WORKSHOP IDENTIFICATION OF ORTHOSIA MOTHS (LEPIDOPTERA)

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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



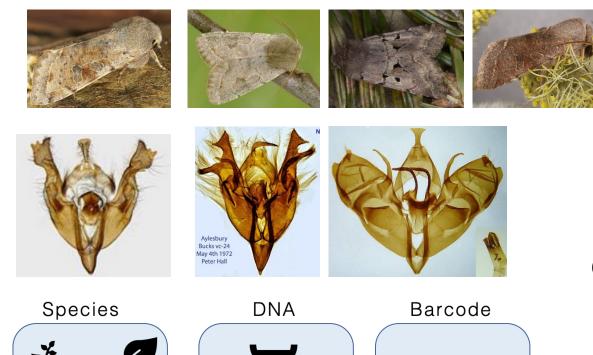
Workflow: Lepidopteran taxonomy

Today

1. external features

2. genitalia

3. barcode



© Lepiforum.de

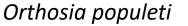
© mothdissection.co.uk



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.







Practise 1: External features

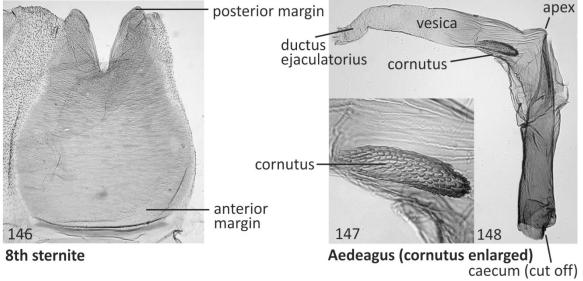


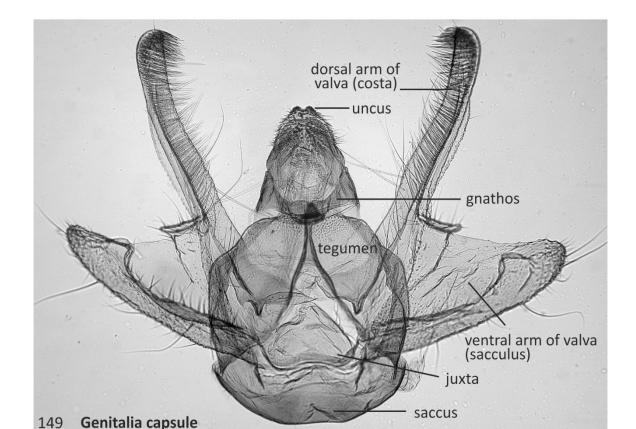
REPRODUCTIVE ORGANS = GENITALIA

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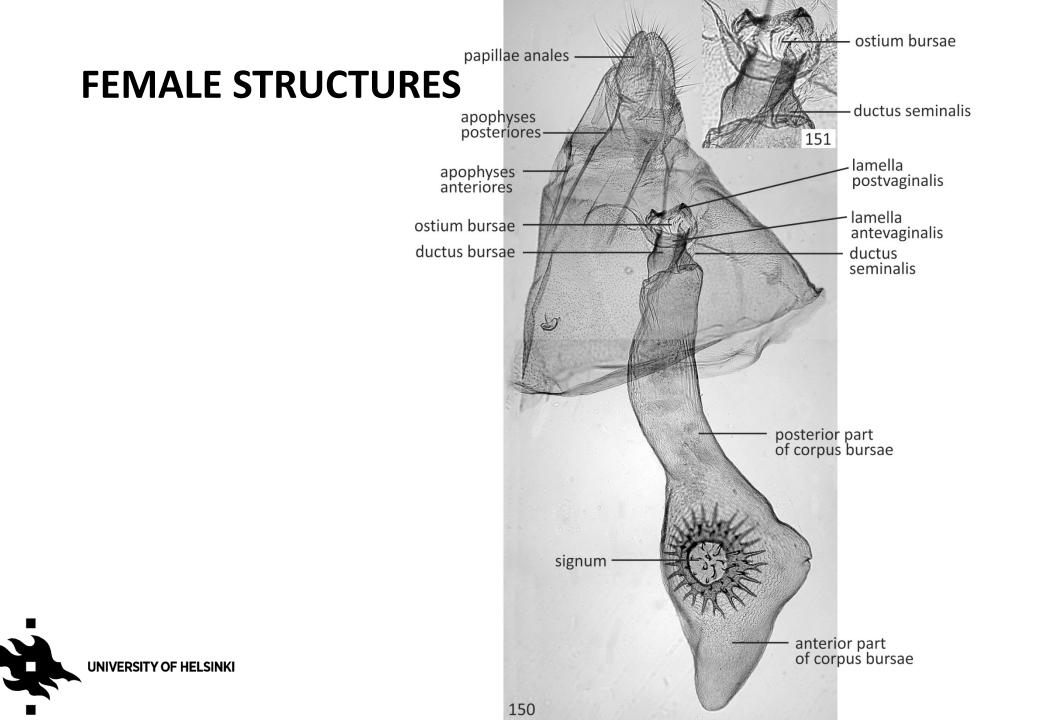
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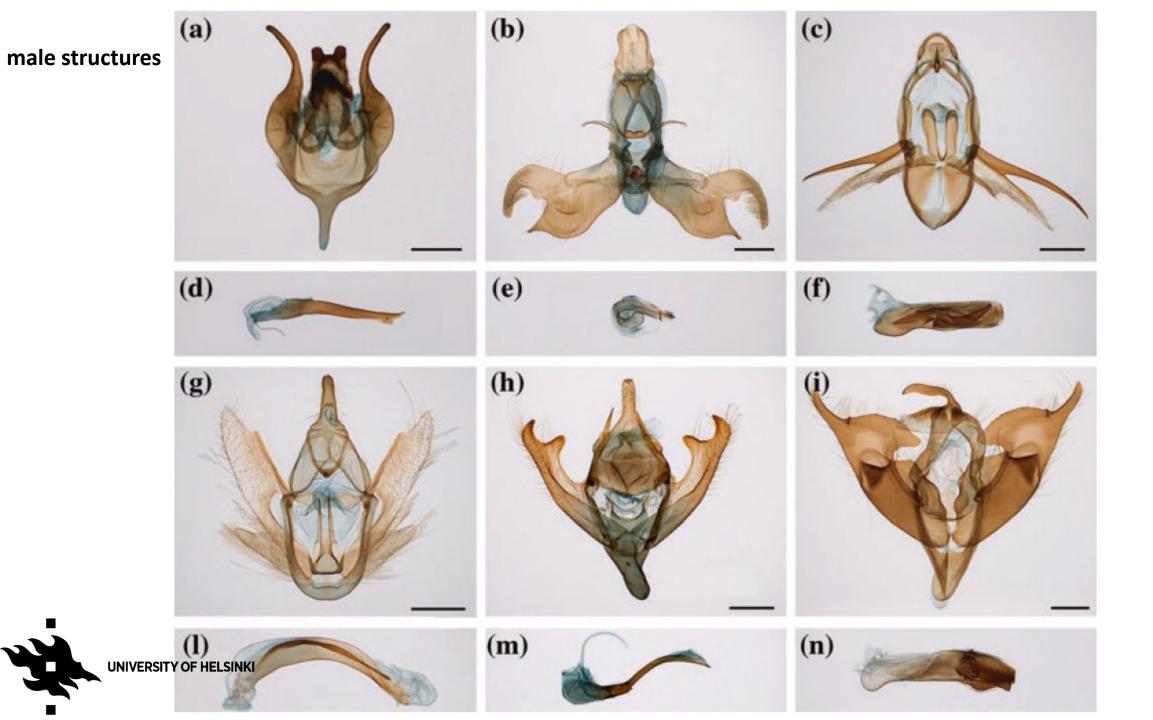
MALE STRUCTURES



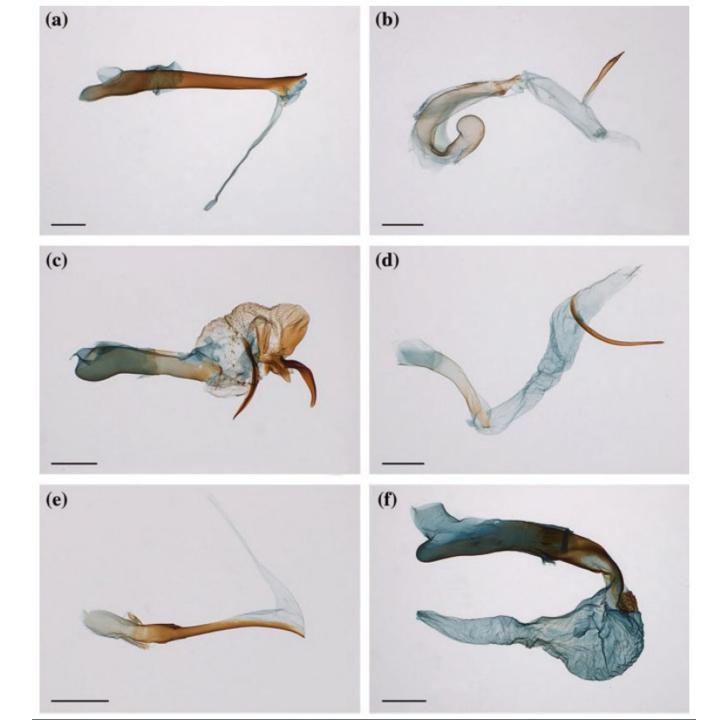




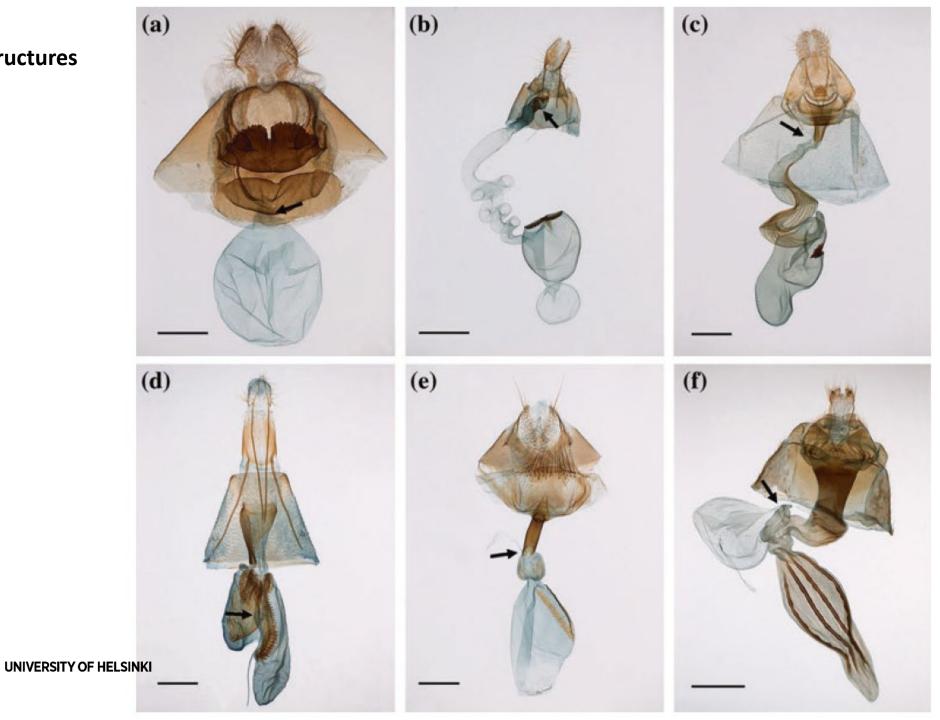


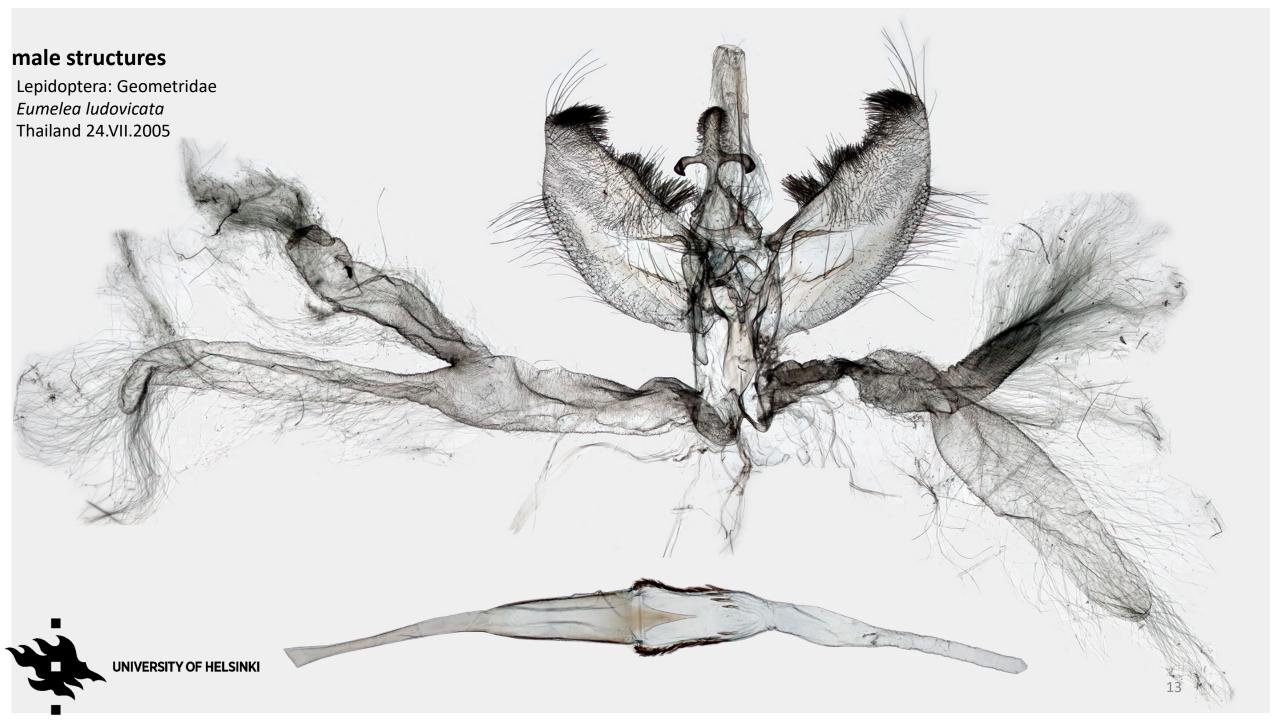


male structures



female structures





Practise 2: Preparation of specimens for KOH treatment



Abdomen removal

KOH treatment

Cleaning

Identification

Storage

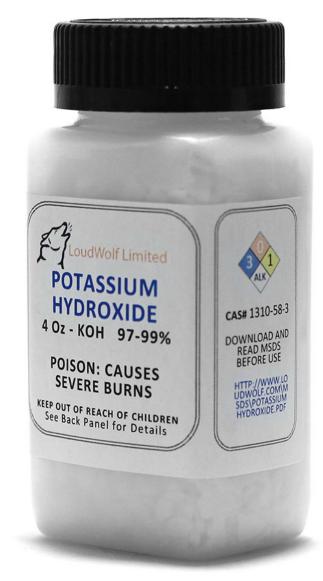


Abdomen removal + labeling



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

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Lunch break

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



Abdomen removal

KOH treatment

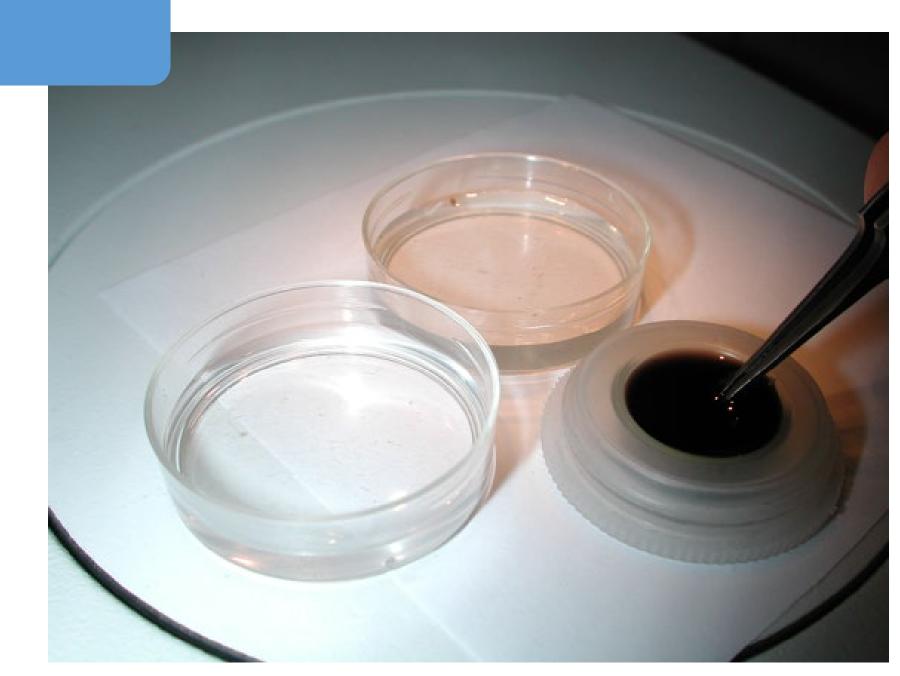
Cleaning

Identification

Storage







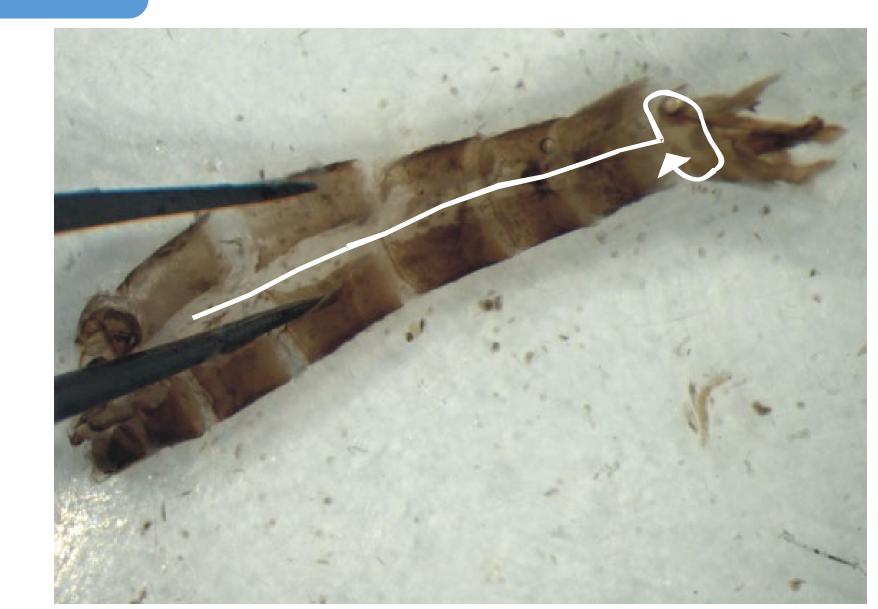


- in water
- pull genitalia gently

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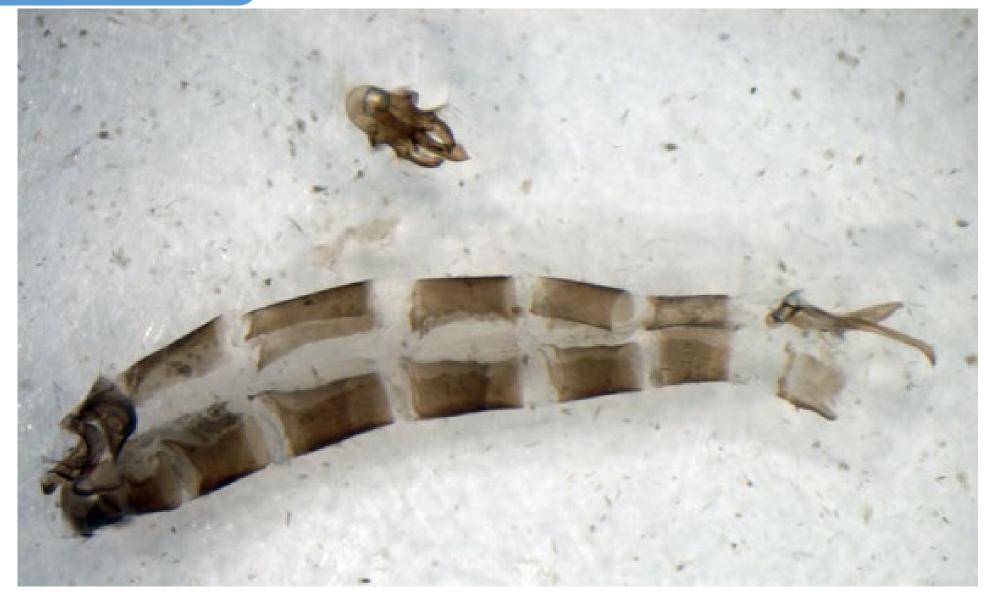


- in water
- remove genitalia





- in water
- remove genitalia





- in water
- clean with paper, brush, forceps





Identification

- Time to work 🙂
- Use the identification literature provided



Orthosia gothica



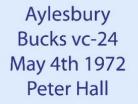
Orthosia incerta



source: https://mothdissection.co.uk ³⁰

Orthosia opima

Orthosia gracilis

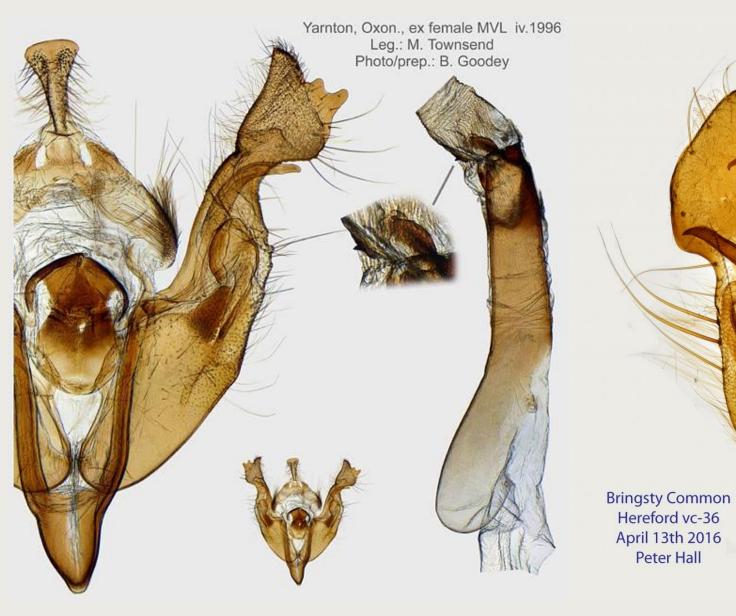


2184 Orthosia opima Northern Drab

73.247 (2186) Orthosia gracilis Powdered Quaker Little Cowarne Herefordshire vc-36 April 2nd 2014 Peter Hall

source: https://mothdissection.co.uk ³¹

Orthosia populeti



Orthosia cruda

Hereford vc-36 April 13th 2016 Peter Hall

73.245 (2182) Orthosia cruda **Small Quaker**

source: https://mothdissection.co.uk 32

Orthosia cerasi

Orthosia miniosa



RESULTS

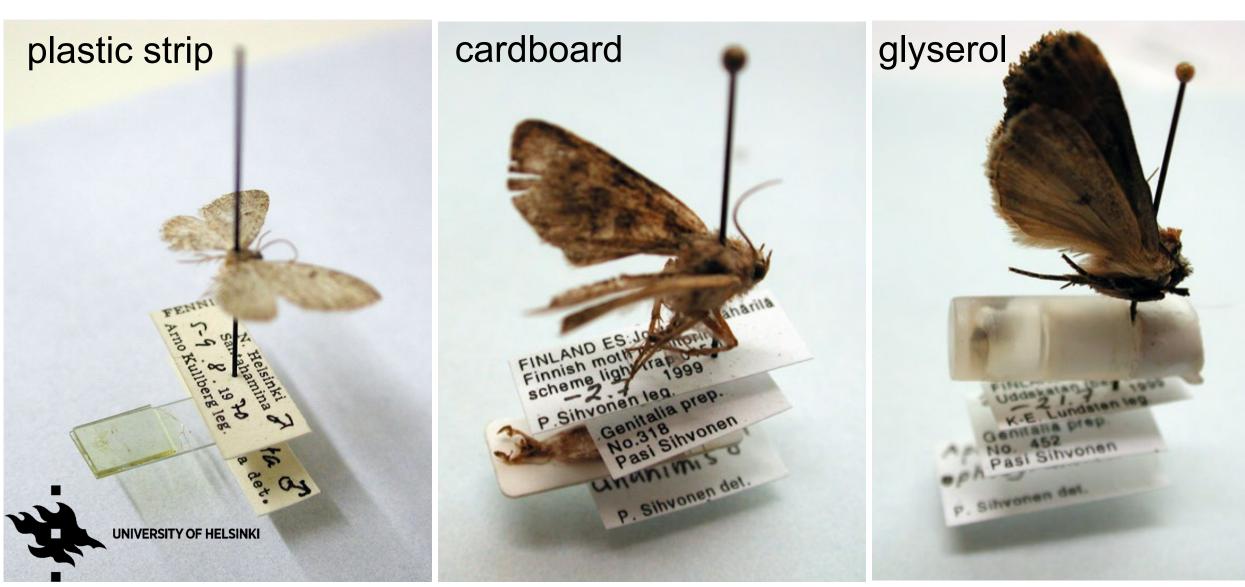
- General discussion
- Variation in external appearance; what characters you used?
- Variation in reproductive organs; what characters you used?

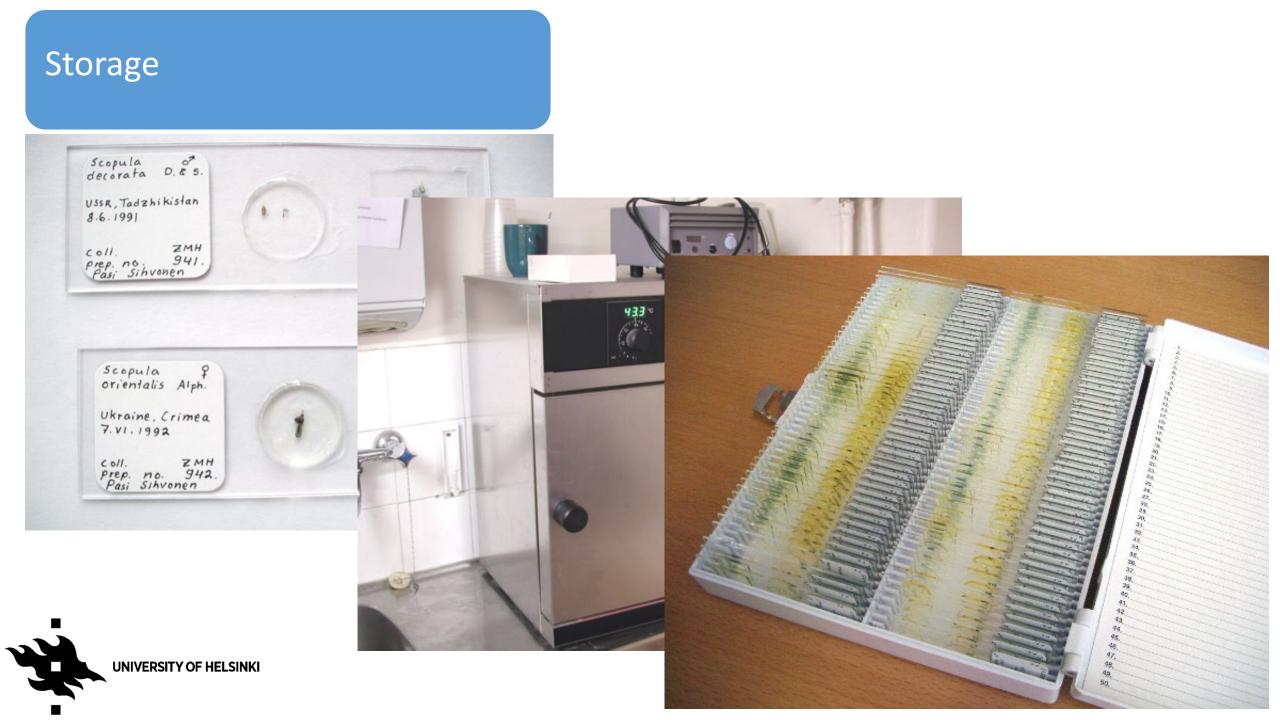


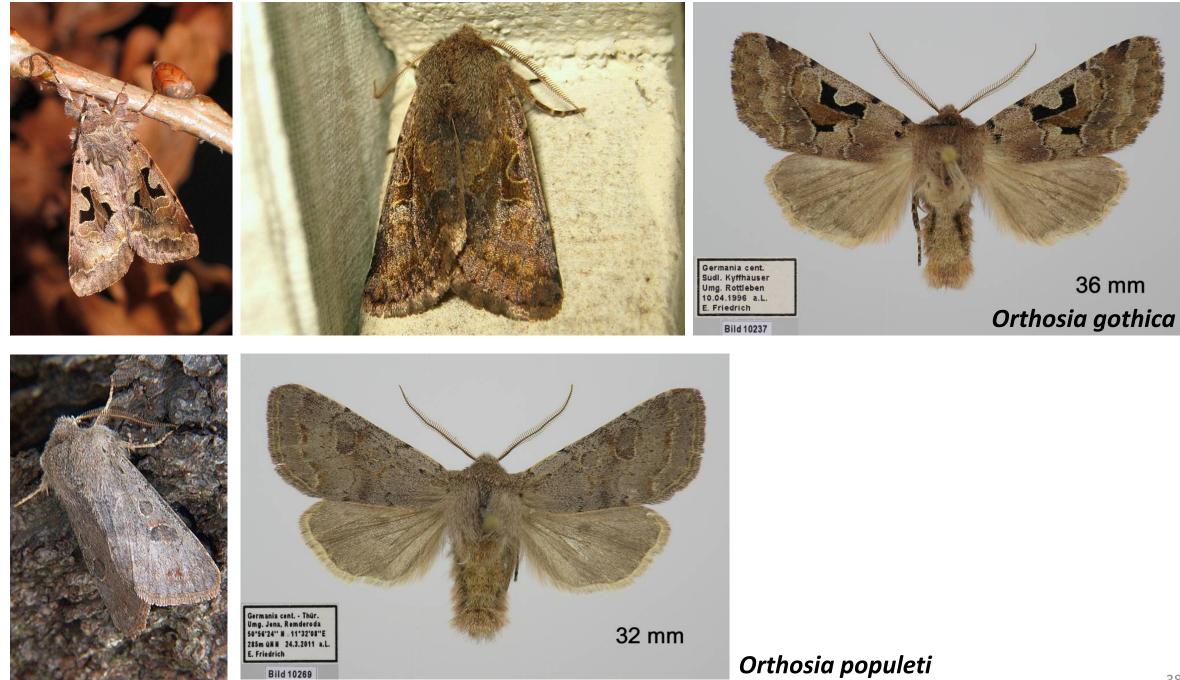
Correct answers











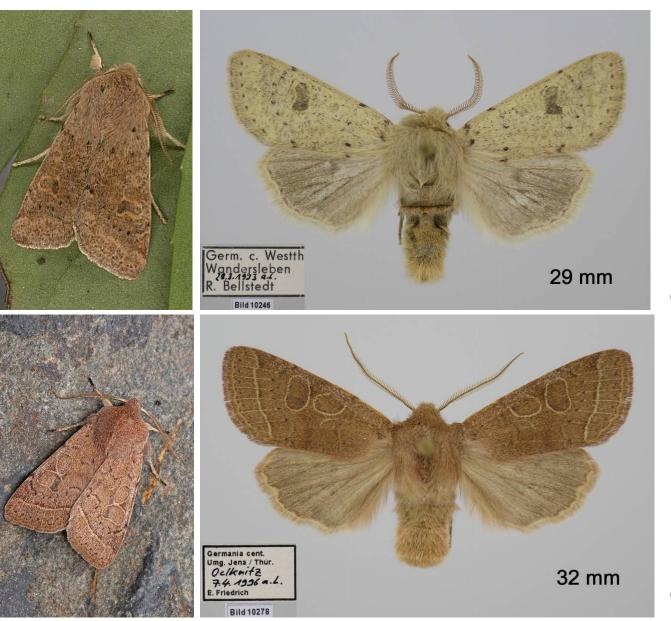




Orthosia opima



Orthosia incerta



Orthosia cruda

Orthosia cerasi







Orthosia miniosa

Orthosia gracilis

Practise 3: Identification based on DNA barcodes





BARCODE OF LIFE DATA SYSTEM V4

Advancing biodiversity science through DNA-based species identification.

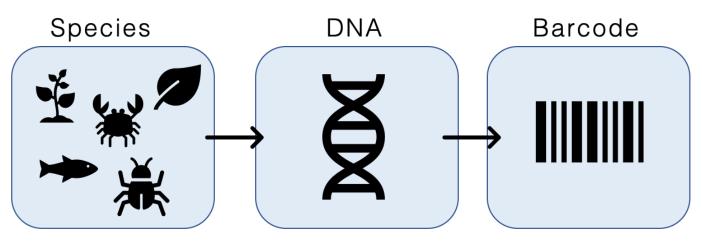
EXPLORE THE DATA

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.

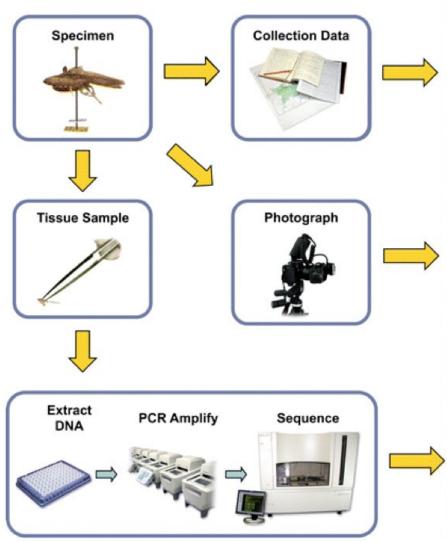
DNA barcode

• 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 <u>https://v4.boldsystems.org/</u>
- Metabarcode data handled on http://www.mbrave.net/

Workflow for DNA barcoding



		pp from the Russian Far	East [LYRFE]
Specimen Ider	tifiers		(imigeone.)
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Institute / Fiel	id Num: PaA-08-558	Collection Code :	
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phylum (Arthropoda	Tissue Type :	
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order :	Lapidoptena	Sex :	M.
family: subfamily:	Lymantridae Lymantrinae	Reproduction : Life Stage :	A
genus :	Lymanthia	Note :	·····
species :	Lynamia datar		
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BARCODE EXERCISE 1

Everyone will get a barcode region of COI gene

- 1. Identify the gene > <u>https://v4.boldsystems.org/index.php</u> > 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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