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Title: Traditional Morphometrics of Adult Mosquitoes

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes**

- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

- 3. Filming location:** Will the filming need to take place in multiple locations? **No**

- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 11

Number of Shots: 30

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

REQUIRED: Summarize your research focus and the main questions it aims to answer.

- 1.1. **Estelle Martin:** Our research investigates how anthropogenic disturbances, including habitat fragmentation, urbanization, and sociodemographic variables, influence mosquito community composition, abundance, and fitness.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Describe the research contexts or systems in which this protocol can be applied.

- 1.2. **Estelle Martin:** Traditional morphometrics measures physical trait lengths and can be applied when direct fitness assessments of fecundity or survival are not feasible.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

What does this protocol allow researchers to study or measure?

- 1.3. **Estelle Martin:** Mosquito body size predicts longevity, blood meal size, fecundity, and pathogen resistance, serving as a proxy for vectorial capacity and disease transmission risk.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Describe the most important consideration or challenge when performing this protocol.

- 1.4. **Estelle Martin:** The most important challenge when performing this protocol is ensuring complete trait removal from brittle tissues using standardized anatomical landmarks.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Are there any additional methods or analyses that can be performed following this procedure?

- 1.5. **Estelle Martin:** Additional analyses that can be performed following this procedure include geometrical morphometrics to investigate variation in trait shape.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

NOTE: All SCOPE and SCREEN shots are filmed by the videographer

2. Mosquito Dissection and Imaging

Demonstrator: McNamara

- 2.1. Obtain an individual female *Culex nigripalpus* mosquito anesthetized on ice [1] and transfer it into a 90-millimeter Petri dish [2]. Place the dish under a dissecting microscope equipped with a digital camera [3].
 - 2.1.1. Talent sitting with the test tube or cage containing mosquitoes on the workbench.
 - 2.1.2. Talent transferring a single female *Culex nigripalpus* mosquito into a 90 mm Petri dish.
 - 2.1.3. Talent placing the Petri dish under the dissecting microscope.
- 2.2. Adjust the magnification based on the species and individual insect [1]. Focus the camera so that the mosquito's entire body is visible [2] and fills most of the field of view [3].
 - 2.2.1. Talent adjusting the microscope magnification.
 - 2.2.2. SCOPE: Show the mosquito coming into focus until the full body fills most of the camera field of view.
 - 2.2.3. SCOPE: Shot of the mosquito in sharp focus.
- 2.3. Use micro-scissors to dissect the hind left leg at the coxa [1], the left wing at the jugum [2], the left antenna at the antennifer [3], and the proboscis at the clypeus [4]. Then, transfer the dissected tissues onto a slide mounted with double-sided tape [5].
 - 2.3.1. SCOPE: Talent dissecting the hind left leg at the coxa with micro-scissors.
 - 2.3.2. SCOPE: Talent dissecting the left wing at the base of the jugum with micro-scissors.
 - 2.3.3. SCOPE: Talent dissecting the left antenna at the antennifer with micro-scissors.
 - 2.3.4. SCOPE: Talent dissecting the proboscis at the clypeus with micro-scissors.
 - 2.3.5. SCOPE: Talent transferring the dissected tissues onto the slide mounted with double-sided tape.

2.4. Adjust the remaining mosquito body so its left side is visible [1]. Remove additional limbs if needed so the mosquito sits flush with the Petri dish [2]. Capture an image of the mosquito's left side with the microscope camera [3].

2.4.1. SCOPE: Talent repositioning the mosquito body so the left side is visible.

2.4.2. SCOPE: Talent removing additional limbs so the mosquito sits flush with the Petri dish.

2.4.3. SCOPE: Capture the side-view image of the mosquito with the microscope camera.

2.5. Without adjusting magnification or focus, remove the Petri dish containing the mosquito and replace it with the slide containing the dissected antenna, proboscis, wing, and hind leg [1]. Capture an image of the dissected tissues with the microscope camera [2].

2.5.1. Talent replacing the Petri dish with the slide containing the dissected tissues.

2.5.2. SCOPE: Capture the image of the dissected antenna, proboscis, wing, and hind leg.

2.6. Without adjusting magnification or focus, remove the slide containing mosquito tissues and replace it with the calibration slide stage micrometer [1]. Capture an image of the calibration slide with the microscope camera [2].

2.6.1. Talent replacing the tissue slide with the calibration slide stage micrometer.

2.6.2. SCOPE: Capture the image of the calibration slide. **NOTE: If required use "FileSlide-scale-without-measurements.tif, as an inset**

3. Mosquito Trait Measurement

3.1. Open the images collected during mosquito dissection in the AmScope AmLite Recording and Analysis program [1].

3.1.1. SCREEN: Open the collected dissection images in the AmScope AmLite Recording and Analysis program.

3.2. With the measurement tool set to pixels, measure a 1-millimeter section of the calibration scale on the calibration slide image [1]. Record the number of pixels per millimeter for the magnification and focus of this image [2].

3.2.1. SCREEN: Select the measurement tool, set it to pixels, and measure a 1 mm section of the calibration scale on the calibration slide image.

3.2.2. SCREEN: Record the pixels per mm value for the image. **NOTE: If required use Slide-scale-with-measurements.tif as inset**

- 3.3. Measure and record the thorax length on the side-view image from the anterior of the thorax to the posterior of the scutellum [1]. Assess and record the abdomen length from the anterior of the scutellum to the posterior of the abdomen [2]. Use a multi-point measuring tool for traits with bends or joints when necessary [3].
 - 3.3.1. SCREEN: Measure and record the thorax length on the side-view image.
 - 3.3.2. SCREEN: Assess and record the abdomen length on the side-view image.
 - 3.3.3. SCREEN: Use a multi-point measuring tool or multiple separate measurements on a bent or jointed trait.
- 3.4. Determine the dissected antenna length from the terminal segment of the antenna to the base of the antennifer [1]. Then, measure the wing length from the axial incision to the apical margin [2]. Assess the hind leg length as the sum of the tibia and femur lengths [3]. Measure the proboscis length from the base of the clypeus to the termination of the labium [4].
 - 3.4.1. SCREEN: Measure the dissected antenna length from the terminal segment to the base of the antennifer.
 - 3.4.2. SCREEN: Assess the wing length from the axial incision to the apical margin.
 - 3.4.3. SCREEN: Determine the hind leg length as the sum of the tibia and femur lengths.
 - 3.4.4. SCREEN: Measure the proboscis length from the base of the clypeus to the termination of the labium.
- 3.5. Divide each pixel measurement by the pixels per millimeter value obtained from the calibration slide image to calculate the trait lengths in millimeters [1].
 - 3.5.1. SCREEN: Calculate the trait lengths in millimeters by dividing each pixel measurement by the recorded pixels per mm value in the Excel.

Results

4. Results

4.1. The thorax measured 714.08 pixels [1], and the abdomen measured 1528.63 pixels [2].

4.1.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the red measurement line near to the head region of the mosquito labelled 714.08 pixels*

4.1.2. LAB MEDIA: Figure 3A. *Video editor: Highlight the red measurement line towards the tail end labelled 1528.63 pixels*

4.2. The left tibia and femur measured 1900.49 pixels [1], the left antennae measured 1063.09 pixels [2], the left wing measured 1749.00 pixels [3], and the proboscis measured 964.14 pixels [4].

4.2.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the red measurement line on the long leg labelled 1900.49 pixels*

4.2.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the red measurement line on the curved antenna labelled 1063.09 pixels*

4.2.3. LAB MEDIA: Figure 3B. *Video editor: Highlight the red measurement line on the wing labelled 1749.00 pixels*

4.2.4. LAB MEDIA: Figure 3B. *Video editor: Highlight the red measurement line on the proboscis labelled 964.14 pixels*

4.3. Morphometric assessment of *Culex nigripalpus* collected from large forest plots [1] included measurements of total body length, leg length, wing length, antennal length, and proboscis length [2].

4.3.1. LAB MEDIA: Table 1.

4.3.2. LAB MEDIA: Table 1. *Video editor: Show the entire table while highlighting the columns showing "total body length", "leg length", "wing length", "antennal length", and "proboscis length" respectively while "total body length", "leg length", "wing length", "antennal length", and "proboscis length" is mentioned in the VO.*

1. Culex nigripalpus
Pronunciation link: No confirmed link found
IPA: /'kju:lɛks ˌnɪɡrɪ'pælpəs/
Phonetic Spelling: KYOO·leks nig·rih·PAL·puhs
2. Anesthetized
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetize>
IPA: /əˈnɛsθəˌtaɪzd/
Phonetic Spelling: uh·NES·thuh·tyzed
3. Petri
Pronunciation link: <https://www.merriam-webster.com/dictionary/Petri%20dish>
IPA: /'pi:tri/
Phonetic Spelling: PEE·tree
4. Micro-scissors
Pronunciation link: No confirmed link found
IPA: /'maɪkroʊ ˌsɪzərz/
Phonetic Spelling: MY·kroh SIZ·erz
5. Coxa
Pronunciation link: <https://www.merriam-webster.com/dictionary/coxa>
IPA: /'kɒksə/
Phonetic Spelling: KOK·suh
6. Jugum
Pronunciation link: <https://www.merriam-webster.com/dictionary/jugum>
IPA: /'dʒu:gəm/
Phonetic Spelling: JOO·guh-m
7. Antenna
Pronunciation link: <https://www.merriam-webster.com/dictionary/antenna>
IPA: /æn'tɛnə/
Phonetic Spelling: an·TEN·uh
8. Antennifer
Pronunciation link: No confirmed link found
IPA: /æn'tɛnɪfər/
Phonetic Spelling: an·TEN·ih·fer
9. Proboscis
Pronunciation link: <https://www.merriam-webster.com/dictionary/proboscis>
IPA: /proʊˈbɒsɪs/
Phonetic Spelling: proh·BOSS·is
10. Clypeus
Pronunciation link: <https://www.merriam-webster.com/dictionary/clypeus>
IPA: /'klɪpiəs/
Phonetic Spelling: KLIP·ee·uhs
11. Micrometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/micrometer>
IPA: /maɪˈkrɒmɪtər/
Phonetic Spelling: my·KROM·uh·ter

12. Thorax
Pronunciation link: <https://www.merriam-webster.com/dictionary/thorax>
IPA: /'θɔræks/
Phonetic Spelling: THOR·aks
13. Scutellum
Pronunciation link: <https://www.merriam-webster.com/dictionary/scutellum>
IPA: /skju:'tɛləm/
Phonetic Spelling: skyoo·TEL·uhm
14. Abdomen
Pronunciation link: <https://www.merriam-webster.com/dictionary/abdomen>
IPA: /'æbdəmən/
Phonetic Spelling: AB·duh·muhn
15. Axial
Pronunciation link: <https://www.merriam-webster.com/dictionary/axial>
IPA: /'æksɪəl/
Phonetic Spelling: AK·see·uhl
16. Apical
Pronunciation link: <https://www.merriam-webster.com/dictionary/apical>
IPA: /'eɪpɪkəl/
Phonetic Spelling: AY·pih·kuhl
17. Tibia
Pronunciation link: <https://www.merriam-webster.com/dictionary/tibia>
IPA: /'tɪbiə/
Phonetic Spelling: TIB·ee·uh
18. Femur
Pronunciation link: <https://www.merriam-webster.com/dictionary/femur>
IPA: /'fi:mər/
Phonetic Spelling: FEE·mer
19. Labium
Pronunciation link: <https://www.merriam-webster.com/dictionary/labium>
IPA: /'leɪbiəm/
Phonetic Spelling: LAY·bee·uhm
20. Morphometric
Pronunciation link: <https://www.merriam-webster.com/dictionary/morphometric>
IPA: /,mɔrfə'mɛtrɪk/
Phonetic Spelling: mor·fuh·MET·rik
21. Antennal
Pronunciation link: <https://www.merriam-webster.com/dictionary/antennal>
IPA: /æn'tɛnəl/
Phonetic Spelling: an·TEN·uhl