

# WORKSHOP

## IDENTIFICATION OF *ORTHOZIA* MOTHS (LEPIDOPTERA)

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PBIO/IPS-161 BIOLOGICAL COLLECTIONS 2020



# Program

9-12 Introduction

Practise 1: Identification based on external features

Practise 2: Preparation of specimens for KOH treatment

12-13 Lunch

13–15 Practise 2 (cont.): Identification based on reproductive organs

15–16 Practise 3: Identification based on DNA barcodes

(learning diaries)

# Workflow: Lepidopteran taxonomy

- Not so rare starting point: a heap of mixed Lepidoptera
- **How to group them to species?**
- Several ways
  - external features
  - reproductive organs (genitalia)
  - barcodes

# Workflow: Lepidopteran taxonomy

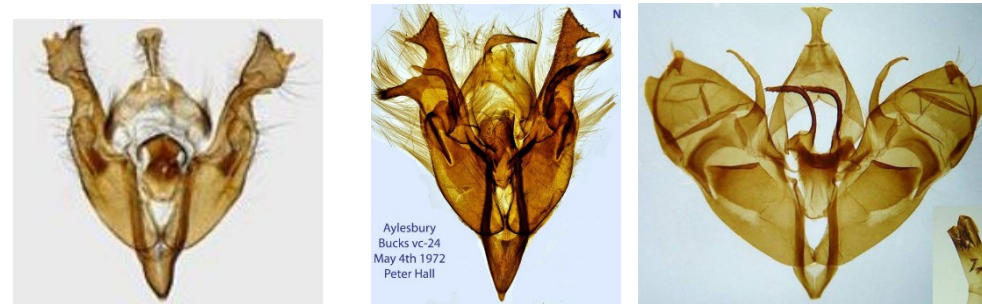
Today

1. external features



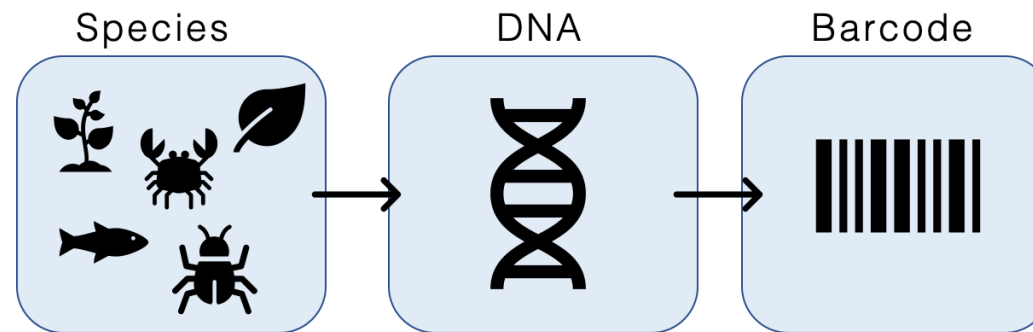
© Lepiforum.de

2. genitalia



© mothdissection.co.uk

3. barcode

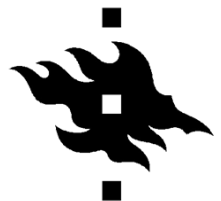


# Target group: noctuid genus *Orthosia* (owlet moths)

- Species of this genus hibernate in pupae, but the full-grown adult is already developed.
- Advantage: fast start, before most migratory birds are hunting them.
- 8 species in Finland, 5 of those are common. All of them can be identified using external characters or general appearance.



*Orthosia populeti*

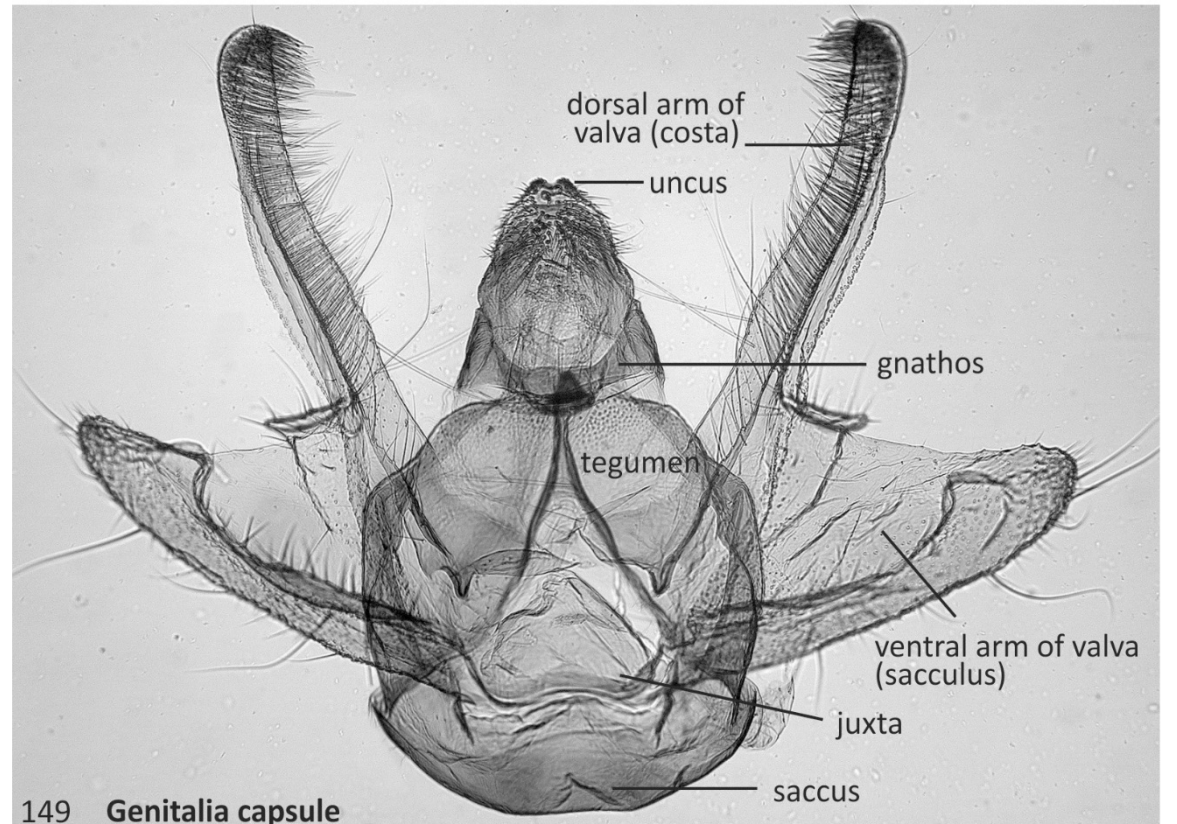
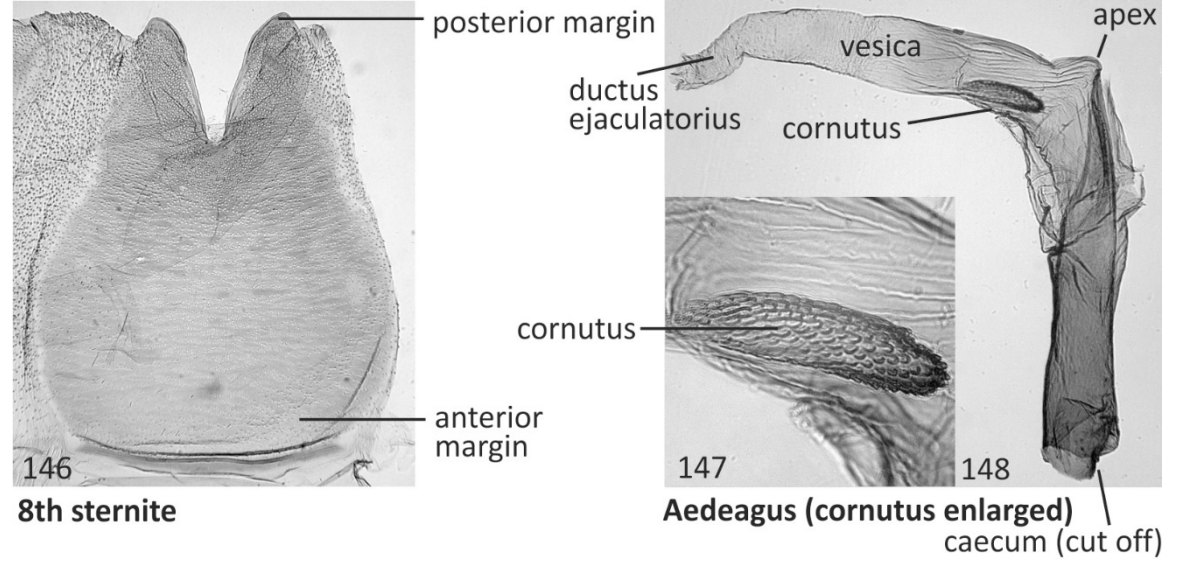


# Practise 1: External features

**REPRODUCTIVE ORGANS  
= GENITALIA**

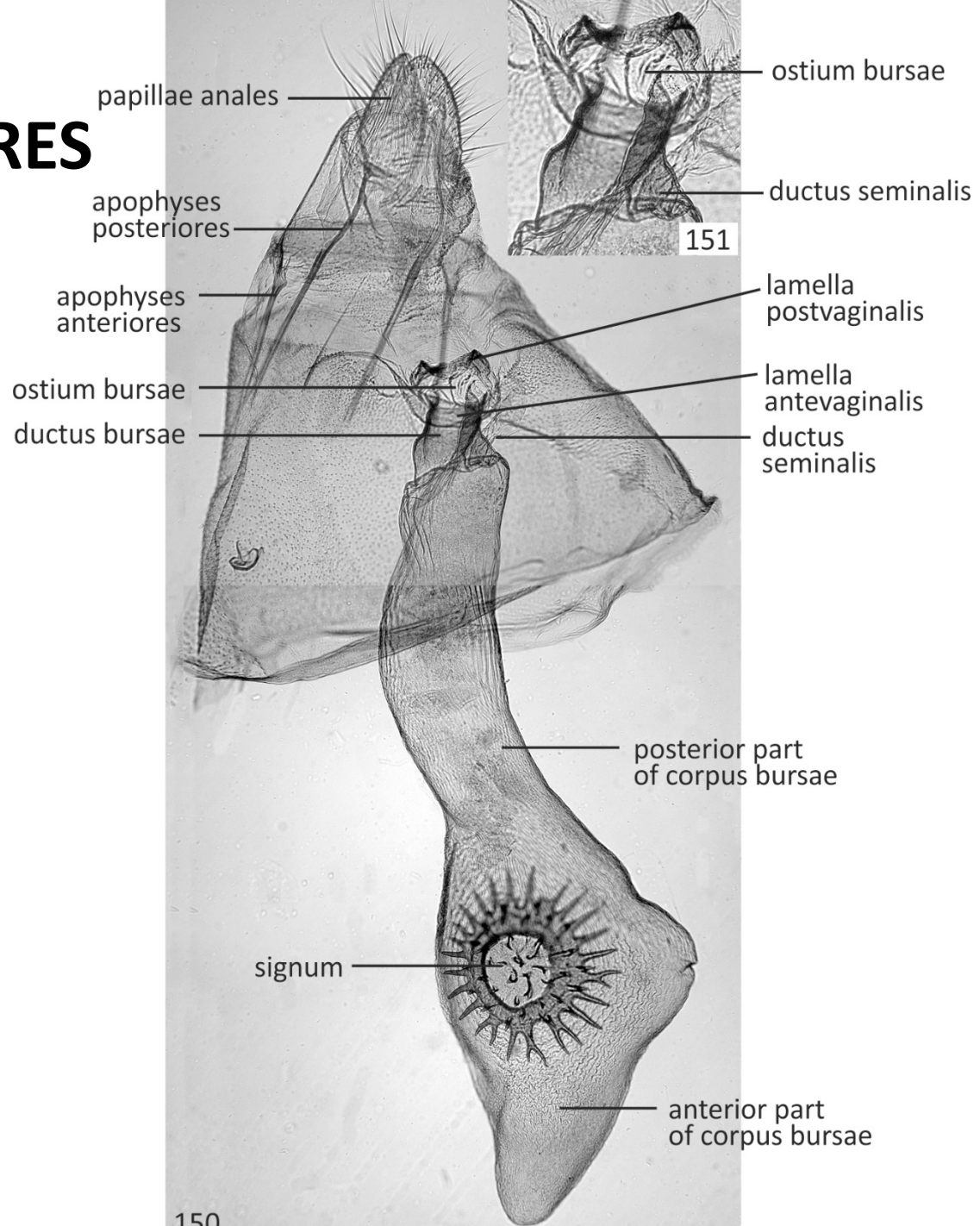


# MALE STRUCTURES

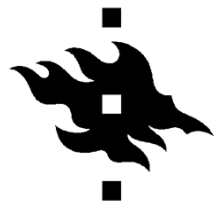
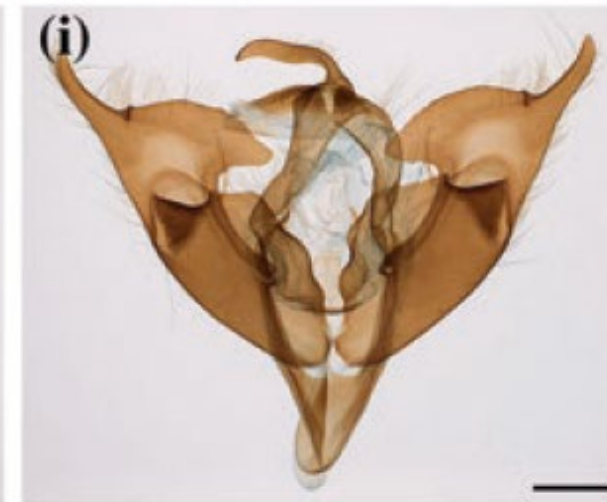
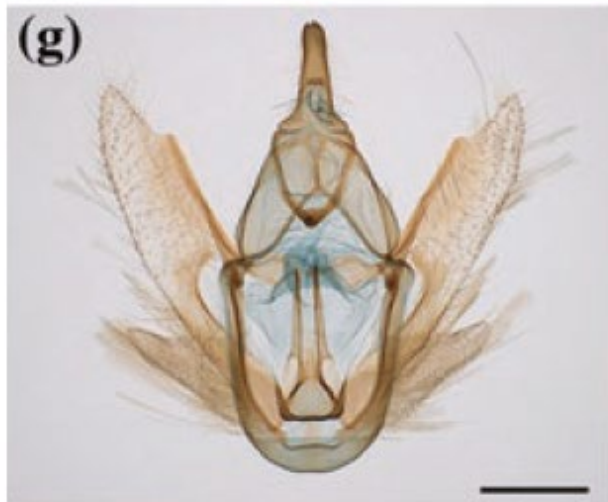
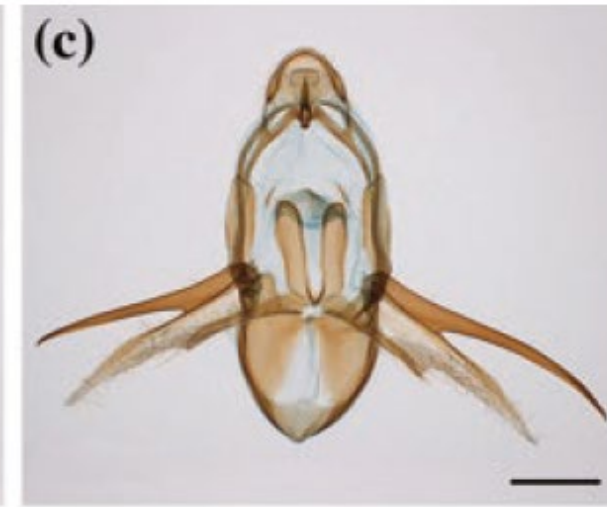
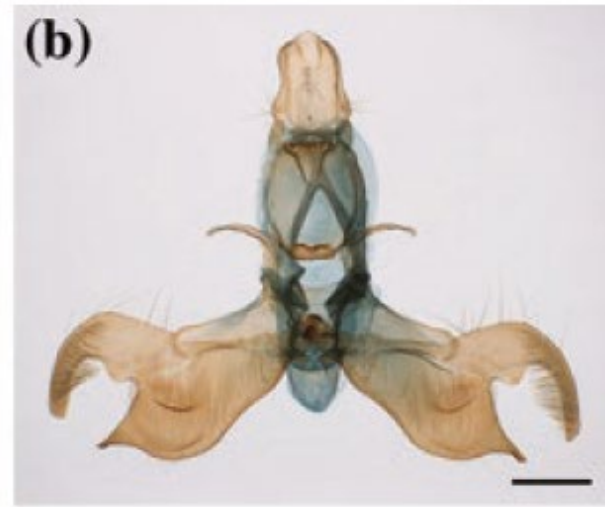
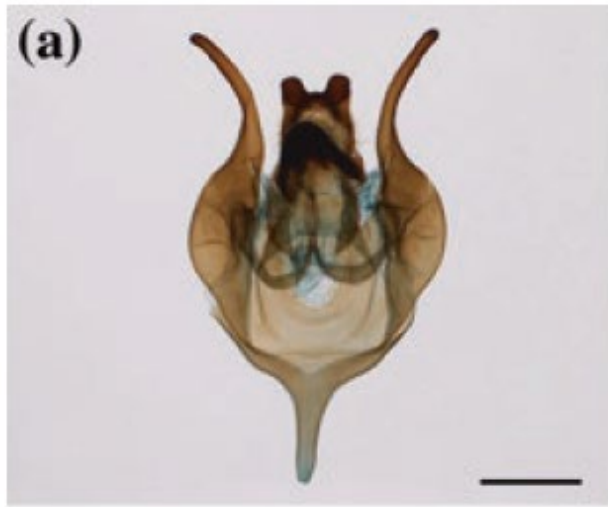




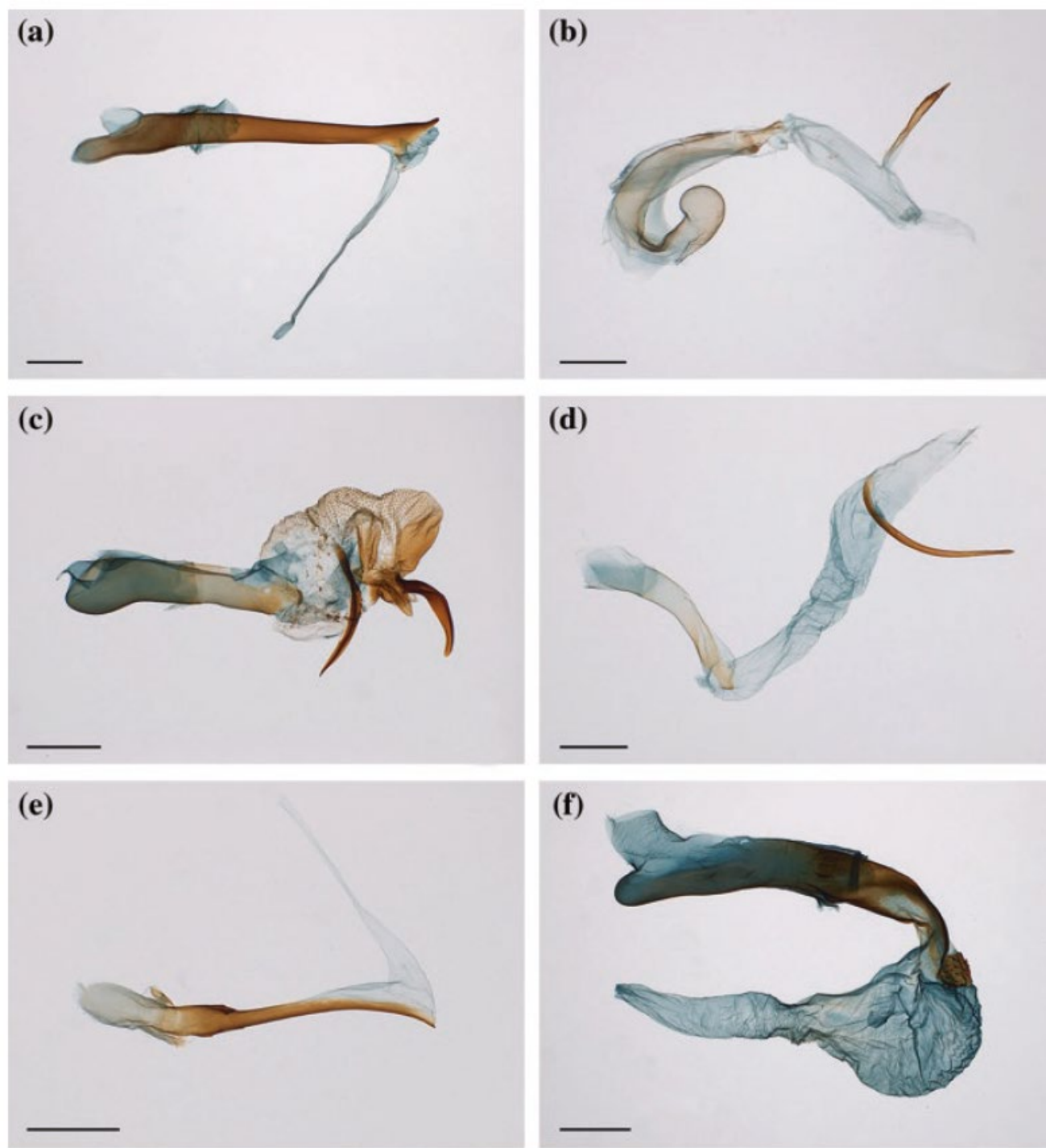
# FEMALE STRUCTURES



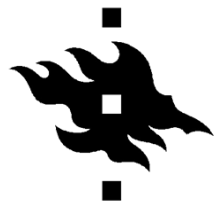
male structures



male structures



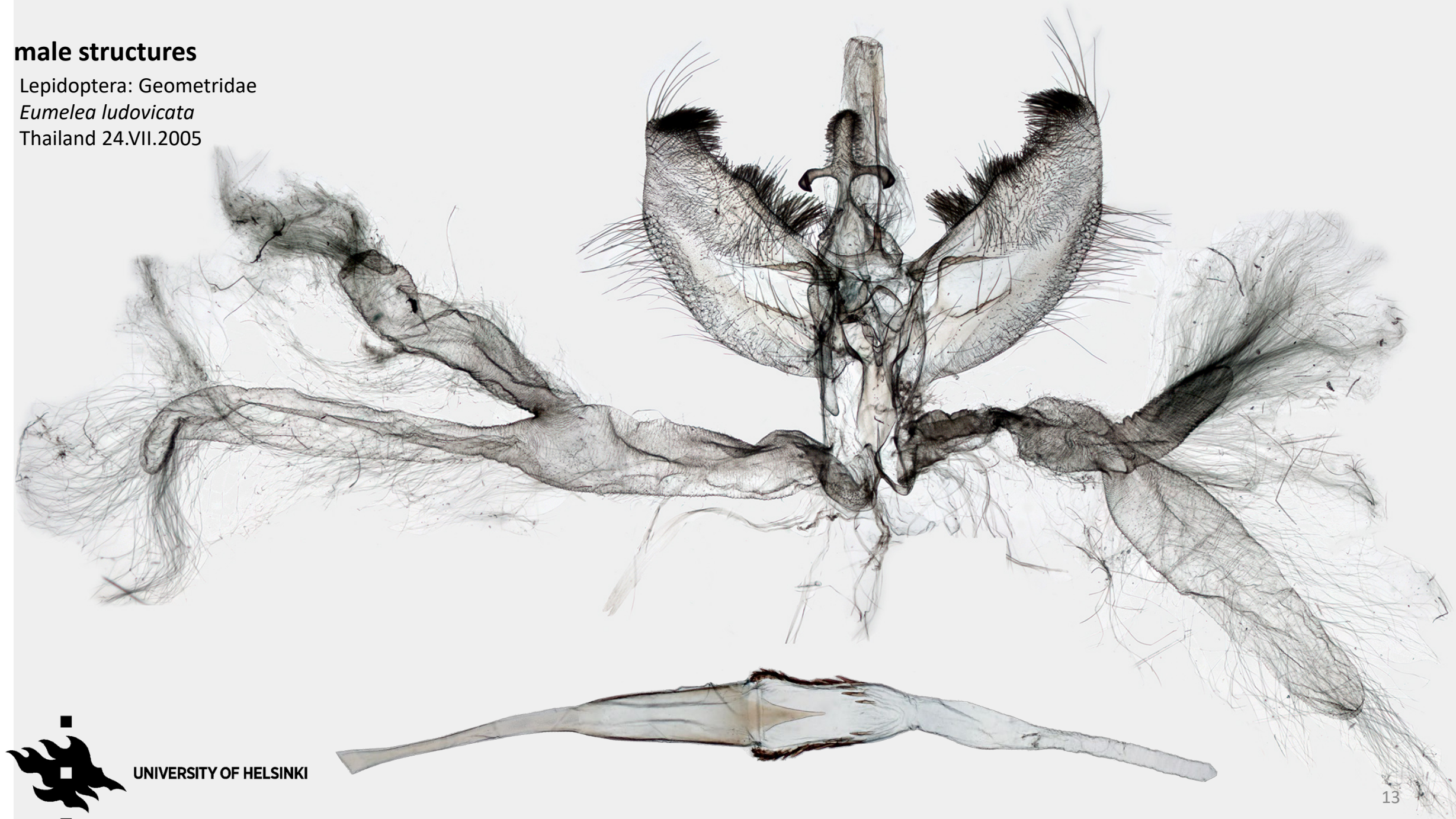
female structures





# male structures

Lepidoptera: Geometridae  
*Eumelea ludovicata*  
Thailand 24.VII.2005



# Practise 2: Preparation of specimens for KOH treatment

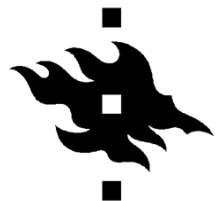
Abdomen removal

KOH treatment

Cleaning

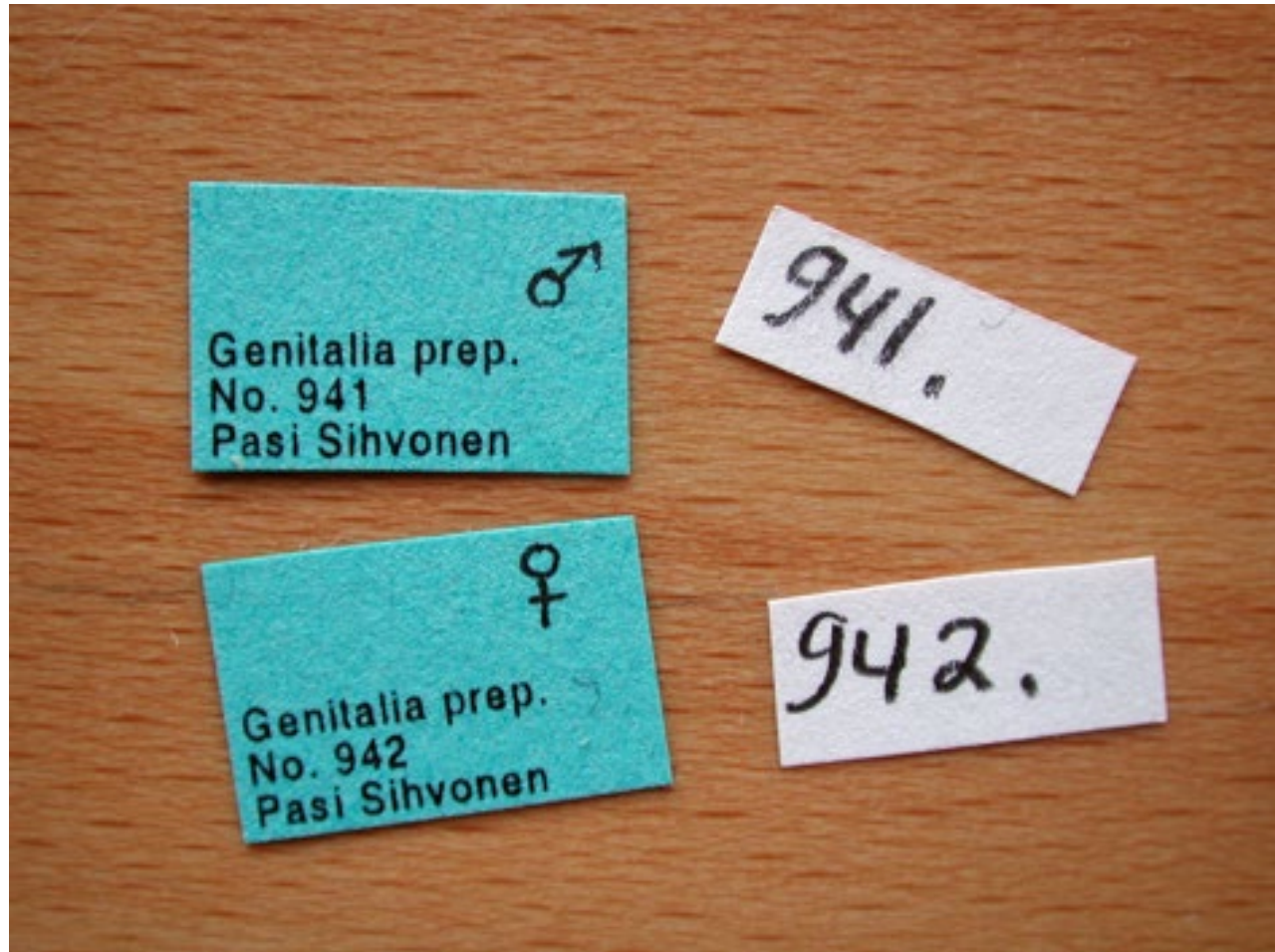
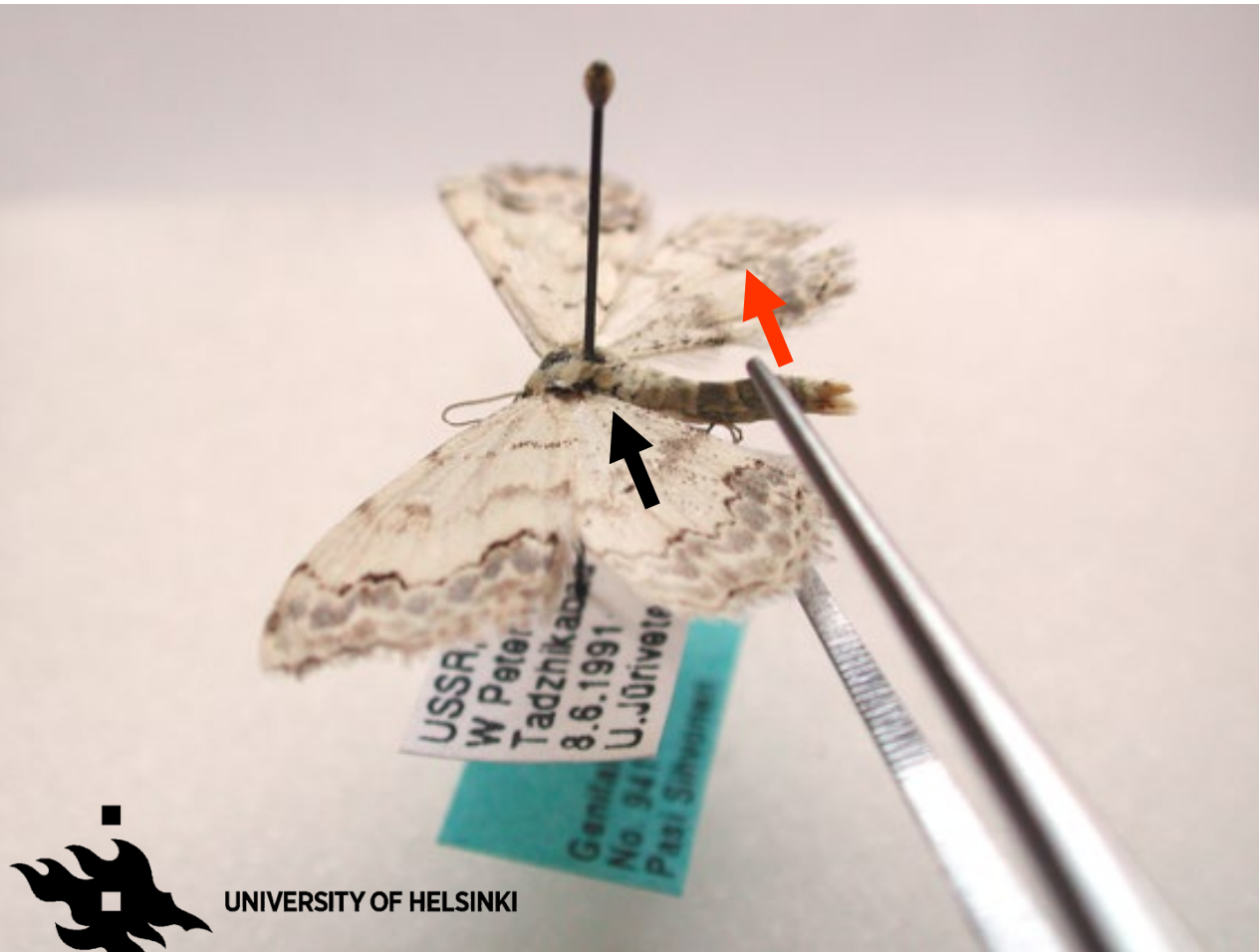
Identification

Storage





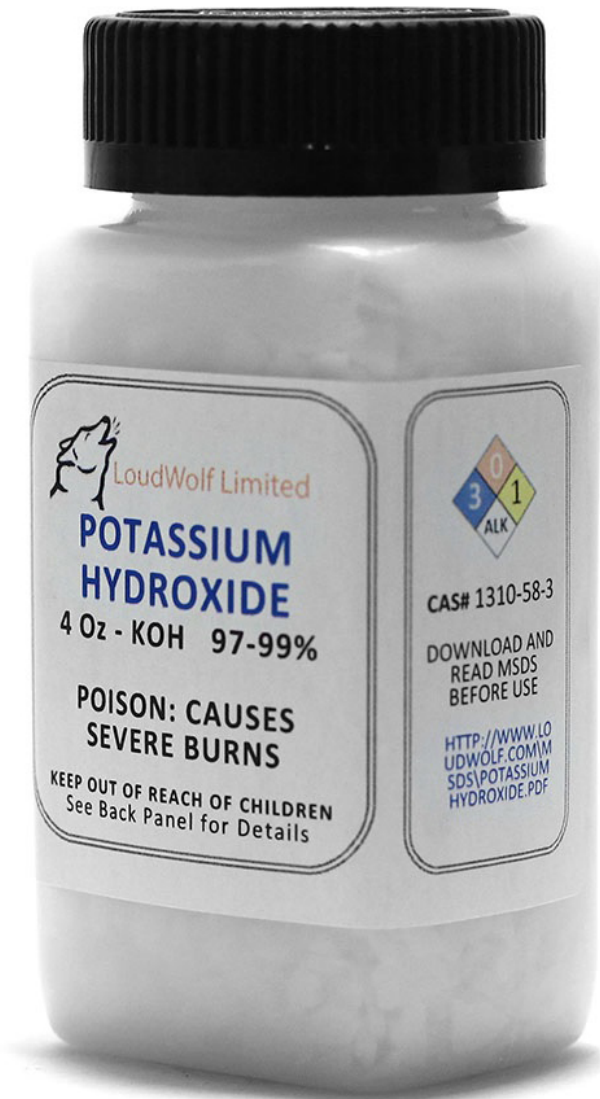
# Abdomen removal + labeling



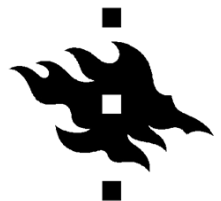


# Genitalia structures

- abdomen treated with 10% KOH or similar
- fat removed, structures become soft



# KOH treatment



# KOH treatment

- 5-10 mins in heat block or overnight in room temperature



Lunch break



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(learning diaries)

# Practise 2 (cont.): Identification based on reproductive organs

Abdomen removal

KOH treatment

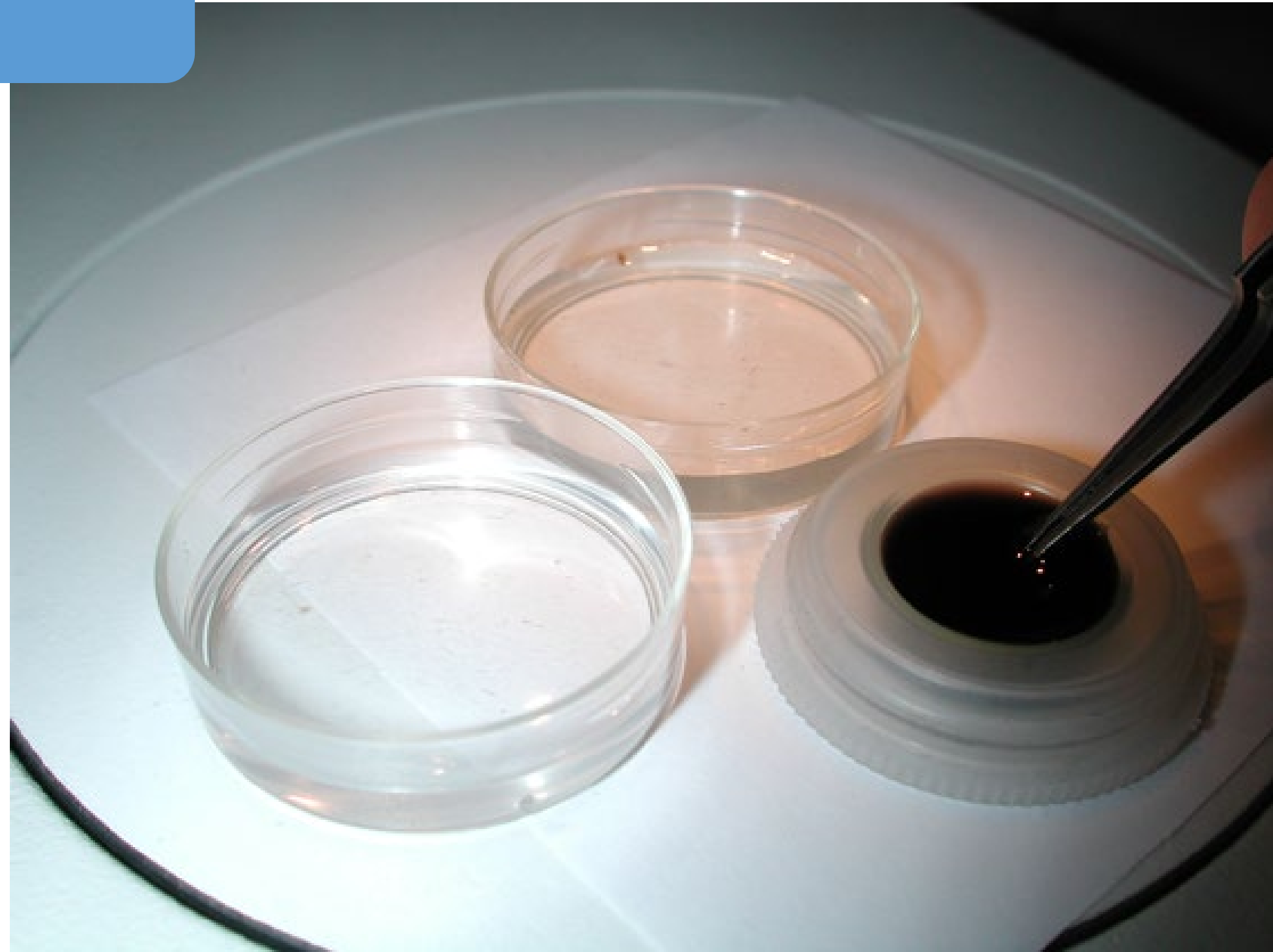
Cleaning

Identification

Storage



# Cleaning





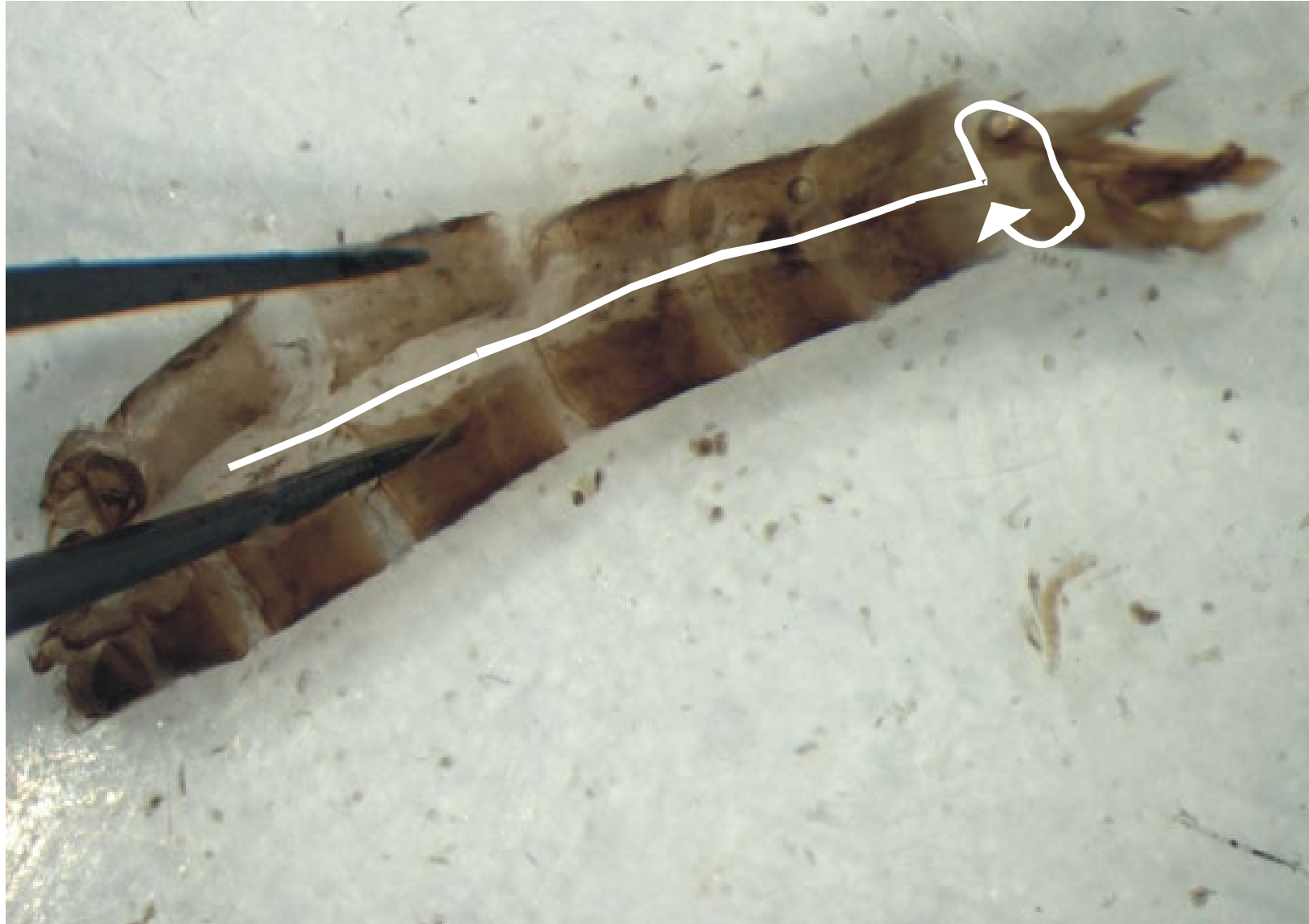
# Cleaning

- in water
- pull genitalia gently



# Cleaning

- in water
- remove genitalia



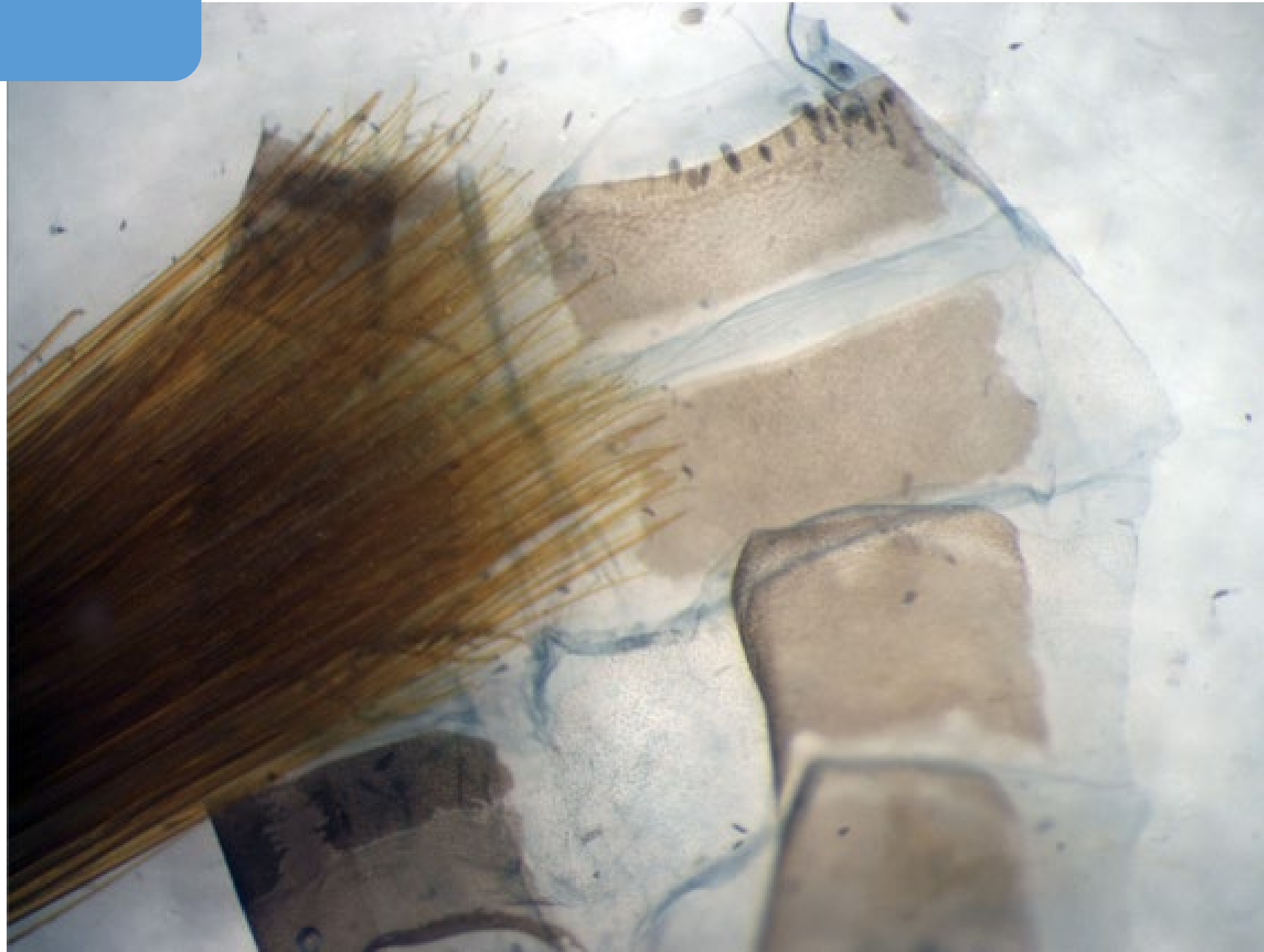
# Cleaning

- in water
- remove genitalia



# Cleaning

- in water
- clean with paper, brush, forceps



# Identification

- Time to work 😊
- Use the identification literature provided

## *Orthosia gothica*



Great Britain  
Hereford  
13.IV.2016  
Peter Hall

## *Orthosia incerta*

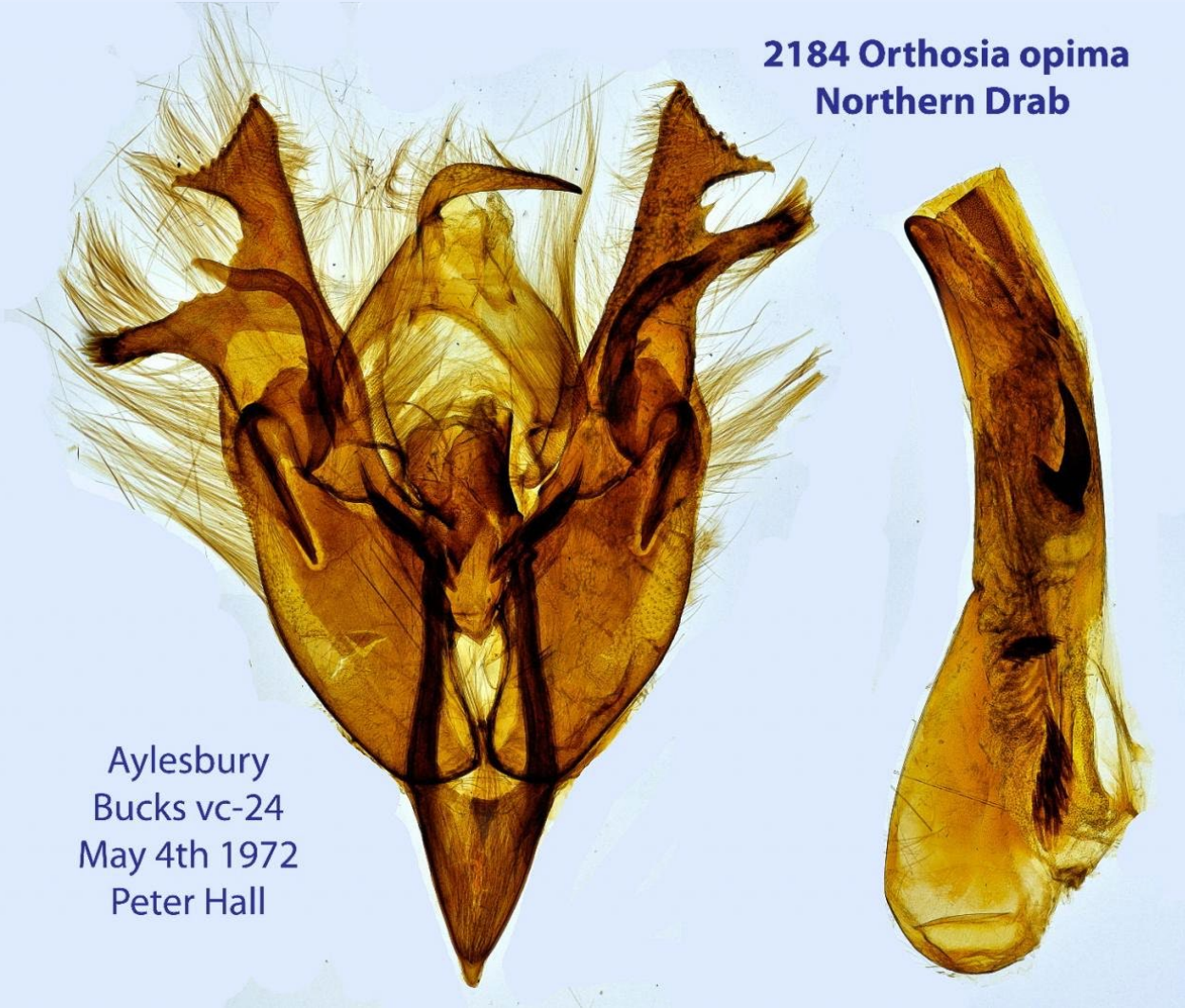


Clouded Drab  
*Orthosia incerta*  
Shaggs, Dorset  
iv.2007  
Det.: L.J. Hill  
N.B. Aedeagus not to scale.



# *Orthosia opima*

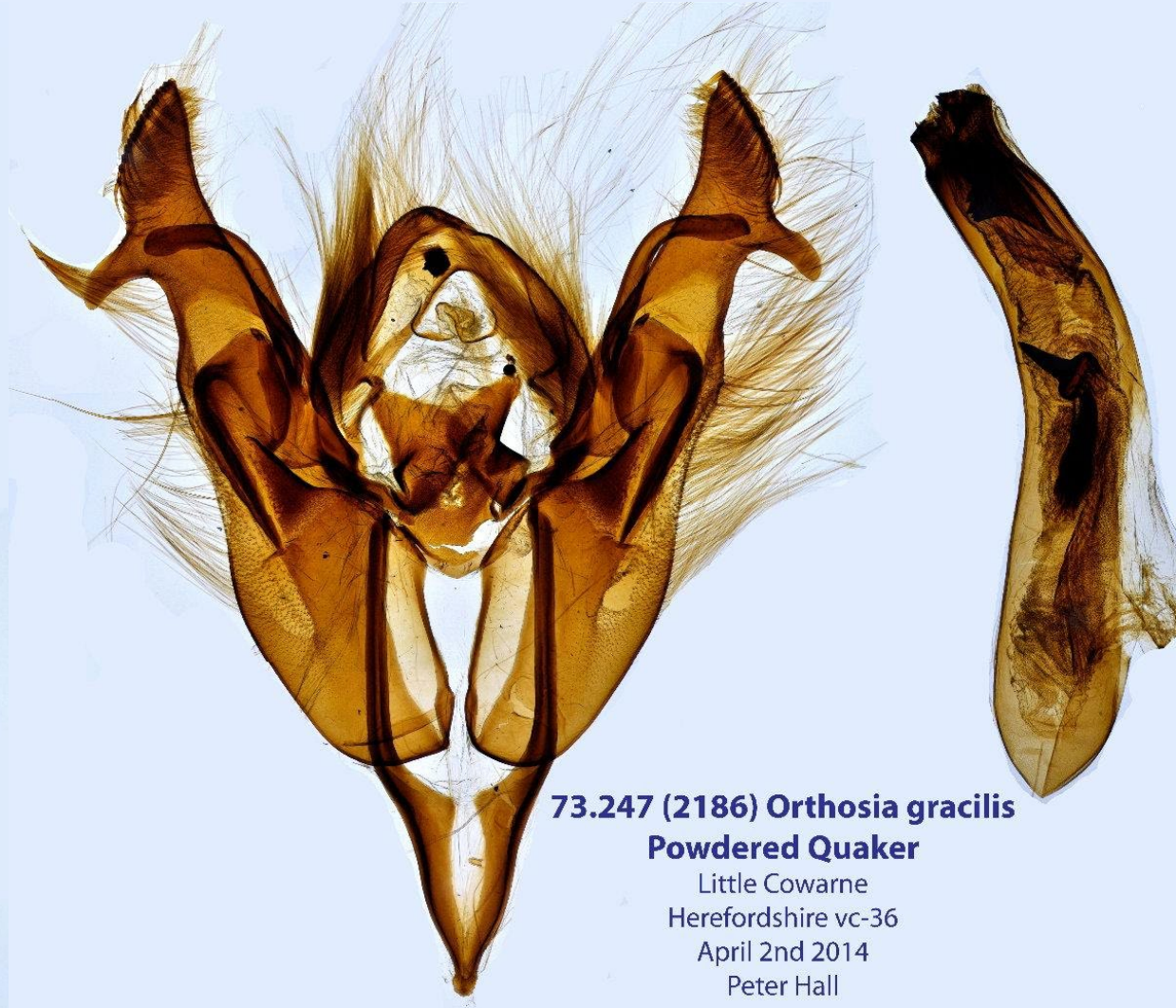
2184 *Orthosia opima*  
Northern Drab



Aylesbury  
Bucks vc-24  
May 4th 1972  
Peter Hall

# *Orthosia gracilis*

73.247 (2186) *Orthosia gracilis*  
Powdered Quaker

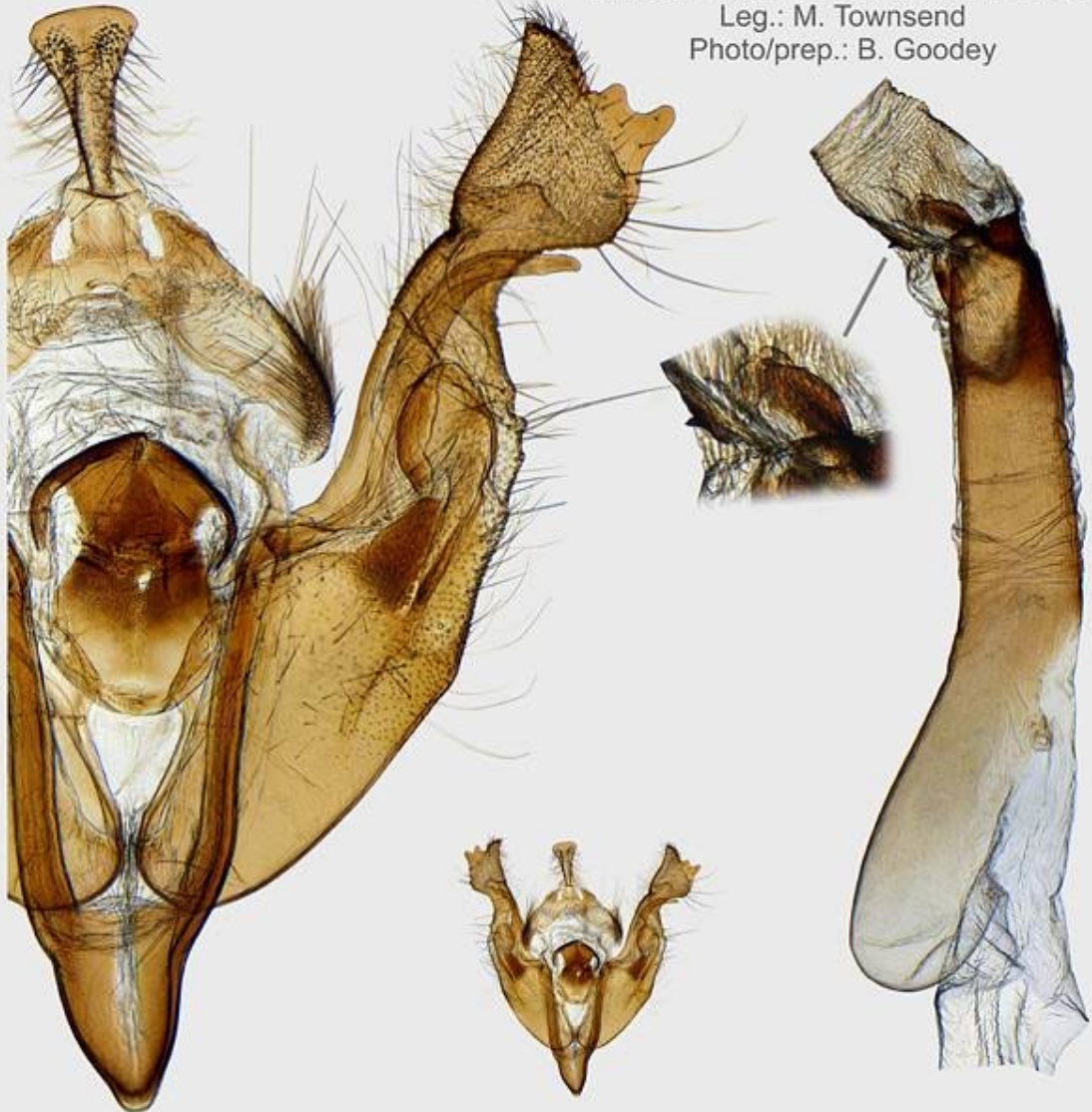


Little Cowarne  
Herefordshire vc-36  
April 2nd 2014  
Peter Hall



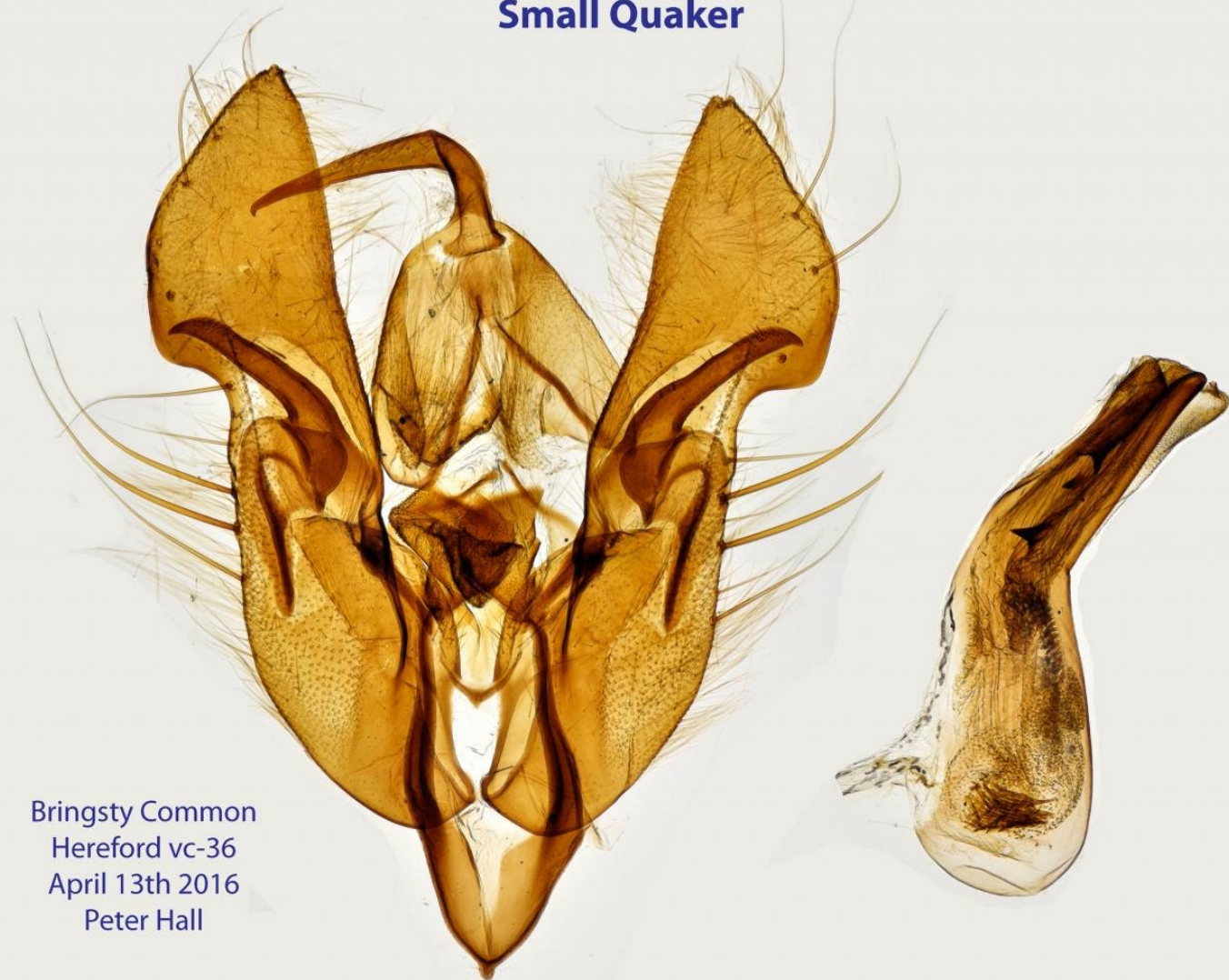
# *Orthosia populeti*

Yarnton, Oxon., ex female MVL iv.1996  
Leg.: M. Townsend  
Photo/prep.: B. Goodey



# *Orthosia cruda*

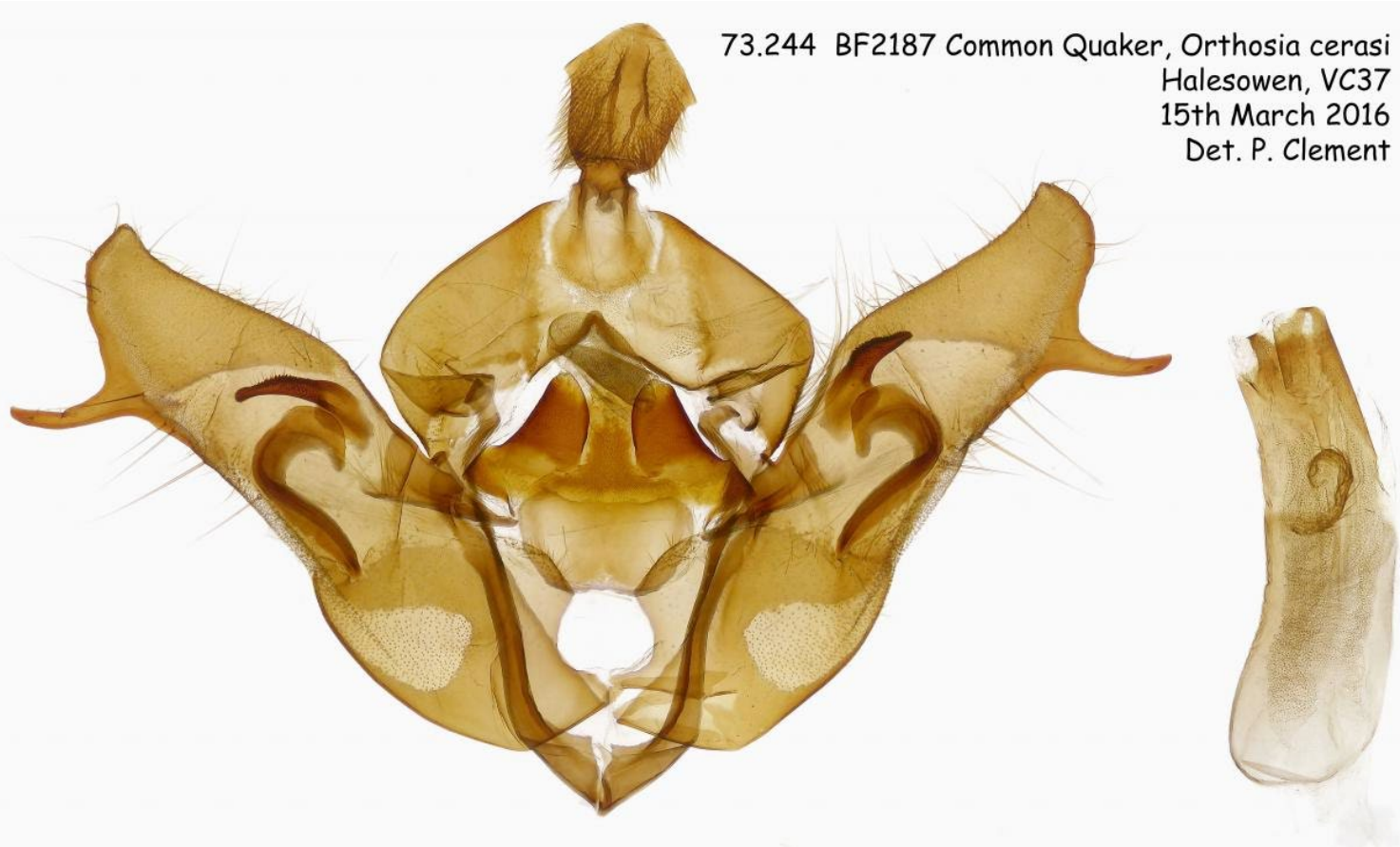
73.245 (2182) *Orthosia cruda*  
Small Quaker



Bringsty Common  
Hereford vc-36  
April 13th 2016  
Peter Hall



## *Orthosia cerasi*



## *Orthosia miniosa*



# RESULTS

- General discussion
- Variation in external appearance; what characters you used?
- Variation in reproductive organs; what characters you used?

# Correct answers

# Storage

plastic strip



cardboard

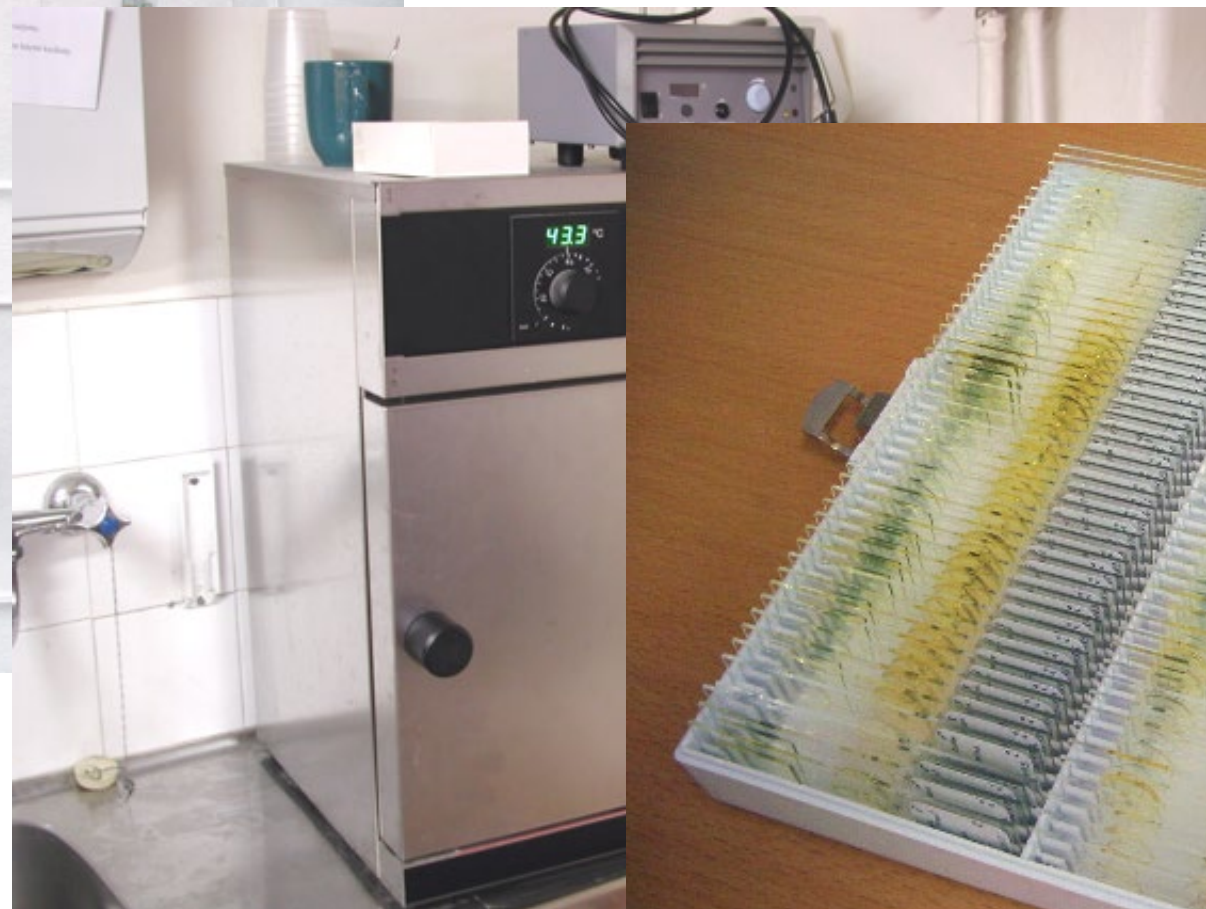


glyserol

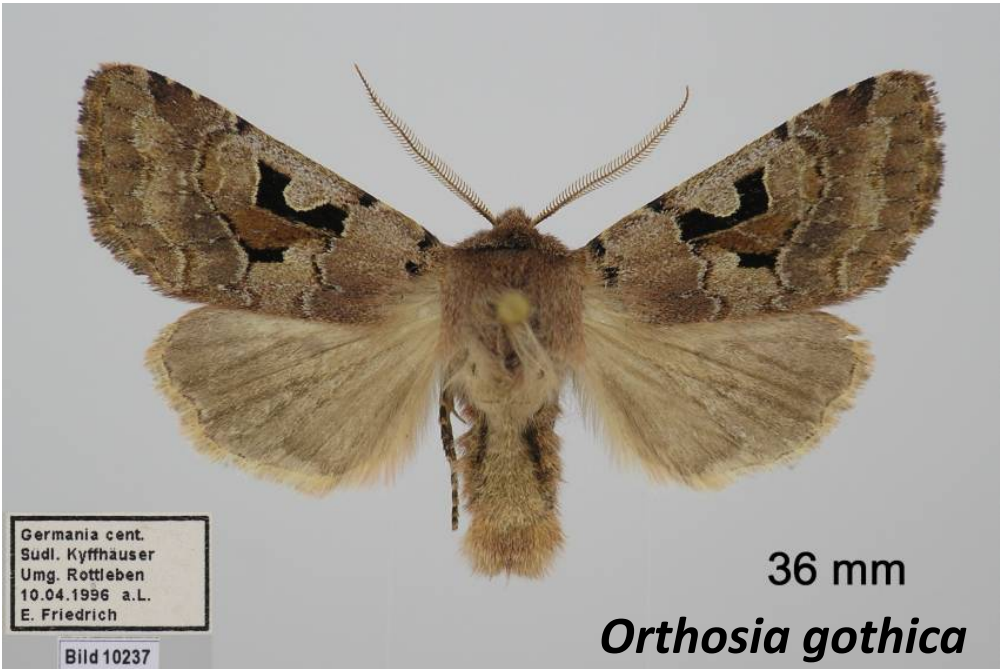




# Storage











*Orthosia opima*



*Orthosia incerta*





*Orthosia cruda*



*Orthosia cerasi*





*Orthosia miniosa*



*Orthosia gracilis*

# Practise 3: Identification based on DNA barcodes

# BARCODE OF LIFE DATA SYSTEM <sup>v4</sup>

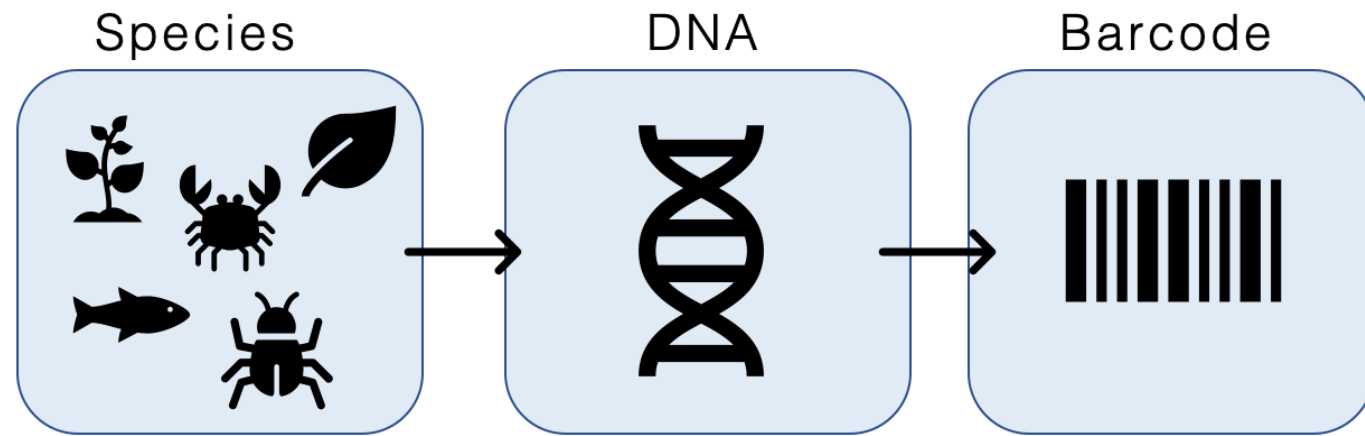
Advancing biodiversity science through DNA-based species identification.

[EXPLORE THE DATA](#)

## DESIGNED TO SUPPORT THE GENERATION & APPLICATION OF DNA BARCODE DATA

BOLD is a cloud-based data storage and analysis platform developed at the Centre for Biodiversity Genomics in Canada. It consists of four main modules, a data portal, an educational portal, a registry of BINs (putative species), and a data collection and analysis workbench.

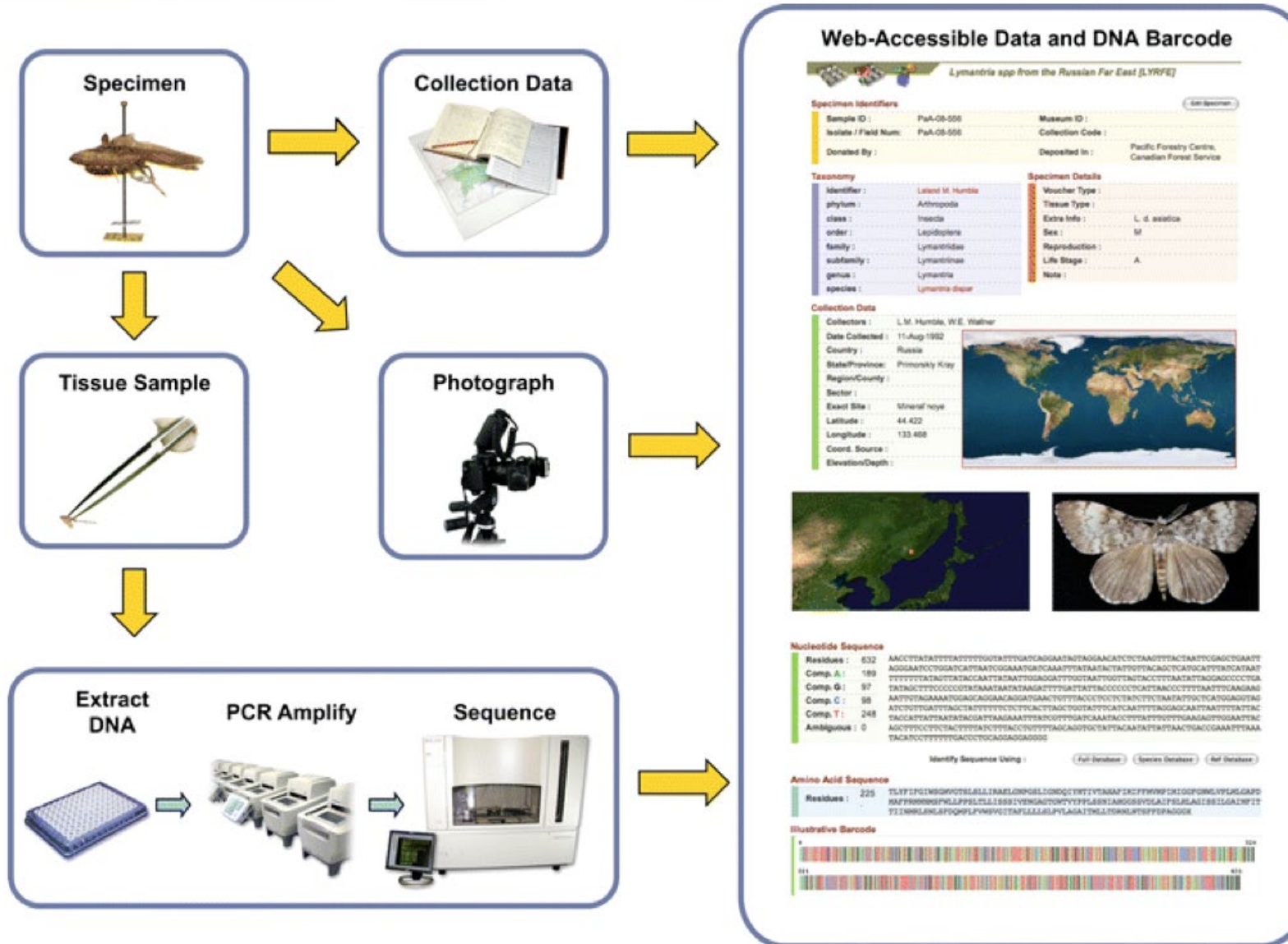
# DNA barcode



- DNA-based species identification
- Uses a short section of DNA from a specific gene or genes (in animals part of mitochondrial COI gene)
- Needs a reference library
- Useful in species identification, taxonomy, conservation biology, food industry, illegal trading, matching together different stages of life cycle, food webs
- Barcode data handled on <https://v4.boldsystems.org/>
- Metabarcoding data handled on <http://www.mbrave.net/>



# Workflow for DNA barcoding



# BARCODE EXERCISE 1

Everyone will get a barcode region of COI gene

1. Identify the gene > <https://v4.boldsystems.org/index.php> > identification
  1. Examine results page, including tree and BIN
  2. Report identification (order, family, species)
  3. What is the nearest neighbour? What is the maximum genetic distance?

# BARCODE EXERCISE 2 [note: skipped, not to be included in learning diaries]

Log in

- **username: to be provided**
- **password: to be provided**

2. Make genetic analysis > record search

1. "Vanessa atalanta" > Select BIN page of one sample. Report "Distance to nearest neighbour" and "Nearest member taxonomy" (=Genus and species).
2. "Vanessa" > Select all > Make taxon ID tree > How many BINs?

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(learning diaries)