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A pictorial step-by-step guide to detect phlebotomine sand flies (Diptera: Psychodidae) in entomological catches

Introduction

Phlebotomine sand flies (Diptera: Psychodidae) are inconspicuous insects that are easily missed during sorting within diverse entomological bycatch. The type of trapping device used significantly influences the taxonomic composition of specimens accumulated in the trap net or collection cup. Light traps are commonly used to sample nocturnal insects attracted to light. However, they often catch a wide variety of insects, which makes it difficult to locate and identify sand flies among bycatch. Scanning for **distinct morphological features** of sand flies might ease the sorting process (check Characteristic Feature Table).

This **step-by-step guide** aims to provide a visual guidance for detection of sand flies, using a Central European trapping site, where typically moths (Lepidoptera) and true flies (Diptera) dominate the collections. However, the same approach can be applied to various environmental settings, with different species composition.



Diptera and Lepidoptera-dominated light trap catch

Lab/tutorial photographs © David Sainitzer

Scan the QR Code



Find guidance for live sand fly trapping and handling (for phlebovirus or *Leishmania* isolation) at <https://climos-project.eu/climos-training-material/>

Getting started!

Before starting, prepare trap content as explained below and check the Characteristic Feature Table, which will support the sorting process!

Take trap net from freezer.

Turn it neck down and accumulate all content in the neck.



1



Open neck and spread content carefully on a white paper sheet.

2



3

Make sure to remove all insects from the net!

Scan the QR Code

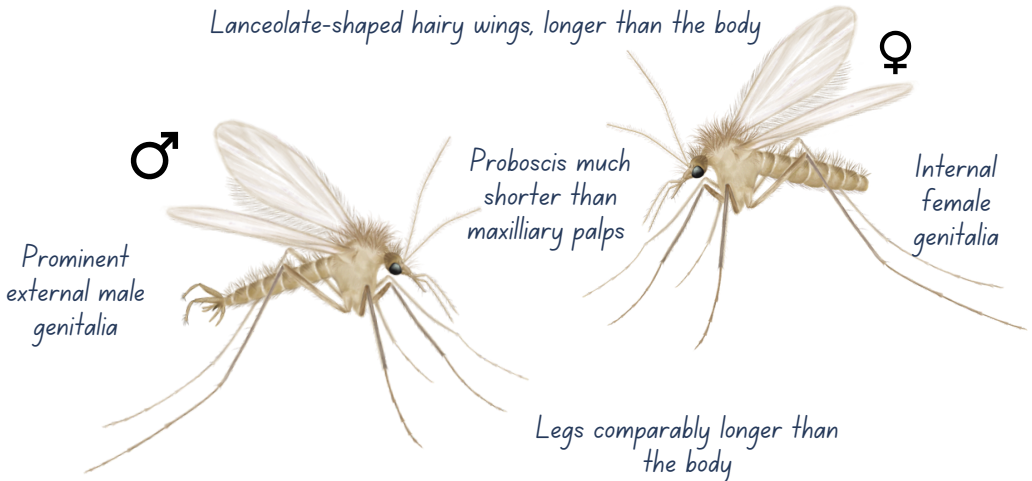


Find guidance on preparation for sand fly trapping and relevant gear in our CLIMOS video series on <https://climos-project.eu/climos-training-material/>

Characteristic Feature Table

Check all suspected specimens for characteristic features under the stereomicroscope for confirmation.

Feature	Description
General appearance	Soft-bodied, fragile, and densely haired; overall morphology gives a “dusty” look under light.
Body length	1.5–3.5 mm – very small and delicate compared to most other dipterans.
Color	Pale yellow to light brown; body densely covered with fine hairs that may detach during storage.
Wings	Large, narrow, lanceolate; held upright (“V” shape) when at rest.
Eyes	Large, dark, and distinctly separated.
Antennae	Long, slender, with 16 segments and a bead-like (moniliform) appearance.
Proboscis	Short, not extending beyond the head (unlike mosquitoes).
Thorax	Humped and densely hairy, giving a compact appearance.
Legs	Very long and slender relative to body size.
Sexual dimorphism	Prominent male genitalia (paired claspers and aedeagus) at the end of abdomen.



Let's start!

STEP 1

Use entomological tweezers to **spread content equally** and work fast but precisely to minimize nucleic acid degradation that might affect molecular analyses!





STEP 2

Start sorting by **removing largest insects** (>2 cm). In case bycatch is needed for reference collections, set aside and store properly (e.g. 70% ethanol, dry at -20°C / -80 °C, as appropriate).



STEP 3

Continue sorting specimens down to an approximate size of 5 mm. At this stage, detailed species identification is not required; **sorting** according to **morphotype** is sufficient to obtain an overview of the catch composition. Do not discard any insects yet!





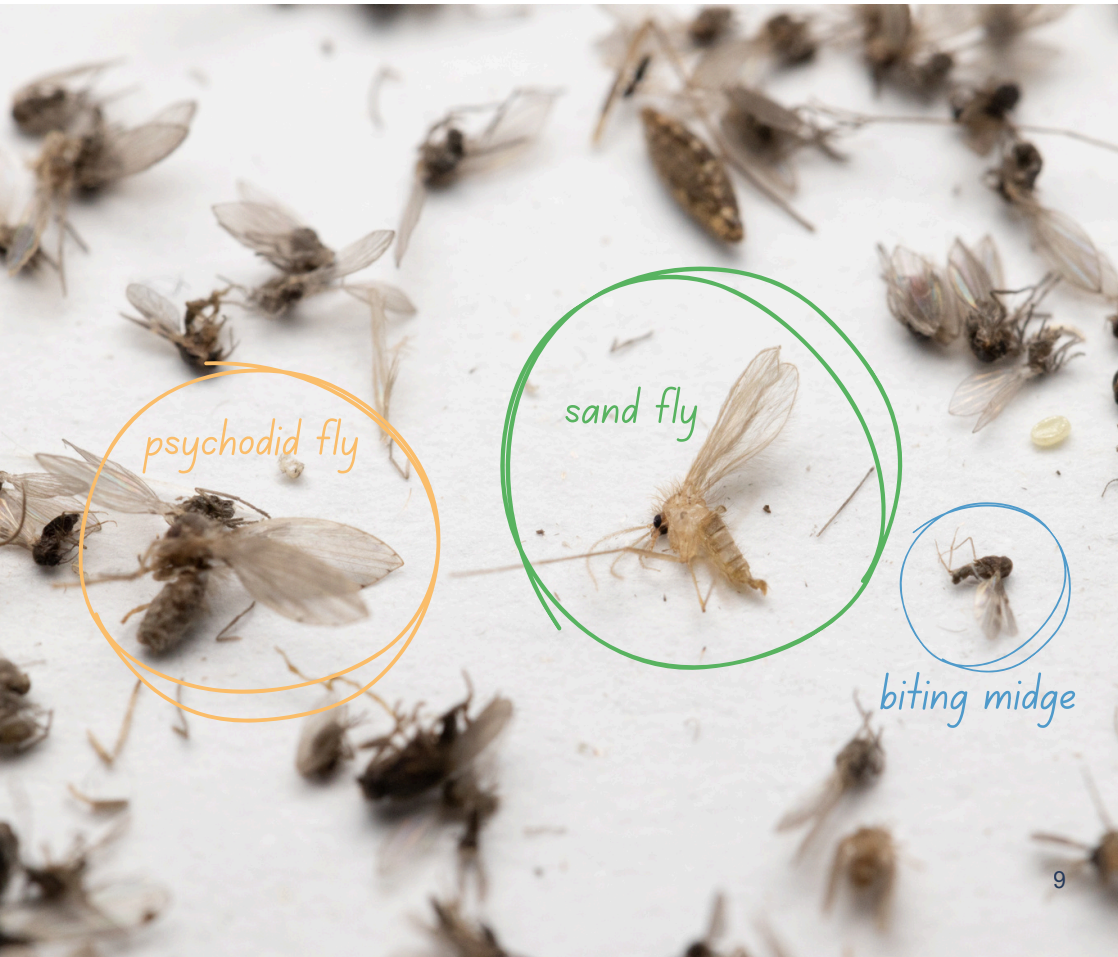
STEP 4

Focus on the remaining small (<5 mm) insects. This fraction will primarily consist of small dipteran or other insects that are often difficult to discriminate/distinguish. Perform a quick general scan, followed by individual inspection of each specimen. Have a look at the **Characteristic Feature Table** (page 4).

STEP 5

Several dipteran insects of similar size may resemble **sand fly-like habitus**. For example, biting midges (Ceratopogonidae) (blue) or psychodid flies (yellow).

Set specimens with sand fly-like appearance/morphology aside and compare them with pictures (e.g. step 5 and 6). Sand flies will be lighter in color (green) compared to most other small dipteran insects that appear rather black.



wings broken



legs fully attached

wings curled
up and not
erect



legs mostly
missing



appearance
much darker,
probably due to
various storage
conditions



end of abdomen
missing,
no sex
discrimination
possible!



STEP 6

Check potential sand fly specimens carefully under the **stereomicroscope!** Missing wings, legs or abdomens are regularly observed and make the process challenging (see various sand flies above). If size guide, Characteristic Feature Table and reference pictures do not help, take a photo and send it to an expert!

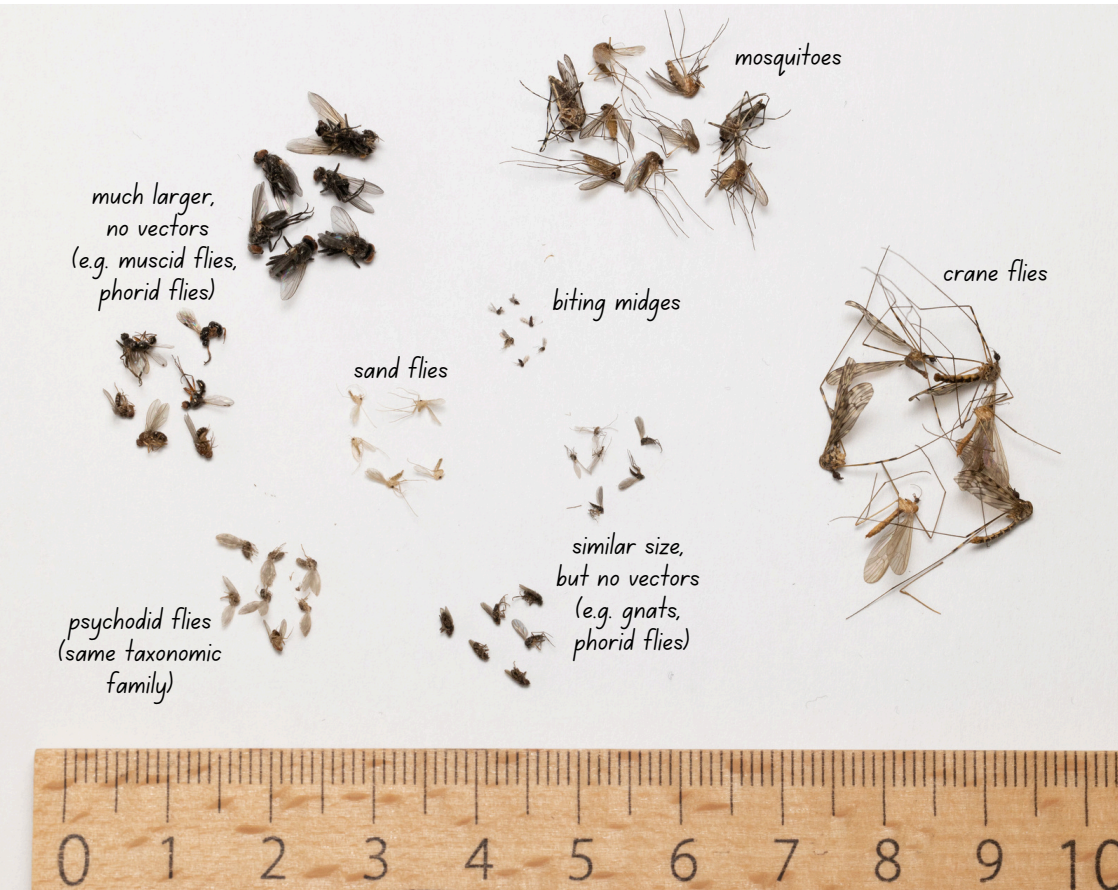
Scann the QR Code



For guidance through dissection, mounting, morphological identification and more visit <https://climos-project.eu/climos-training-material/>

Size Guide

Detecting sand flies within a mixed catch can be challenging due to their minute size. As illustrated here, **sand flies** are among the smallest insects in typical light-trap collections, measuring approximately **1.5–3.5 mm**. Comparing relative body sizes of trapped specimens can greatly assist in locating potential sand flies during sorting, particularly with other well-known vector species, such as mosquitoes or biting midges.





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About CLIMOS

CLIMOS aims to reduce the impact of climate change on the spread of vector-borne and zoonotic diseases, using Eco-health and One Health approaches.

The project focuses on understanding how climate and environmental factors affect sand fly populations and the diseases they transmit across Europe. By developing an Early Warning System and decision support tools, CLIMOS will help improve climate and health predictions, infection risk forecasts, and adaptation strategies.

Composed of 29 partners, this project brings together researchers, practitioners, health institutions, technology platform designers, and at-risk communities to conduct innovative and applied research seeking to be better prepared for current and future impacts of climate and environmental changes on human and animal health, using sand flies and the multiple sand fly-borne diseases that they transmit as a model system.

This approach will combine the benefits of traditional and innovative vector surveillance and disease control disciplines with climate change projection and impact modeling, ultimately leveraging their benefits to deliver data-driven policy-relevant tools which will be of greater value to the scientific community, accessible to stakeholders, and applicable at local scales.

Partners



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CLIMOS project is part of the Climate Change and Health Cluster. Six Horizon Europe projects, BlueAdapt, CATALYSE, CLIMOS, HIGH Horizons, IDAlert, and TRIGGER.