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Title of resource
GN_30: Dissecting salivary glands of field collected *S. damnosum* s.l.
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DOI
N/A
Description
Protocol for field collection and salivary gland dissection of blackflies (*S. damnosum* s.l.). This protocol was developed as part of the Gnatwork Transformative Science project "Understanding host heterogeneity to vector-borne disease exposure: Implications for modelling disease transmission and improving the design of control interventions".
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Resource history
N/A

GN_30: Dissecting salivary glands of field collected Simulium damnosum s.l.

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Introduction

This protocol is complementary to our protocol "GN_29: Measuring the human IgG and IgM immune response against salivary antigens of *Simulium damnosum* s.l. by ELISA as a proxy of exposure to blackfly bites". Though labour-intensive, salivary gland dissection of bloodsucking arthropods is routinely performed in several labs. Laboratory maintenance of colonies of these arthropods facilitates and standardizes the dissection procedure. However, the need for fast flowing water in the life cycle of blackflies complicates its long term maintenance in laboratory settings. Therefore, in order to obtain salivary antigens and setup an immunoassay of which the results can help improving transmission dynamics models of onchocerciasis, host-seeking female blackflies of the *S. damnosum* s.l. complex were dissected immediately after collection in the field. Although dissecting salivary glands is a standard lab procedure, it has not yet been described in a stepwise fashion for blackflies collected and dissected in the field. Therefore, the proper storage conditions of the blackflies and the glands, and the subsequent dissection techniques are described in detail below. Identification of the collected blackflies can be done using either morphology or molecular techniques. For morphological identification we refer to [1]; for molecular identification of blackflies we refer to our publication where it is explained in detail.

Material

- ✓ Polypropylene collection tubes (7.5 cm × 1.2 cm)
- ✓ Freezer
- ✓ Crushed ice
- ✓ 95 % ethanol
- ✓ Tris-NaCl buffer (TBS; 20mM Tris, 150mM NaCl, pH 7.5)
- ✓ Stereomicroscope with good lighting
- ✓ Microscope glass slides
- ✓ Pasteur pipet / syringe
- ✓ Two tweezers
- ✓ Stainless steel micro-dissection needles
- ✓ Eppendorf tubes

Methods

1. Collect host-seeking blackflies using standard Human-Landing Catches techniques [2] in polypropylene collection tubes (7.5 cm × 1.2 cm; Sigma-Aldrich, T1911)
2. Place the tubes with collected blackflies in a cool box for storage until dissecting
3. Dissect blackflies on day of collection under a stereomicroscope
4. Anesthetize the flies by placing them in a -20° C freezer for 10 min

   ****Do not leave them in the freezer longer to avoid them dying****

   ****Dissect flies on the day of collection to make sure their salivary glands are not dried out and are still in optimal condition****

   ****Optionally flies can also be killed by adding 95 % ethanol in the collection tube, after which they should be immediately transferred to a tube containing TBS and dissected instantly to preserve the salivary gland proteins****
5. Place a drop of TBS on a microscope glass slide using a Pasteur pipet/ syringe
6. Empty one frozen collection tube, and hold the thorax of the blackfly in between the first pair of tweezers

   ****Batches of collection tubes containing the flies can be placed in the freezer; when taken out of the freezer they can be kept on crushed ice until dissected****

   ****Do not pierce the body of the blackfly with the tweezers; this will prevent you from proceeding to the next step****

   ****The first tweezers should be placed surrounding the scutum; placing them lower will prevent separating the head from the thorax****

   ****Be aware that the fly can wake up from the heat of the lamp of the stereomicroscope – try not to let go of the fly as it can fly away****
7. Place a second pair of tweezers behind the head of the fly and slowly separate head from thorax

   ****Do not hold the head with a firm grip but merely place the tweezers behind the head; by holding the thorax with the first pair of tweezers you can pull both tweezers in opposite direction to slowly separate the head from the thorax; this is a delicate step and requires practice****

8. While doing this, two tubular U-shaped salivary glands will appear (Fig 1); they can be attached to one another by a mid-section
9. Using (flattened) dissection needles take the salivary glands one by one out of the drop of TBS and place them in an Eppendorf tube containing TBS (1 gland per 1 µl TBS; make aliquots of 10 or 20 glands per Eppendorf tube)

   ****Flattening the dissection needles using a hammer is optimal in order to avoid piercing the glands****

   ****When the pair of salivary glands is attached to one other, try to separate them before transferring to the tube****

   ****Make sure to work as clean as possible, piercing an eye/ abdomen will contaminate the solution with non-salivary proteins****
10. While dissecting, keep the salivary glands cold (e.g. on crushed ice)
11. Store the aliquots of salivary glands at –20° C until further use
12. Prior to usage (e.g. immunoassays) disrupt the salivary glands by three quick freeze-and-thaw cycles in liquid nitrogen
13. Spin the disrupted glands at 13,500 rpm for 5 min to pellet any debris
14. Collect the supernatant of all aliquots and pool them together
15. Measure the protein concentration of the final pool (e.g. using a nanodrop spectrophotometer at 280 nm)
16. Aliquot the final pool of the salivary glands and use for subsequent analyses

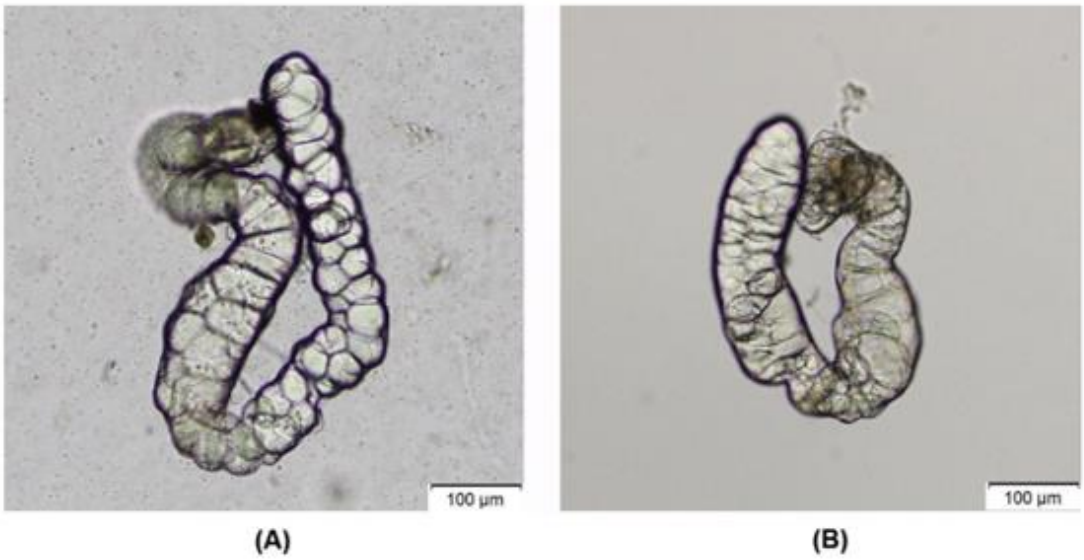

Fig 1. Salivary glands of adult female blackflies taken from [3]**.** (A) *S. nigrogilvum*, (B) *S. nodosum*

References

1. Lamberton PHL, Cheke RA, Walker M, Winskill P, Osei-Atweneboana MY, Tirados I, et al. Onchocerciasis transmission in Ghana: Biting and parous rates of host-seeking sibling species of the Simulium damnosum complex. Parasites and Vectors. 2014;7. doi:10.1186/s13071-014-0511-9

2. Walsh JF, Davies JB, Le Berre R, Garms R. Standardization of criteria for assessing the effect of simulium control in onchocerciasis control programmes. Trans R Soc Trop Med Hyg. 1978;72: 675–676. doi:10.1016/0035-9203(78)90039-1

3. Hempolchom C, Reamtong O, Sookrung N, Srisuka W, Sakolvaree Y, Chaicumpa W, et al. Proteomes of the female salivary glands of Simulium nigrogilvum and Simulium nodosum, the main human-biting black flies in Thailand. Acta Trop. 2019;194: 82–88. doi:10.1016/j.actatropica.2019.03.026