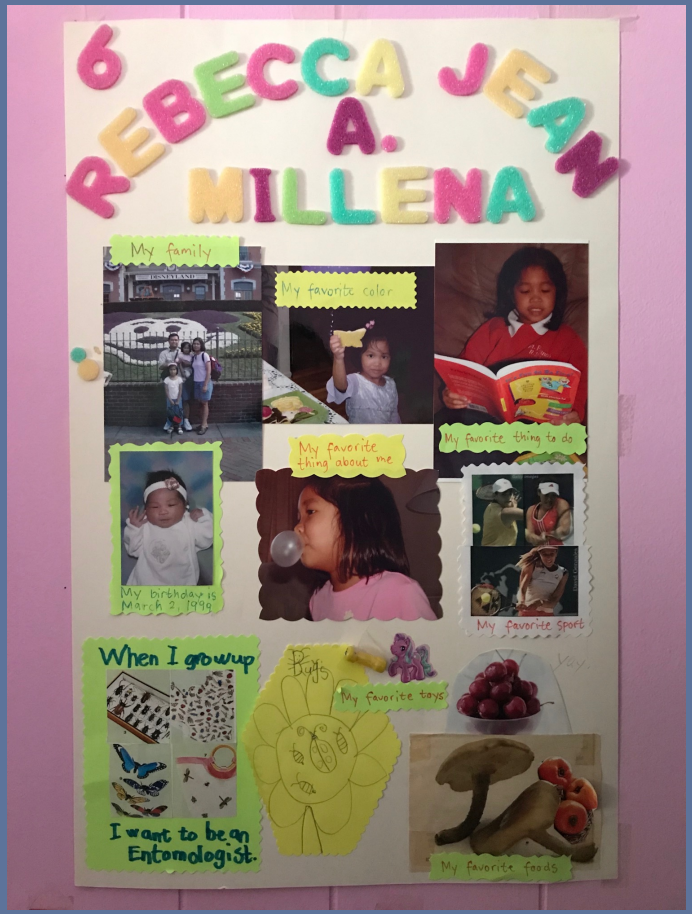
SEQUENCING STREPSIPTERA:
RESOLVING THE EVOLUTIONARY HISTORY OF THE TWISTED-WING PARASITES

RJ Millena – Comparative Biology PhD Candidate, RGGS at AMNH

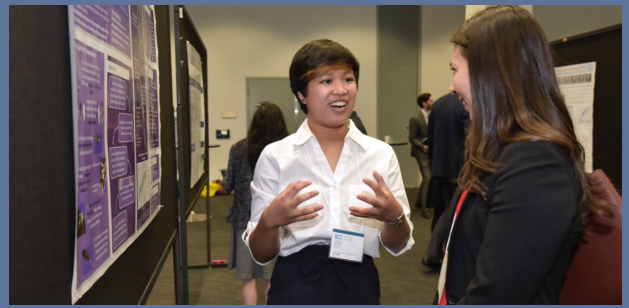
OUTLINE

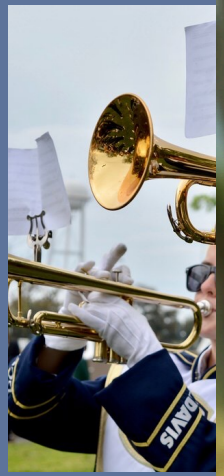
- Who am I?
- What is Strepsiptera?
- What am I doing?

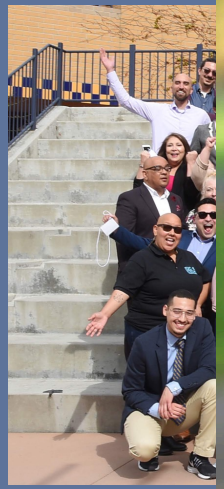

6
REBECCA JEAN
A.
MILLENA
My family
My favorite color
My favorite thing to do
My favorite thing about me
My birthday is March 2, 1999
My favorite sport
When I growup
I want to be an Entomologist.
Boys
My favorite toys
My favorite foods


BECCA JEAN
A.
LENA
favorite color
My favorite thing to do
My favorite sport
My favorite toys
I want to be an Entomologist.
My favorite foods

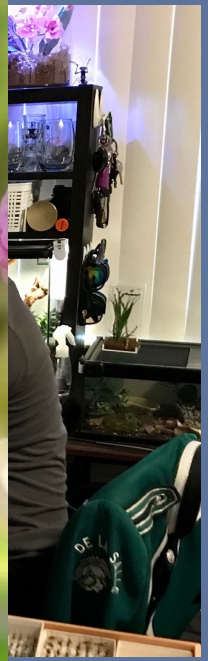

DAVIS
DAVIS



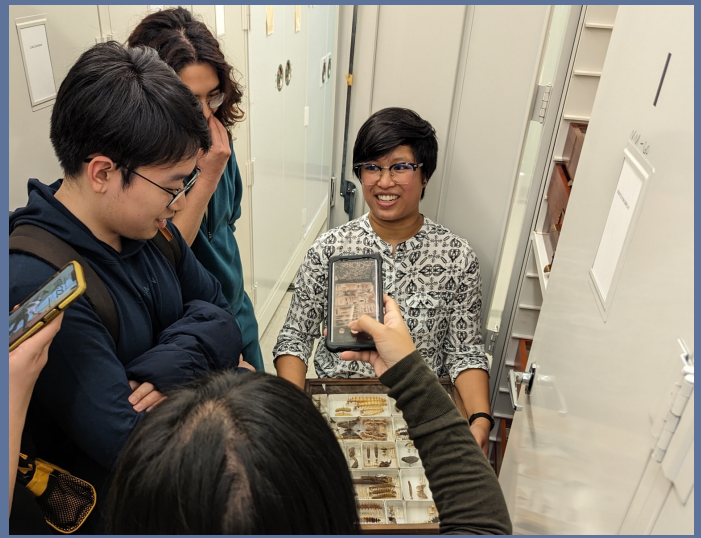




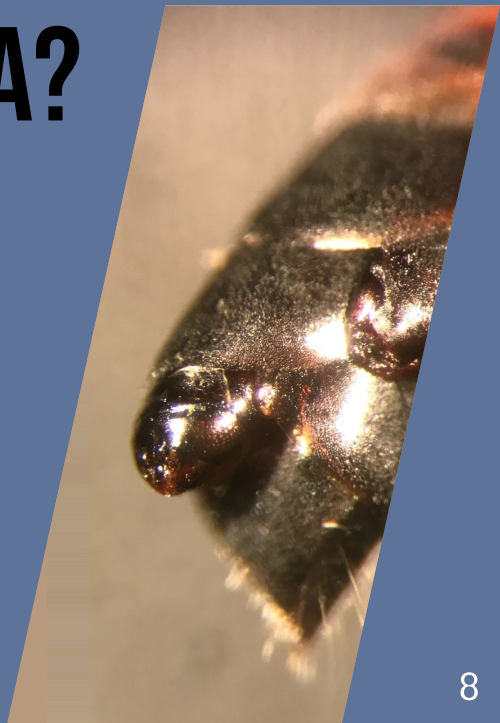

When I grow up I want to be an
Entomologist.

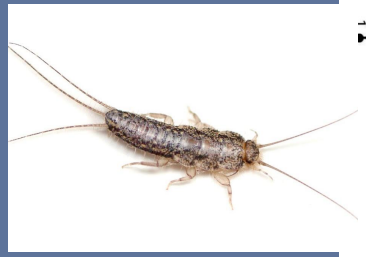


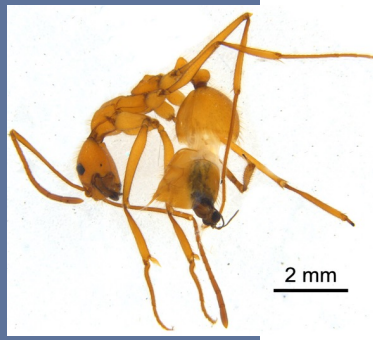

THE AMERICAN MVSEVM OF NATVRAL HISTORY



WHAT IS STREPSIPTERA?



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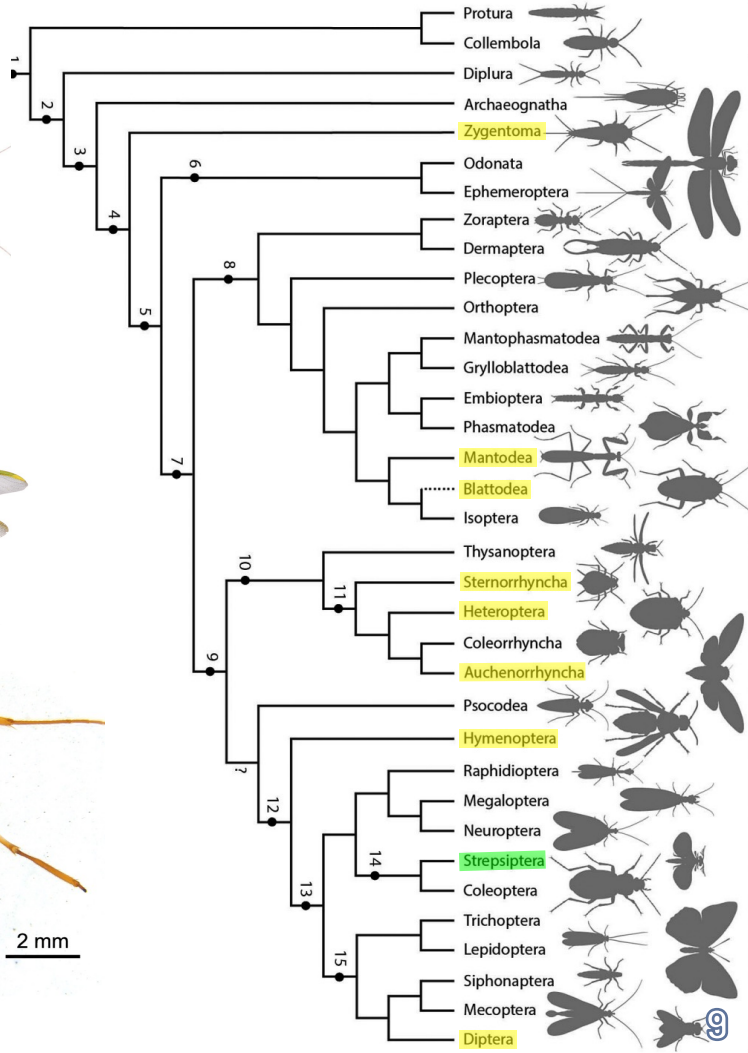
Protura
Collembola
Diplura
Archaeognatha
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Odonata
Ephemeroptera
Zoraptera
Dermaptera
Plecoptera
Orthoptera
Mantophasmatodea
Grylloblattodea
Embioptera
Phasmatodea
Mantodea
Blattodea
Isoptera
Thysanoptera
Sternorrhyncha
Heteroptera
Coleorrhyncha
Auchenorrhyncha
Psocodea
Hymenoptera
Raphidioptera
Megaloptera
Neuroptera
Strepsiptera
Coleoptera
Trichoptera
Lepidoptera
Siphonaptera
Mecoptera
Diptera
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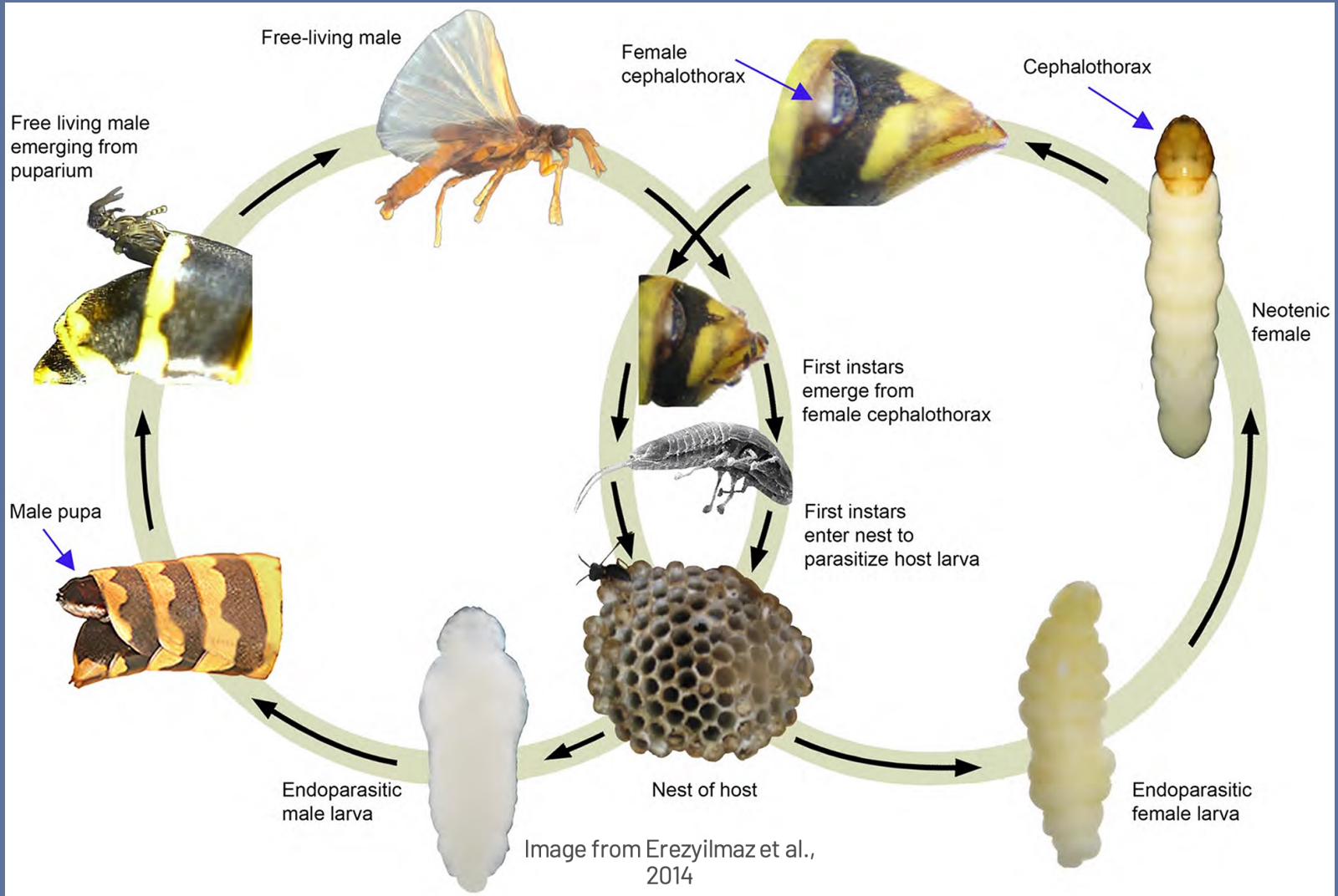
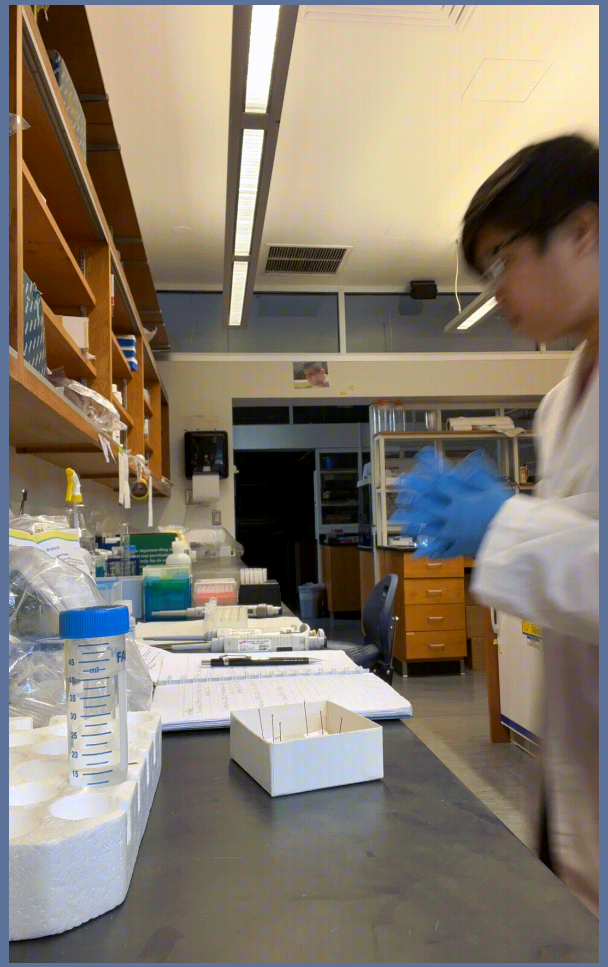
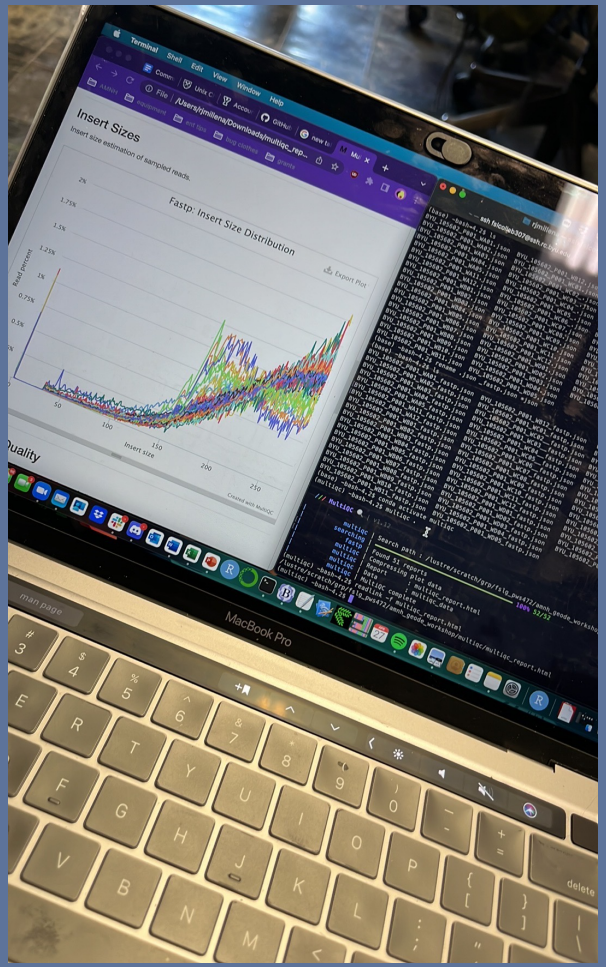


Image from Erezyilmaz et al., 2014




Insert Sizes
Insert size estimation of sampled reads.
Fastp: Insert Size Distribution
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Insert size
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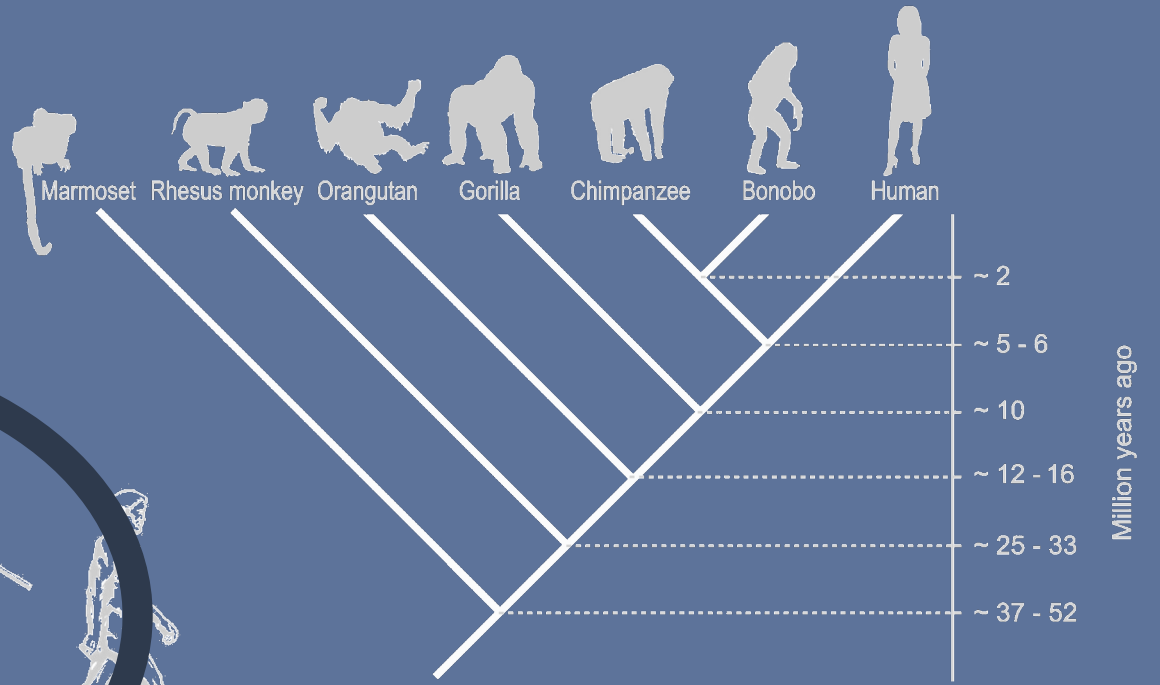
SYSTEMATICS =
EVOLUTION + TAXONOMY

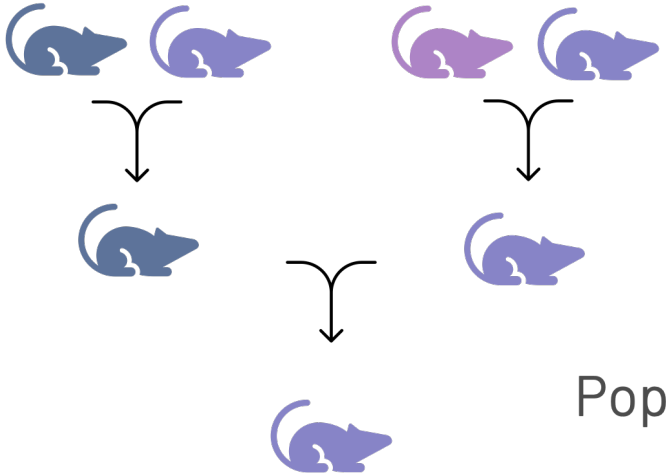
EVOLUTION

Wheeler et al. (2019). *The Conversation,* online article.

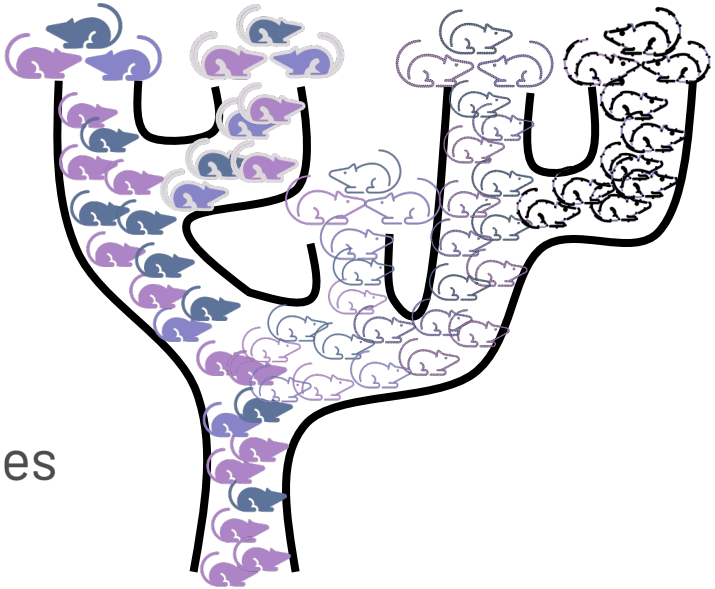


Heijmans et al. (2020). *International Journal of Immunogenetics, 47*(3), 243-260

EVOLUTION: TIMESCALES



Population vs. Species



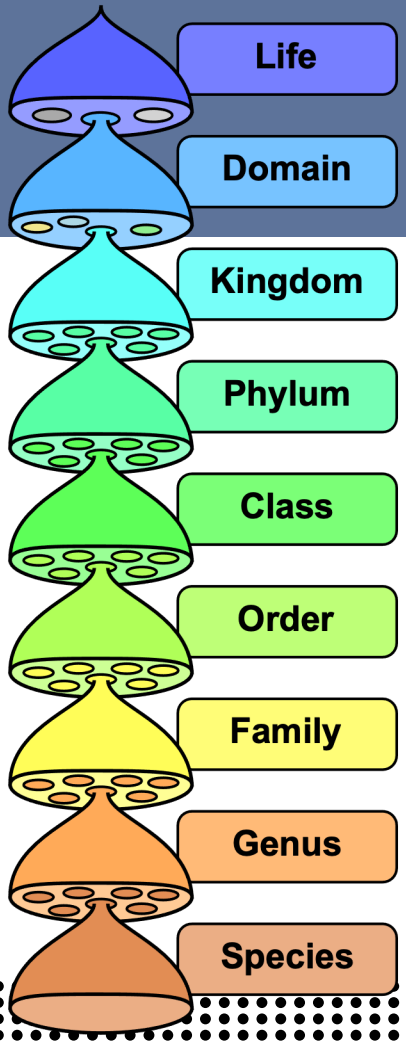
Microevolution

Macroevolution

TAXONOMY

- System of classification completely made up by humans, for humans
- Subclassifications like superorder, tribe, subfamily, etc.
- **D**id **K**ing **P**hilip **C**ome **O**ver **F**or **G**ood **S**paghetti

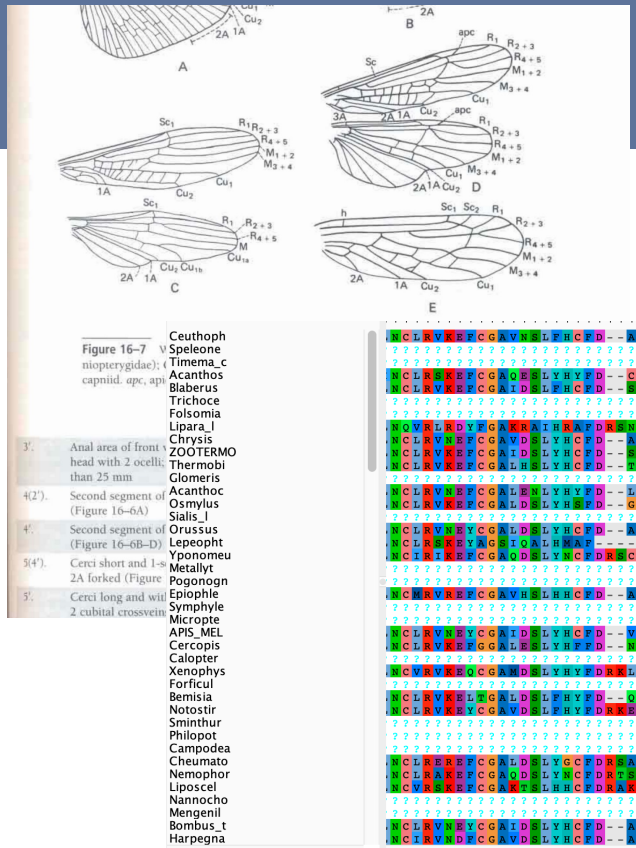
Eukarya > Animalia > Arthropoda > Insecta > Strepsiptera > Xenidae > Xenos > *Xenos peckii*



TAXONOMY

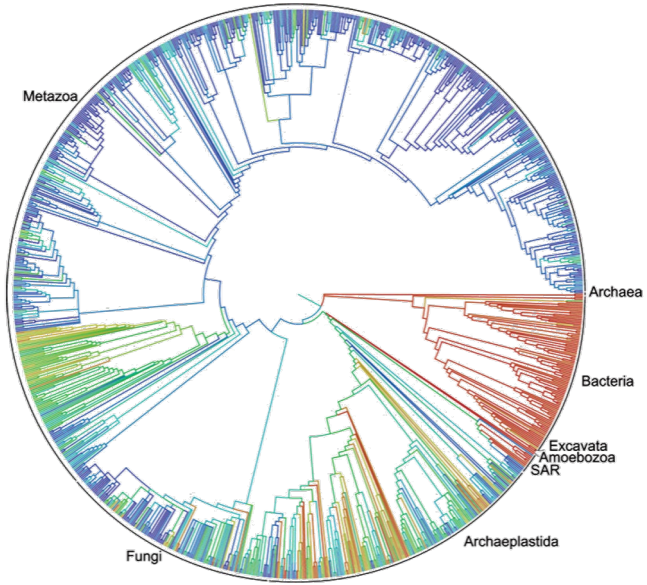
- Traditionally based on morphology (appearance), now can also be based on molecular data (DNA, RNA)
- Taxonomists
  - Identify, name, and describe organisms
  - Perform species delimitation

Borror and DeLong (2005). *Introduction to the Study of Insects.*



SYSTEMATICS

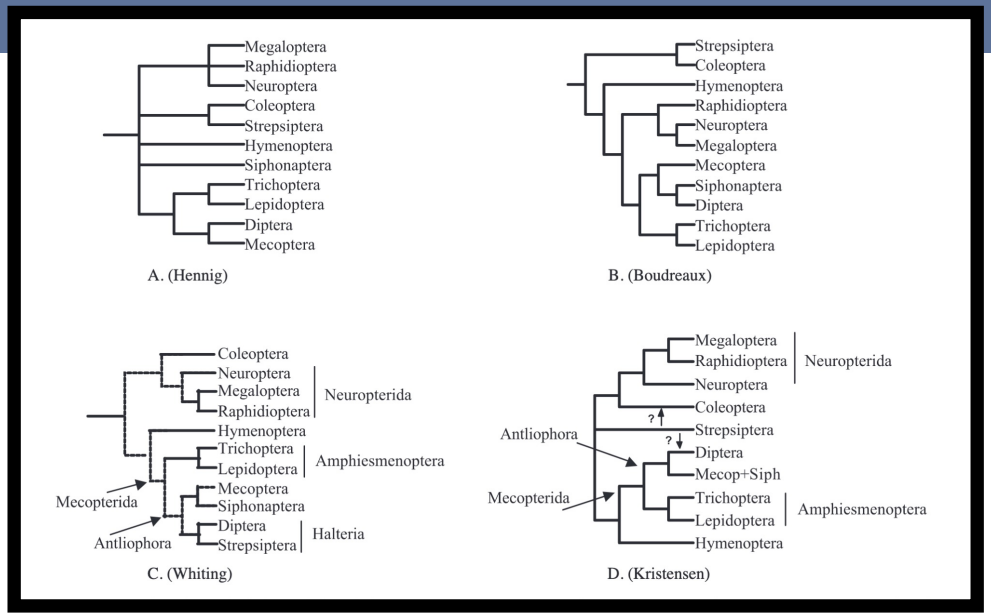
- Evolution + taxonomy
- “The science of naming species and of recovering the relationships between them; the study of describing and analyzing Earth’s biodiversity”
- Phylogenetics



https://tree.opentreeoflife.org/
https://www.onezoom.org/

EVOLUTIONARY QUESTIONS

- Enigmatic placement in the insect radiation
- Unresolved intraordinal relationships
- Ancestral characteristics
- Evolutionary trends



Whiting, M. F. (2002). Zoologica Scripta, 31, 3 – 15

EVOLUTIONARY QUESTIONS

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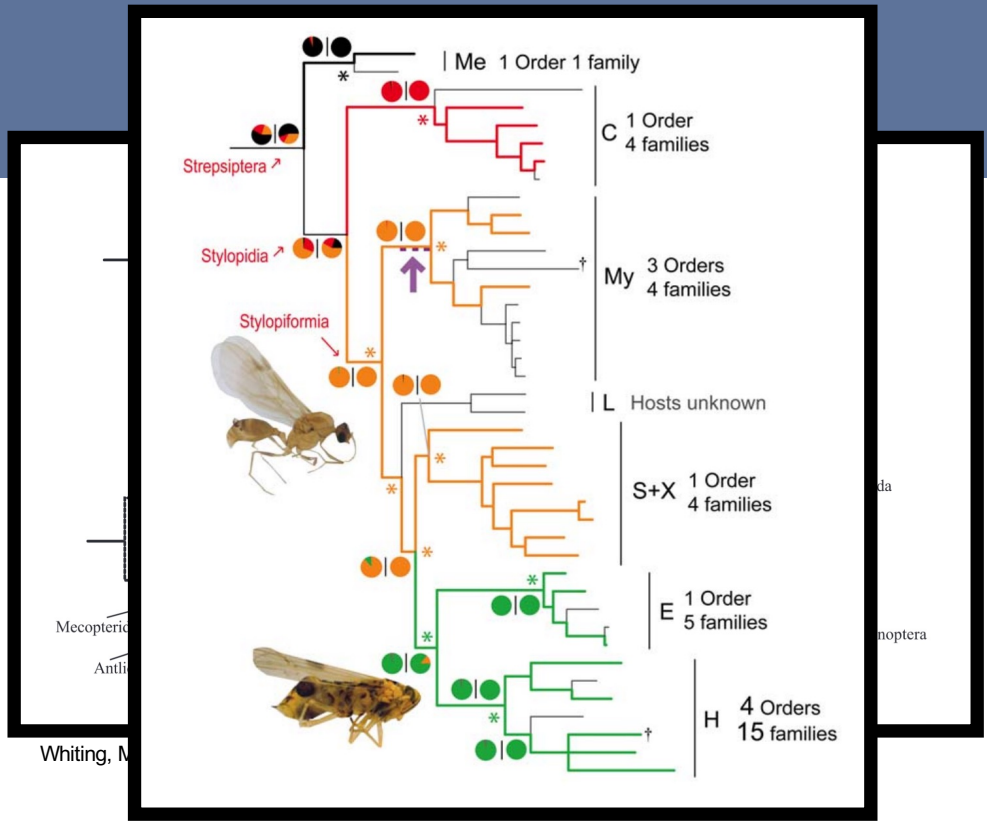

McMahon et al. (2011). PLoS ONE 6(6), e21206.

EVOLUTIONARY QUESTIONS

- Enigmatic placement in the insect radiation
- Unresolved intraordinal relationships
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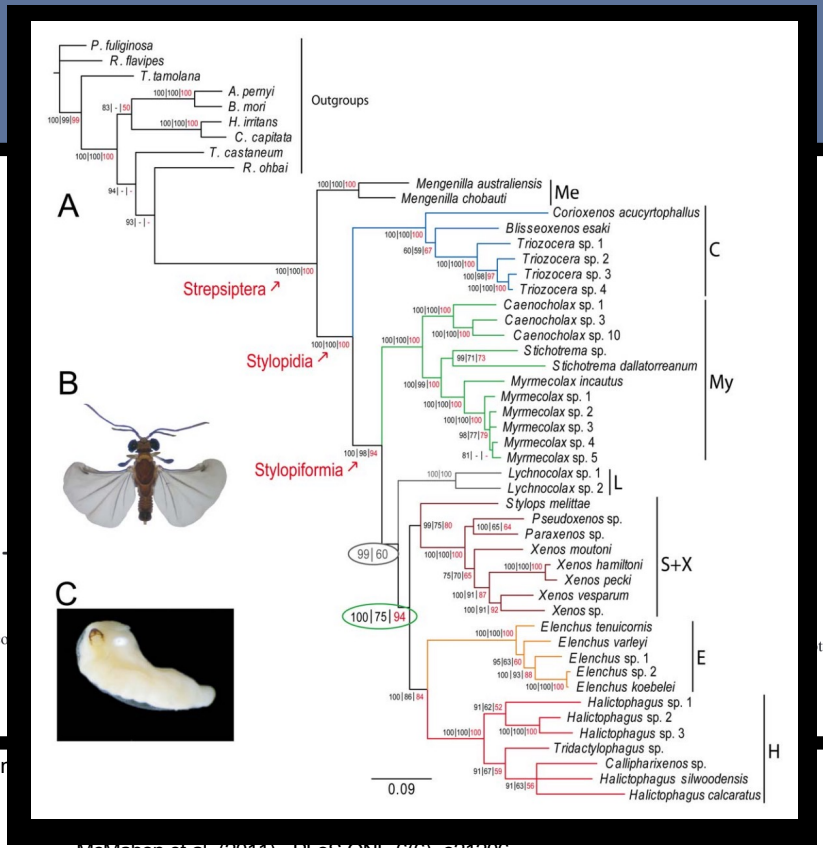


McMahon et al. (2011). PLoS ONE 6(6), e21206.

McMahon et al. (2011). PLoS ONE 6(6), e21206.

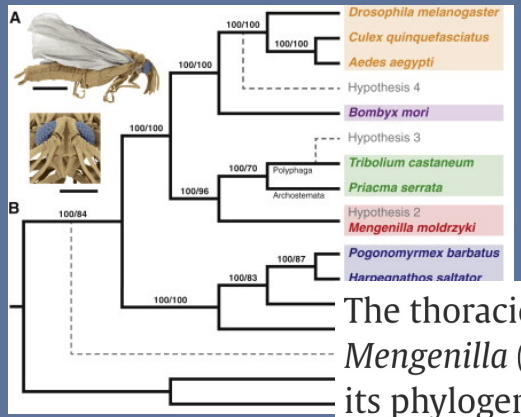
Genomic and Morphological Evidence Converge to Resolve the Enigma of Strepsiptera

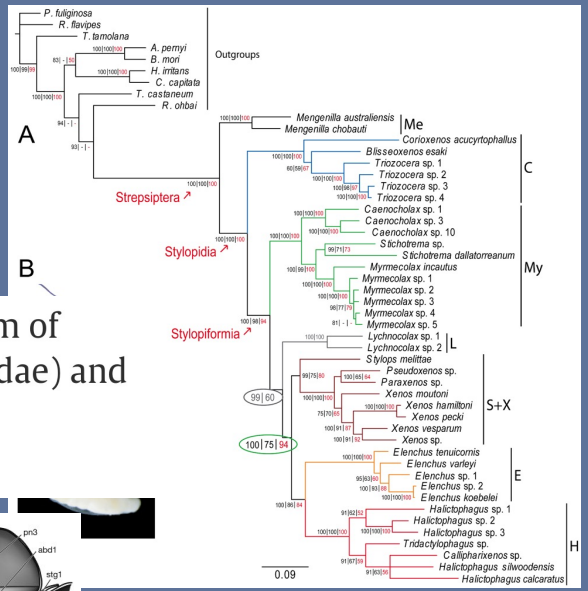
Oliver Niehuis,* Gerrit Hartig, Sonja Grath, Hans Pohl, Jörg Lehmann, Hakim Tafer, Alexander Donath, Veiko Krauss, Carina Eisenhardt, Jana Hertel, Malte Petersen, Christoph Mayer, Karen Meusemann, Ralph S. Peters, Peter F. Stadler, Rolf G. Beutel, Erich Bornberg-Bauer, Duane D. McKenna, and Bernhard Misof*

The First Molecular Phylogeny of Strepsiptera (Insecta) Reveals an Early Burst of Molecular Evolution Correlated with the Transition to Endoparasitism

Dino P. McMahon*, Alexander Hayward, Jeyaraney Kathirithamby*

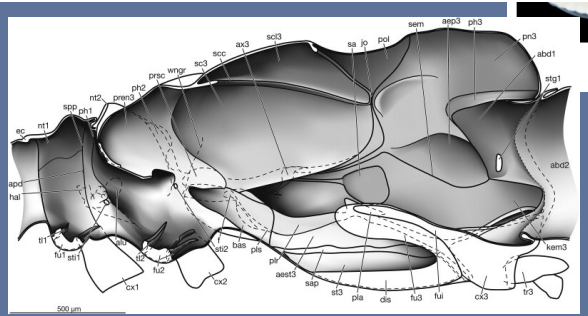
Department of Zoology, University of Oxford, Oxford, United Kingdom





The thoracic skeleto-muscular system of *Mengenilla* (Strepsiptera: Mengenillidae) and its phylogenetic implications

Monica Koeth [a 1], Frank Friedrich [b], Hans Pohl [a], Rolf Georg Beutel [a]



DISSERTATION

01 GENOMES

Strepsiptera in insect evolution + the evolution of strepsipteran genomes

Sequencing three whole genomes for three strepsipteran species

02 PHYLOGENY

The evolution of strepsipteran genes + evolutionary relationships in the order

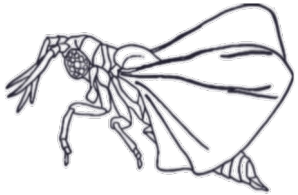
Constructing a genus-level phylogeny of Strepsiptera

03 MORPHOLOGY

The evolutionary trends of strepsipteran appearance + structures

Evaluating internal morphology in the males of Strepsiptera

01



CHARACTERIZING THE GENOMIC ELEMENTS OF STREPSIPTERA

01 GENOMES

Main Question:

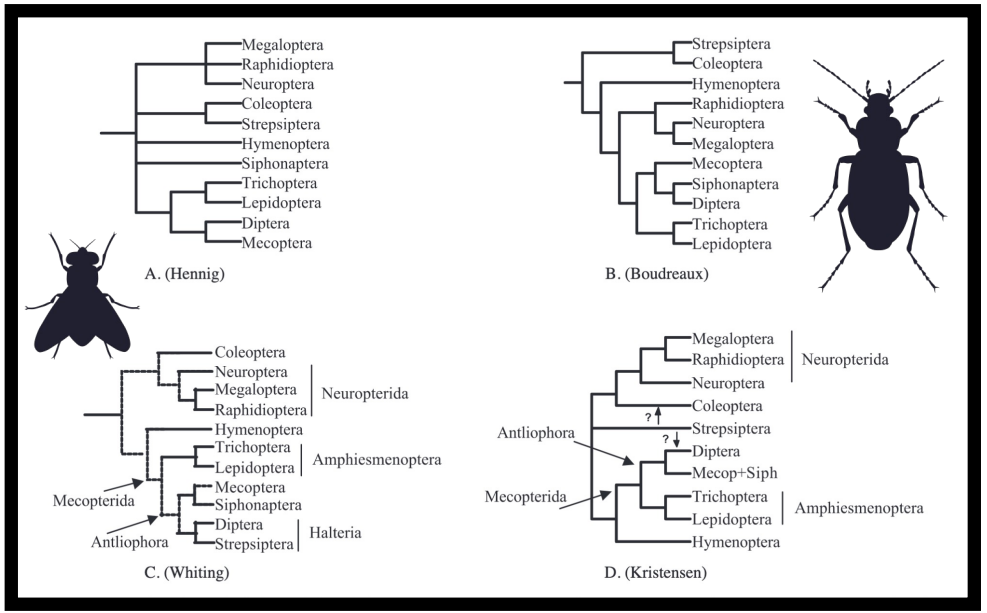
How do the genomes of Strepsiptera compare to its hypothetical closest relatives?

Aims:

- Sequence three strepsipteran genomes across three families
- Generate chromosome-level assemblies for Strepsiptera
- Perform a comparative analysis on the genomes of Neuropteroidea

01 GENOMES

- Historically disputed position in insect evolution
- Sister to Coleoptera
  - Supported by molecular evidence
- Only a singular strepsipteran WGS
- None examined for composition



Whiting, M. F. (2002). Zoologica Scripta, 31, 3 – 15

01 GENOMES

- **Strepsipteran genomes**
  Xenos peckii (Xenidae)
  Elenchus koebelei (Elenchidae)
  Caenocholax fenyesi (Myrmecolacidae)
- **Comparative analyses**

Coleoptera

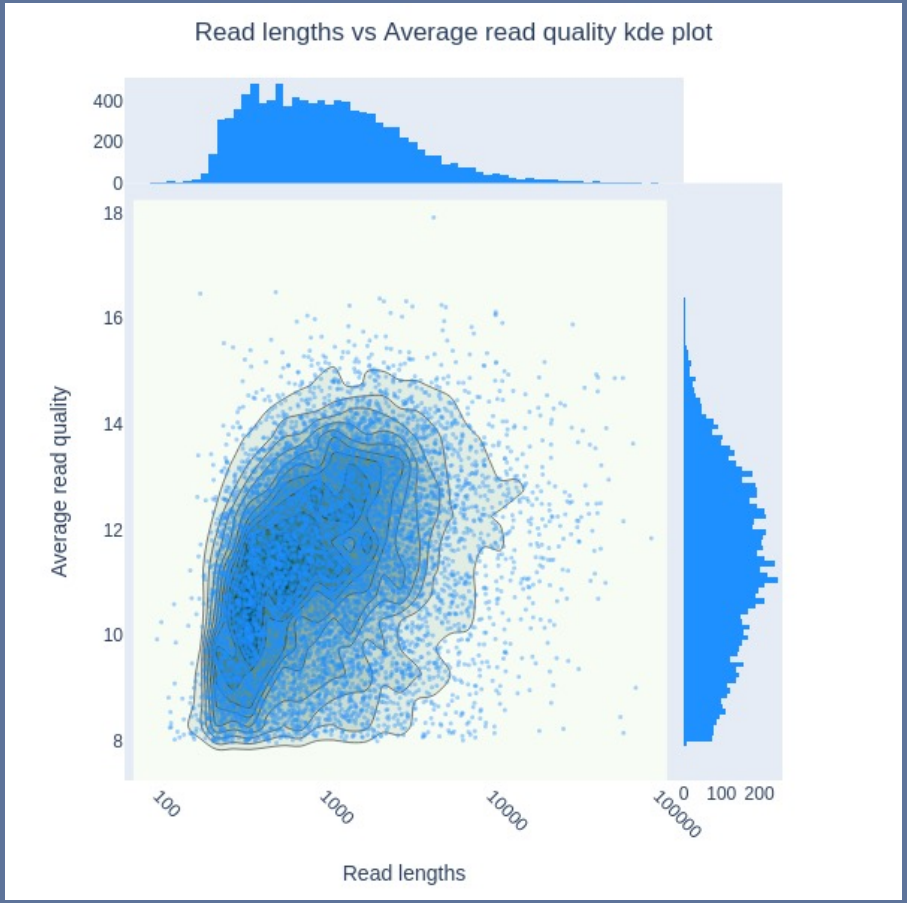
Neuroptera

Megaloptera

01 GENOMES

Preliminary results:

- Flye, retrained with Augustus
- Retrained BUSCO score: 83.0%
  - Initial score of ~35%
- n50: 421387 (421 kb)
- Better in quality assessment than the only published strepsipteran genome
  - n50: ~4000 (4 kb)


Read lengths vs Average read quality kde plot
Average read quality
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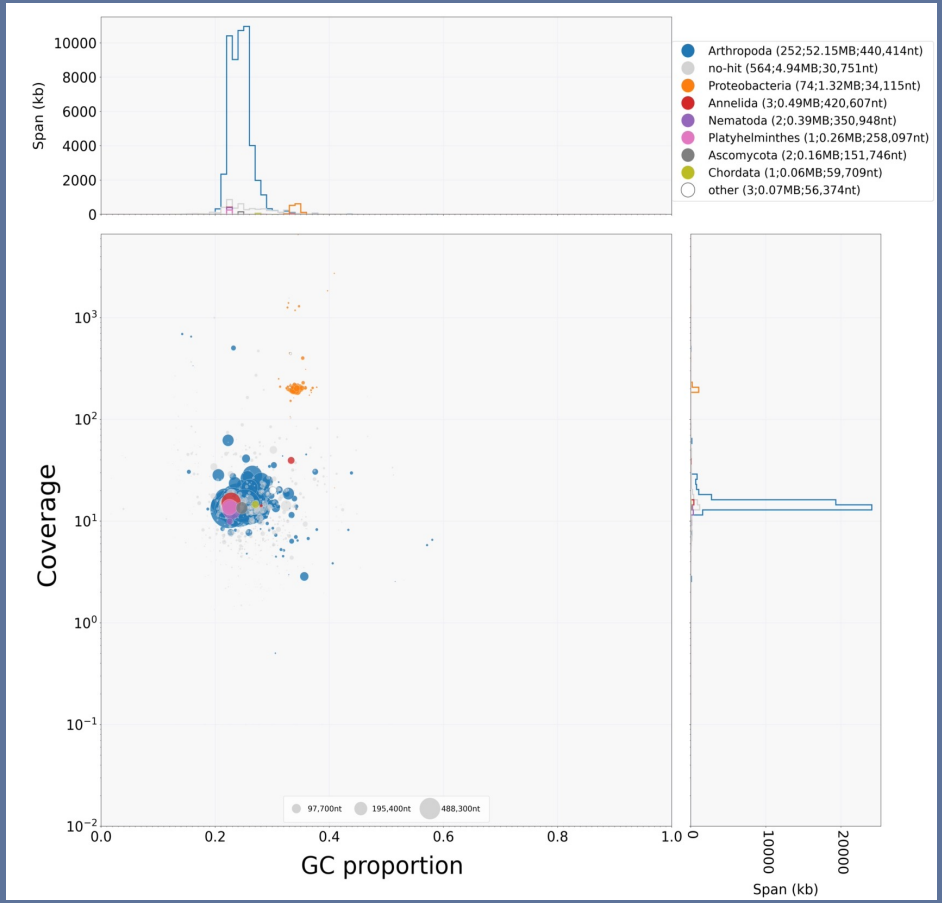
Span (kb)
10000
8000
6000
4000
2000
0
Arthropoda (252;52.15MB;440,414nt)
no-hit (564;4.94MB;30,751nt)
Proteobacteria (74;1.32MB;34,115nt)
Annelida (3;0.49MB;420,607nt)
Nematoda (2;0.39MB;350,948nt)
Platyhelminthes (1;0.26MB;258,097nt)
Ascomycota (2;0.16MB;151,746nt)
Chordata (1;0.06MB;59,709nt)
other (3;0.07MB;56,374nt)
Coverage
10^3
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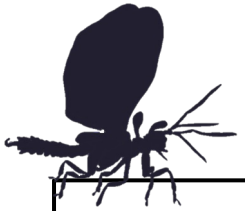
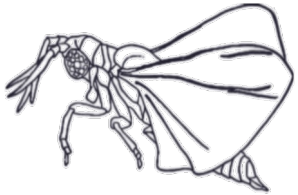
METHODS

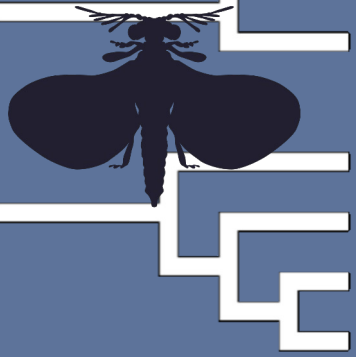


- AIMS
  - THREE GENOMES
    - ONT MINION — *Xenos peckii*, *Elenchus koebelei*
    - PACBIO — *Caenocholax fenyesi*
  - CHROMOSOME-LEVEL ASSEMBLIES
    - ANNOTATIONS — Flye & Augustus
  - COMPARATIVE ANALYSIS
    - COLLINEARITY — VGSC2 & CIRCOS

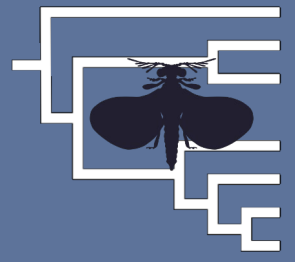
02



DETERMINING THE EVOLUTIONARY RELATIONSHIPS OF STREPSIPTERA



02 PHYLOGENY



Main Question:

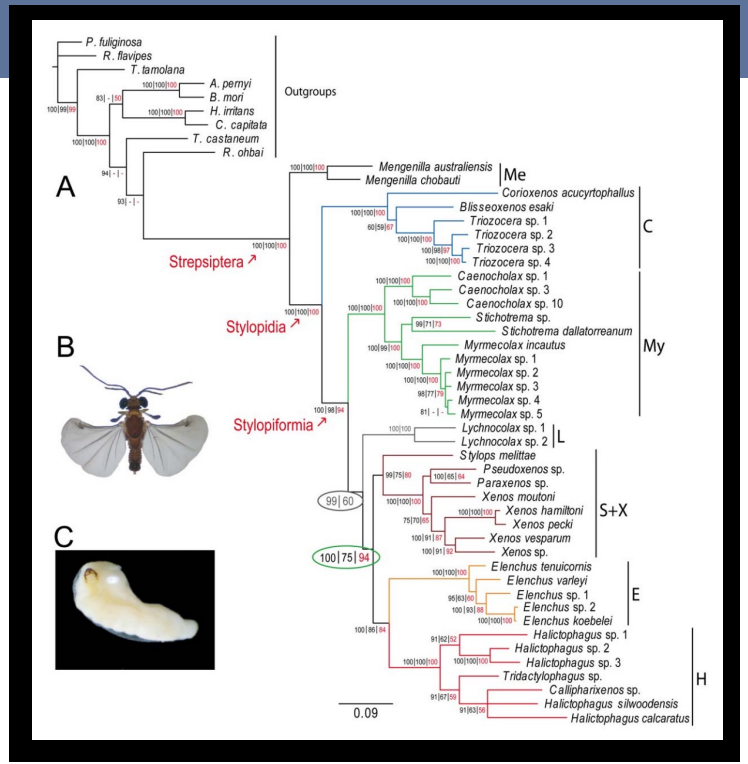
What are the evolutionary relationships of Strepsiptera?

Aims:

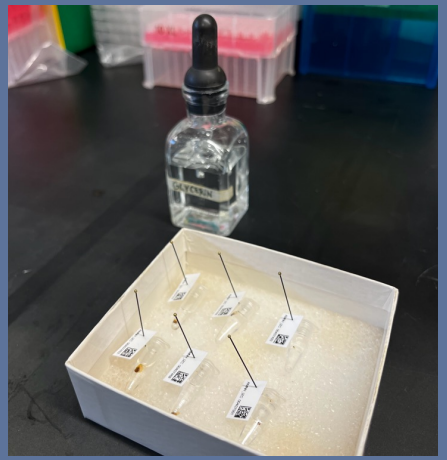
- Resolve lineage relationships at the generic and species level
- Estimate divergence times within the order
- Reconstruct ancestral states for host use and morphology
- Investigate host use and morphological trait evolution and timing

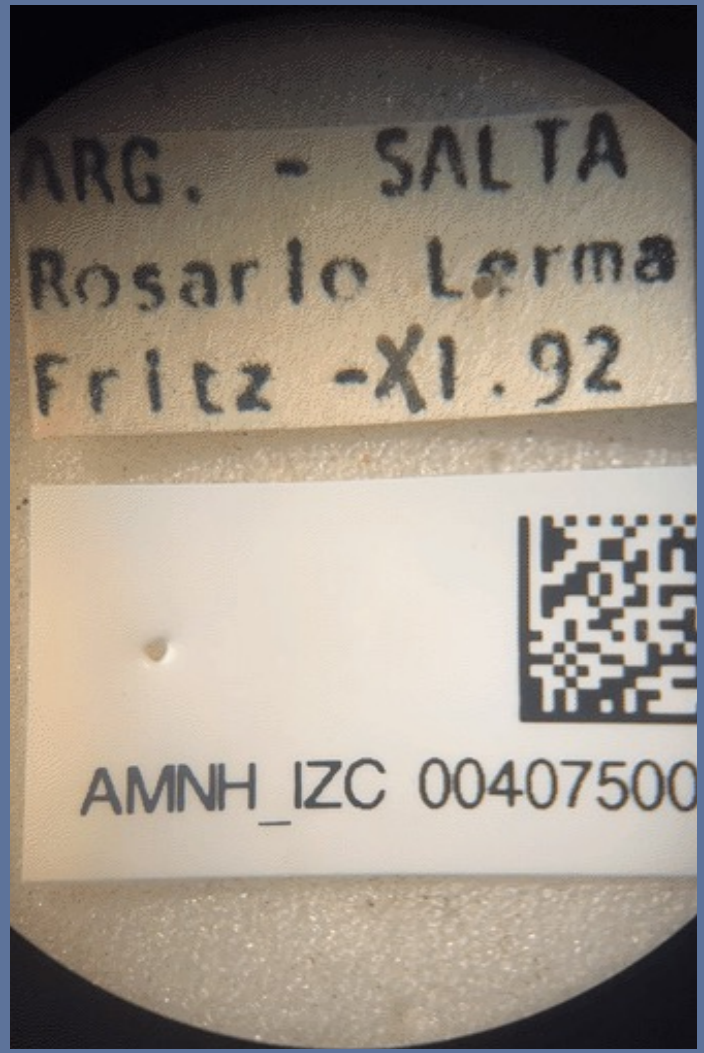
02 PHYLOGENY

- McMahon et al. (2011)
  - Based on 4 genes
  - 41/630 extant taxa
  - 8/10 extant families
- Increasing data = more robust phylogeny?
  - Taxon sampling
  - Genes/loci included
- Anchored hybrid enrichment (AHE) phylogenetics



McMahon et al. (2011). PLoS ONE 6(6), e21206.




ARG. - SALTA
Rosario Lerma
Fritz -XI.92
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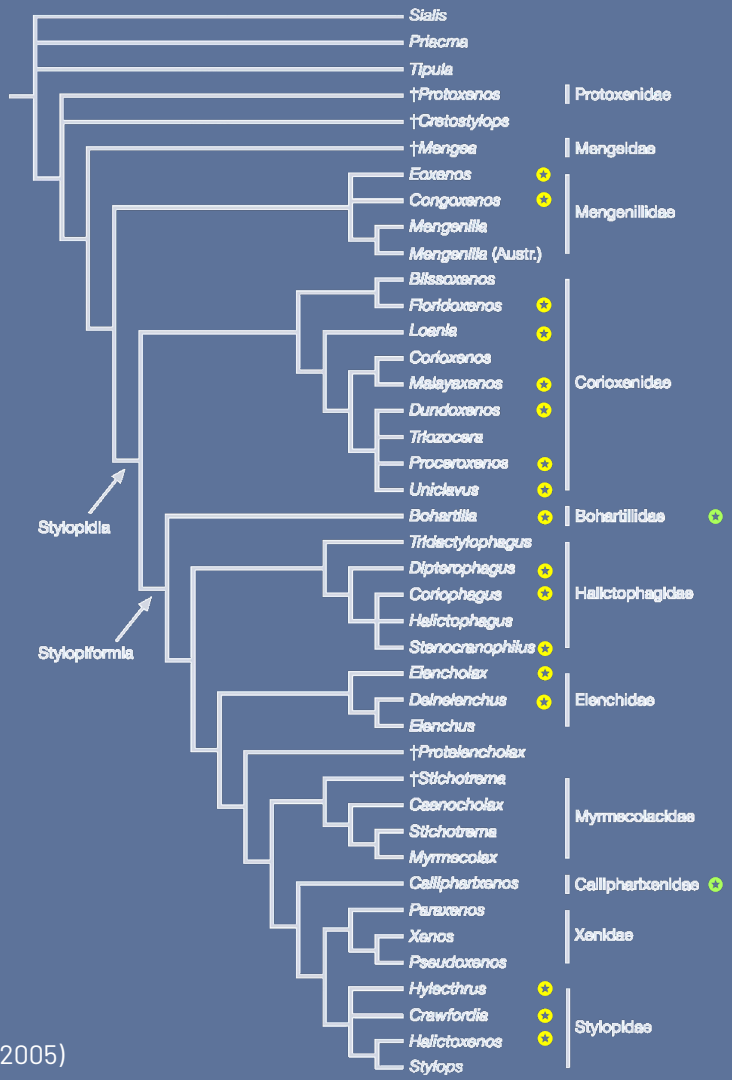
02 PHYLOGENY

- **Reconstructing the phylogeny**
  - Isolate strepsipterans, nondestructive DNA extraction
  - Sequencing the hDNA
  - Anchored hybrid enrichment (AHE)
- **Analyses**
  - Divergence time estimation using fossil calibration priors (BEAST, MCMCtree)
  - Tracing trait evolution (host use, morphological) across the tree

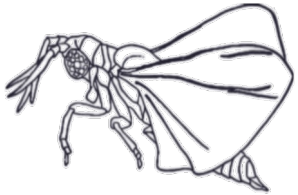


Not shown but will be included:

- Family Phtanoxenidae
  - Genera *Heterobathmilla*, *Kinzelbachilla*, *Phthanoxenos*
- Family Lychnocolacidae
  - Genus *Lychnocolax*
- In Corioxenidae
  - Genus *Eocenoxenos*
- In Myrmecolacidae
  - Genera *Kronomyrmecolax*, *Palaeomyrmecolax*
- In Stylopidae
  - Genus *Jantarostylops*

Image from Pohl & Beutel (2005)

03

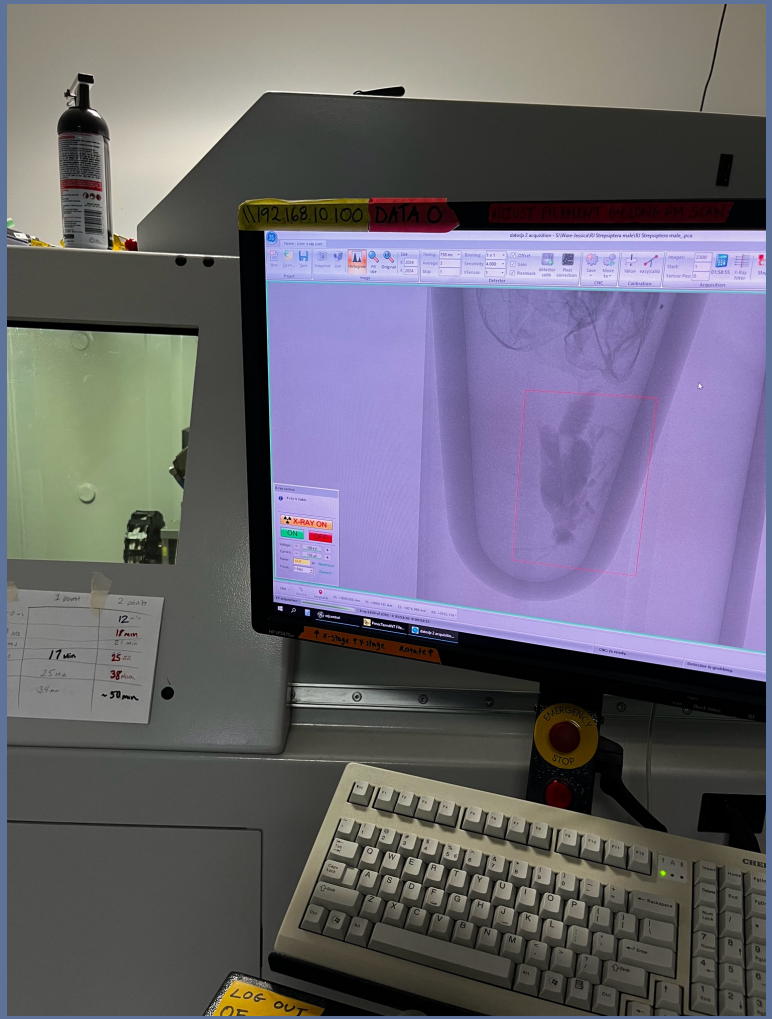


DESCRIBING AND EVALUATING THE VARIATION IN INTERNAL MORPHOLOGY OF STREPSIPTERA

03 MORPHOLOGY

Main Question:
What variation is there in the adult male internal structures of the ten strepsipteran families?

Aims:
- Describe and evaluate variation in internal morphology of male Strepsiptera
- Analyze internal character evolution in a phylogenetic context (using tree from Chapter 2)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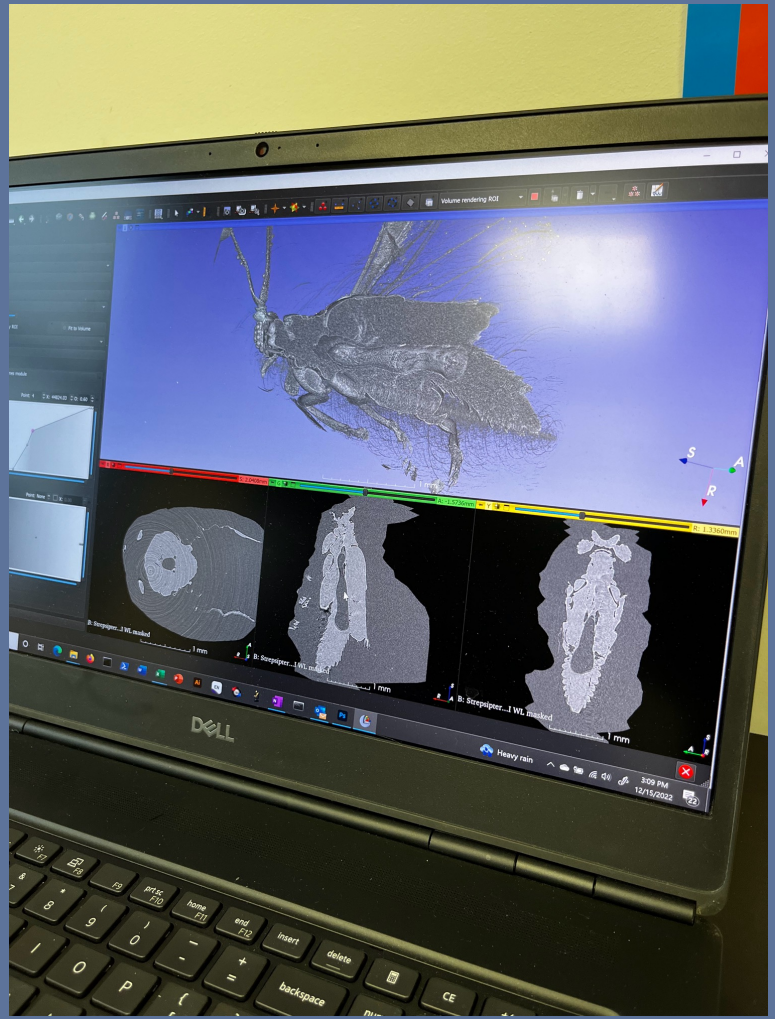
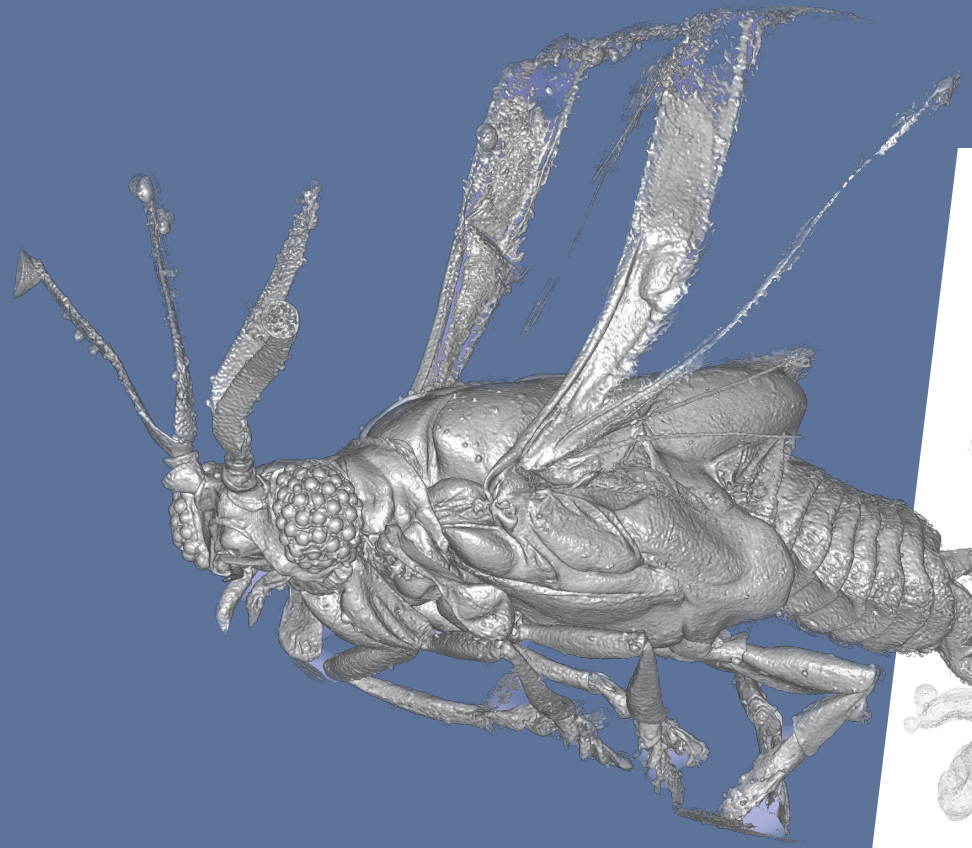
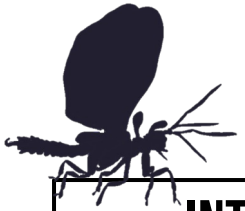

DELL
Heavy rain
3:09 PM
12/15/2022



METHODS

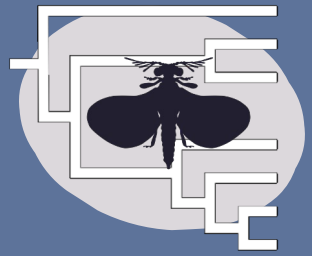


- AIMS
  - INTERNAL MORPHOLOGY
    - µCT — Renders of skeleto-musculature
  - CHARACTER MATRIX
    - KOETH ET AL. (2012); ME — List of internal characters
  - PHYLOGENIES
    - MORPHOLOGY — Parsimony (TNT), Bayesian (MrBayes), ML (RAxML)
    - COMBINED — Bayesian (MrBayes), ML (RAxML)
    - AS CHARACTERS — Traced onto Ch. 2 phylogeny; trait evolution

RECAP

1

- Genomic evolution
- Strepsiptera in the context of insect evolution



2

- Strepsipteran evolution as a whole
- Molecular data

3

- Strepsipteran evolution as a whole
- Morphological data

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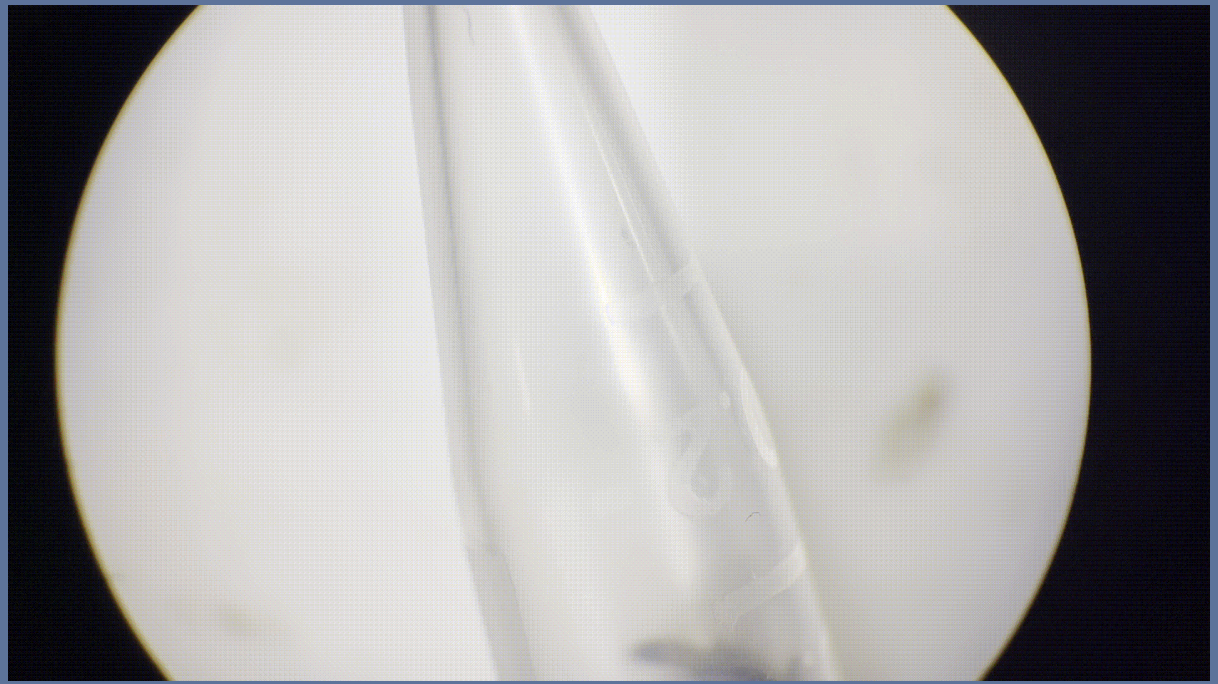

THANK YOU

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