc. in roe deer, red dear and reindeer.

Hypoderma bovis and ***H. lineatum*** in cattle
Bumblebee-like flies, fast, active on sunny days .
Eggs attached to host´s hair, adhesive base of the egg,
usually on legs, invasion and migration of 1st instar,
proteinases (collagenases etc.). Susceptible young calves.
Overwinter: *H. bovis* in epidural fat of spinal canal,
H. lineatum within the wall of esophagus.
Migration host´s back, formation of warbles (granulomas)
Economic losses (disturbance, milk, meet and leather prod.)
Organophosphates and macrocyclic lactones (ivermectin)
before winter! Erradication in W. Europe, incl. CZ.
Risks: reintroduction with cattle, local species from deer.
Humans: accidental hosts, subdermal migratory myases,
(destruction of the eye). Cannot complete development.

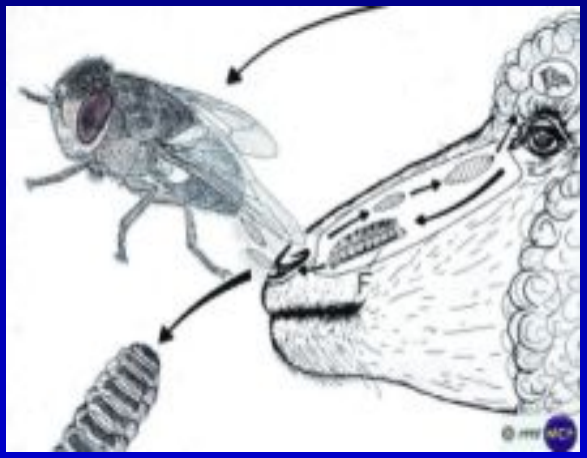
Oestrinae (nasal bot flies)

Oestrus ovis: world-wide, brown-grey fly, viviparous, groups of larvae squirted into the nose or the eye. Sheep and goats: inflammation, sneezing, head shaking. Larval development in frontal (or maxillary) sinuses for months, grown expelled from nose, short-lived adults. Two generations per year in Eastern Europe. Economical losses (Balkan, South Africa, Brazil).

Cephenemyia: several species in deer (incl. reindeer), sinusitis, morbidity. Holarctic, incl. Czech Rep.

Rhinoestrus: large animals, horses, Africa, SE Europe.

Humans: external ophthalmomyasis, irritation.



Warble flies are good and fast fliers, sexes meet on pronounced sites