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UNIVERSITY OF HELSINKI

Use of collections in taxonomic research with a focus on genetic data

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25.9.2020

Biological Collections Course (IPS-161) 5cr

1. Starting session (Jaakko Hyvönen)
2. Storage and curation of collections (Marko Hyvärinen & Aino Juslén)
3. Botanical collections and their proper use (Mikko Piirainen)
4. Biological nomenclature ICNafp (Alexander Sennikov)
5. Biological nomenclature ICZN (Jyrki Muona)
6. International conventions and agreements affecting biological collections (Pedro Cardoso)
7. Use of collections in taxonomic research (Maria Heikkilä & Annina Kantelinen)
8. Documentation, databases, digitalization, open data and biodiversity informatics (Jere Kahanpää & Kari Lahti)
9. Other fields of biological research using collections (Leif Schulman & Aleksi Lehikoinen)
10. Networks and collaboration among natural history museums and collections (Leif Schulman)

+ visits to collections & practicals

Objectives of the lecture

- To understand the importance of biological collections in taxonomic research
- To understand what type of data can be obtained from collection specimens and samples, with a focus on sequence and genome-level characters
- To learn how museums strive to preserve specimens and samples so that they could serve present and future taxonomic research

Lecture outline

- Exercise to be done in pairs/small groups
- Importance of biological collections for taxonomic research
- How to access museum specimens
- Data used in taxonomic studies
  - Morphological characters
  - Molecular characters
  - Storage of specimens and samples
- DNA barcoding and metabarcoding
- Genomics and metagenomics
- Ancient DNA
- Exercise in groups, wrapping up

Musical break

- Pdfs of lecture slides will be available in Moodle

- Supporting literature:

Watson, M. F. & al. (eds.) 2014. Descriptive taxonomy. The foundation of biodiversity research.

- Topics will be revisited in the practical on *Orthosia* moths (Group 1, Tue September 1st)

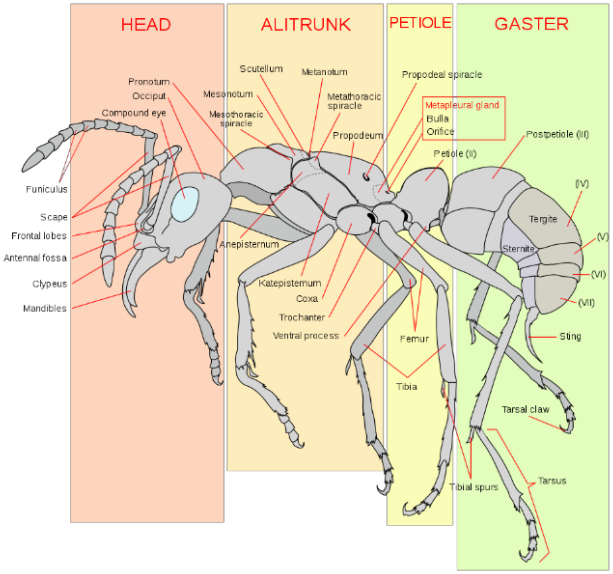
Taxonomic research

- The science of naming, defining (circumscribing) and classifying groups of biological organisms on the basis of shared characteristics
- Understanding biodiversity
- Order of evolution
- Many applications

Data used in taxonomic research

- Characters that tell of common ancestry

Morphology



Wikimedia Commons,
Mariana Ruiz, CC-BY-NC-4.0

Genes

Wikimedia Commons,
DataBase Center for Life Science,
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Also, e.g., physiology, secondary metabolites, behavior, ecology, distribution

harmaaluppo - *Bryoria capillaris*
Jouko Rikkinen, CC-BY-NC-4.0

Data used in taxonomic research

- Often taxonomic classification is based on multiple character types and analysis methods.

- Sometimes there are no clear differences in the morphology/DNA/ecology/other characters of a species and therefore it is good to look at different sources of information.

- Methods and characters used in species delimitation/systematics depend on the group of organisms in question.

ZooKeys 927: 75–97 (2020)
doi: 10.3897/zookeys.927.51142
https://zookeys.pensoft.net

RESEARCH ARTICLE

ZooKeys
Launched to accelerate biodiversity research

Revision of the genus *Hoplodrina* Boursin, 1937 (Lepidoptera, Noctuidae, Xyleninae). I. *Hoplodrina octogenaria* (Goeze, 1781) and its sister species *H. alsinides* (Costantini, 1922) sp. rev. in Europe

Peter Huemer[1], Jean Haxaire[2], Kyung Min Lee[3], Marko Mutanen[3], Oleg Pekarsky[4], Stefano Scalercio[5], László Ronkay[6]

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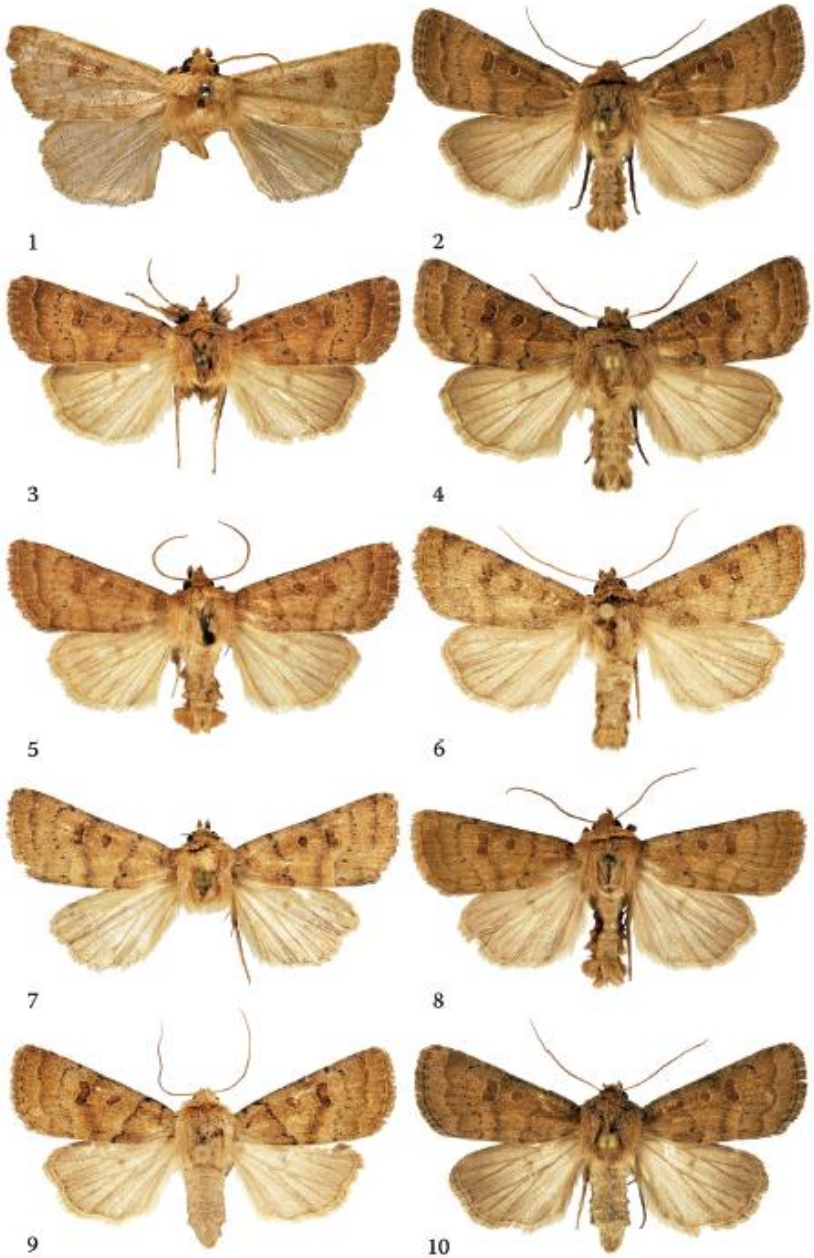
http://zoobank.org/4908DDE1-C3B5-499E-B003-DFB06A132EE6

Citation: Huemer P, Haxaire J, Lee KM, Mutanen M, Pekarsky O, Scalercio S, Ronkay L (2020) Revision of the genus *Hoplodrina* Boursin, 1937 (Lepidoptera, Noctuidae, Xyleninae). I. *Hoplodrina octogenaria* (Goeze, 1781) and its sister species *H. alsinides* (Costantini, 1922) sp. rev. in Europe. ZooKeys 927: 75–97. https://doi.org/10.3897/zookeys.927.51142

Abstract

The taxonomic status of the European *Hoplodrina octogenaria* (Goeze, 1781) is discussed and its partly sympatric sister species, *Hoplodrina alsinides* (Costantini, 1922) **sp. rev.**, is separated and re-described

- Morphology
- DNA barcodes
- ddRAD



Figures 1–10. *Hoplodrina alsinides* (Costantini, 1922), adults in dorsal view. **1** Male, Lectotype of *Caradrina alsinides*, Italy, Emilia-Romagna, Sestola **2** male, Italy, South Tyrol, Sesvenna Mts. **3** male, Romania, Transylvania, slide No.: RL12119 **4** male, Italy, South Tyrol, Sesvenna Mts. **5** male, Austria **6** male, Austria, Carinthia, slide No.: OP1415, BC TLMF Lep 02471 **7** male, Italy, South Tyrol, slide No.: RL10288, BC TLMF Lep 04569 **8** male, Austria, Carinthia **9** female, BC TLMF Lep 04568 **10** emale, Italy, South Tyrol, Sesvenna Mts.

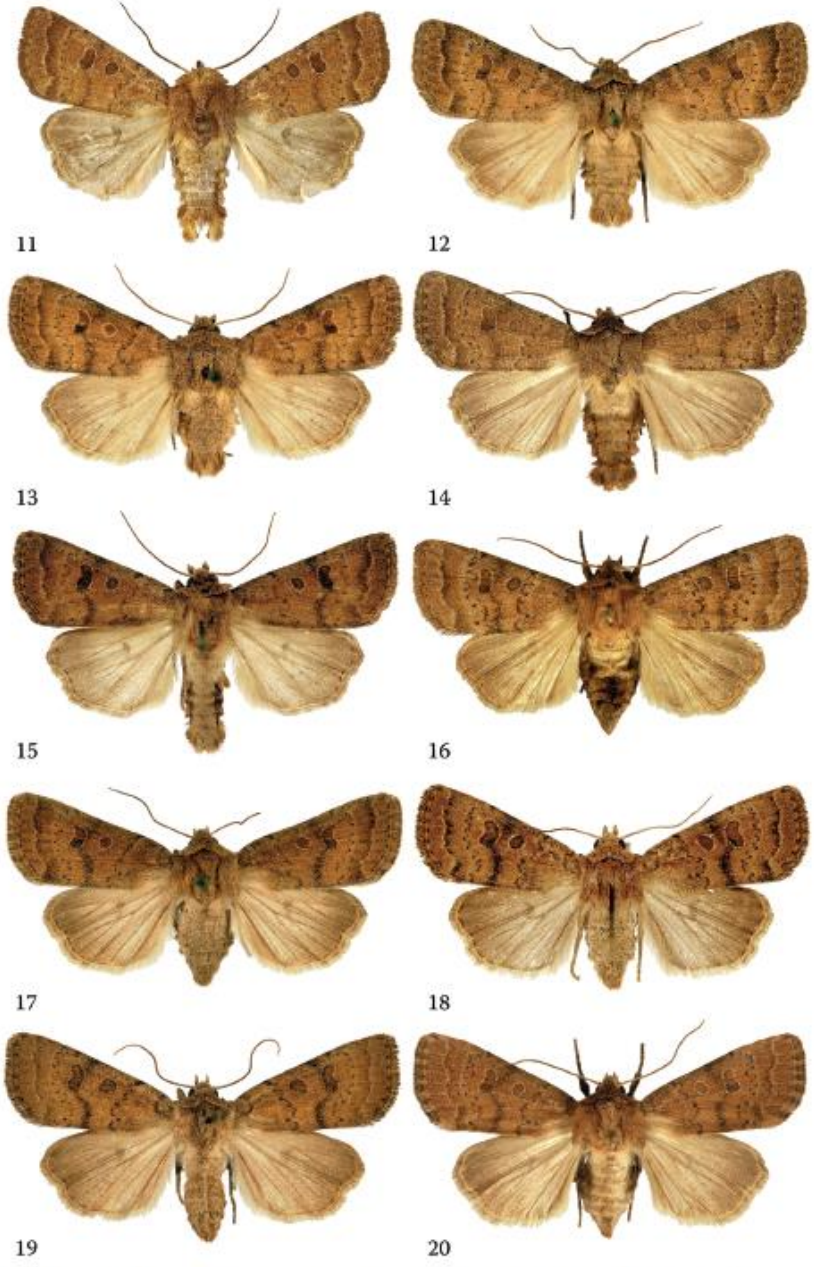


Figure 11–20. *Hoplodrina octogenaria* (Goeze, 1781), adults in dorsal view. **11** Neotype male, Germany, Bayern **12** male, Austria, Carinthia **13** male, Italy, South Tyrol, Sesvenna Mts. **14** male, Italy, South Tyrol, Sesvenna Mts. **15** male, Hungary, Vas County **16** female, Hungary, Pest County **17** female, Austria, Wien **18** female, Hungary, Pest County **19** female, Austria, Burgenland **20** female, Austria, Wien.

Studied 3000 specimens from 12 collections

- **CJHL** Collection Jean Haxaire, Laplume, France
- **CREA-FL** Centro di ricerca Foreste e Legno (Research Centre for Forestry and Wood), Rende, Italy
- **HNHM** Hungarian Natural History Museum, Budapest, Hungary
- **LMK** Landesmuseum Kärnten, Klagenfurt, Austria
- **MCSN** Museo Civico di Storia Naturale, Milano, Italy
- **MNHU** Museum für Naturkunde, Humboldt-Universität, Berlin, Germany
- **NHM** The Natural History Museum (formerly British Museum, Natural History), London, United Kingdom
- **NHMW** Naturhistorisches Museum Wien, Austria
- **RNS** Royal Natural History Museum (Naturhistoriska Riksmuseet), Stockholm, Sweden
- **TLMF** Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria
- **ZMHU** Museum für Naturkunde – Leibniz-Institut für Evolutions- und Biodiversitätsforschung, Berlin, Germany
- **ZSM** Zoologische Staatssammlung, Munich, Germany

Importance of biological collections for taxonomic research

- Biological collections are the cornerstone of taxonomic research for many groups of organisms
- Immense record of biodiversity on Earth, also of biodiversity we have lost and will lose
- Specimens often collected from the most inaccessible parts of the Earth
- Collections specializing in certain groups of organisms or geographical areas
- Institutional and private collections
- The “extended specimen” – videos, sound recordings, photos

How to access collection specimens?

- Specimens and samples can be accessed through visits and loans, increasingly also as virtual loans
- Permission to make dissections or detach parts of the specimen, e.g., for DNA extraction always need to be requested separately.

Visits to museums and specimen loans

- In most cases, firsthand examination of the specimen is preferred.
- Sending specimens is always risky. Specimens could be damaged or lost in the mail.
- If possible, hand-carry loaned material when travelling.
- Travelling to museums around the world costs money and is not always environmentally friendly.
- Some museums require passing a test before you can study their material or show other proof that you have the required expertise to study that kind of material.

Virtual access

- Virtual loans on request
    - Digital photos of specimen and labels, possibly also of associated microscope slides

Virtual access

- Many institutions have programs to digitize collections

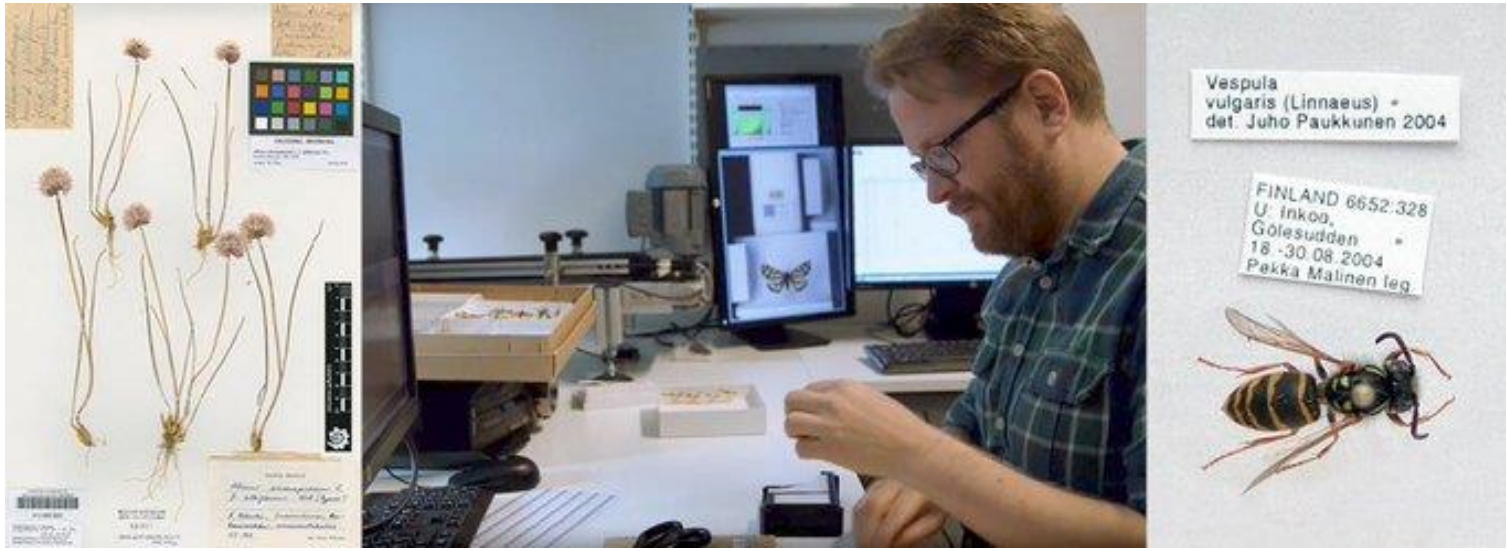


Photo: Luomus

DiSSCo

- Distributed System of Scientific Collections
- 115 European Museums from 21 Countries
- The DiSSCo Research Infrastructure works for the digital unification of all European natural science assets under common curation and access policies and practices that aim to make the data easily Findable, more Accessible, Interoperable and Reusable (FAIR)
- https://www.dissco.eu/
- https://www.dissco.eu/what-is-dissco/

Virtual access

- In the future maybe also for DNA (sequencing on demand)

Virtual access, limitations

- In morphological studies, it is often necessary to handle the specimen yourself, to be able to look at it from different angles and at different magnifications

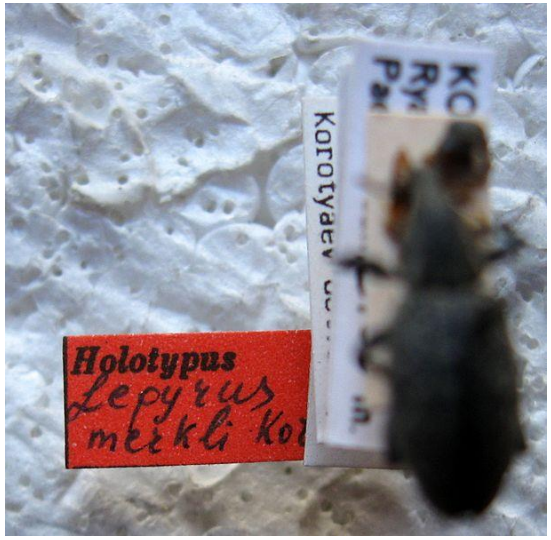
Data used in taxonomic research: Morphological characters

- Extant and extinct organisms (fossils)
- Discrete (e.g., presence/absence) and continuous characters (e.g., measurements)
- Useful in species descriptions, systematics, and in studies of character evolution.

Observation of morphological structures often requires dissection

- Sometimes this cannot be done: e.g., rare specimens, type specimens:
    - Holotype: the single specimen designated by an author as the type of a species. The specimen, or each of a set of specimens, on which the description and name of a new species is based.

Wikimedia commons: CC BY-SA 4.0
Hedbergia abyssinica holotype
Muséum national d'Histoire naturelle, Paris (France) Spécimen P032763



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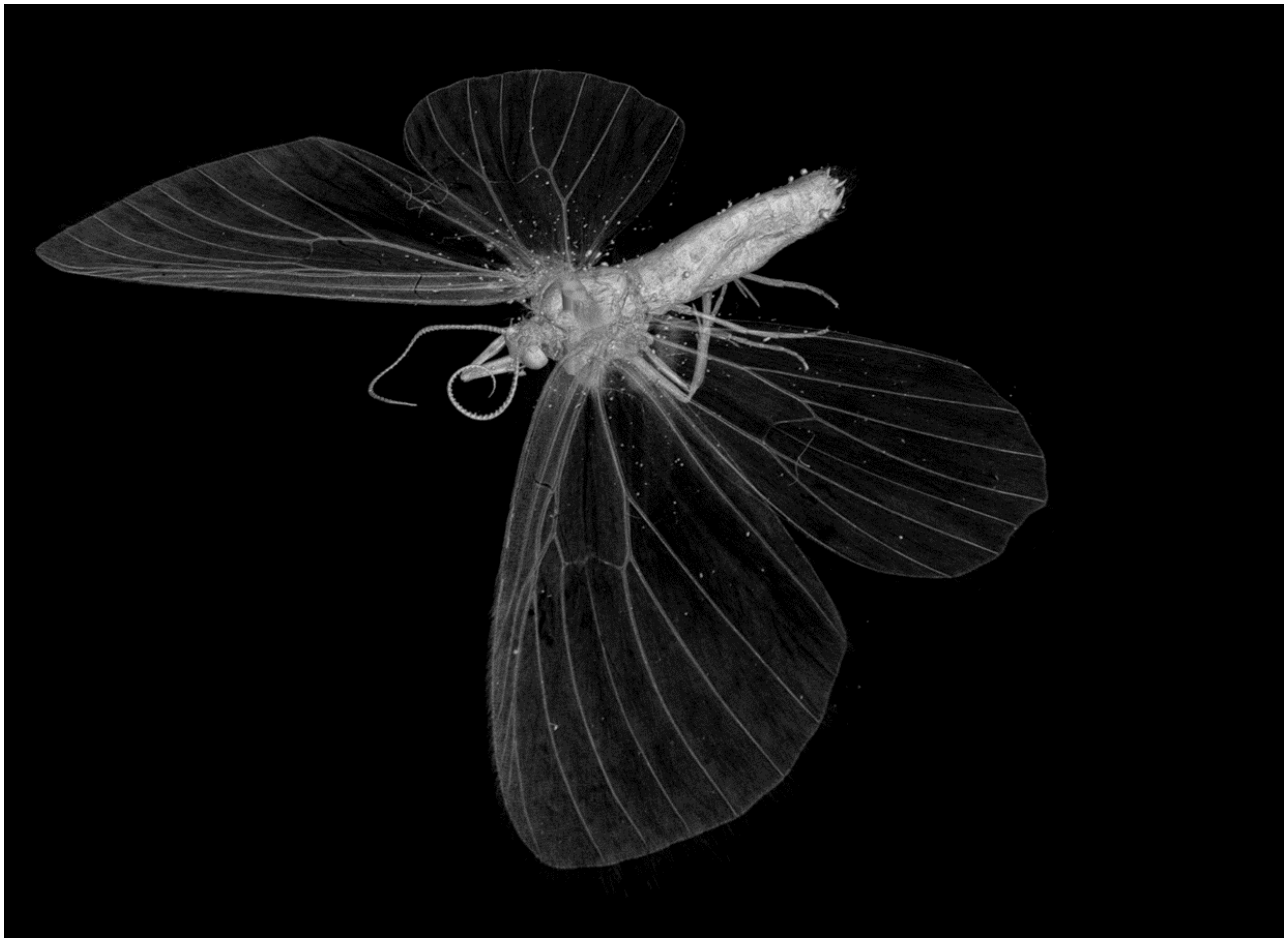
Label of the holotype of *Lepyrus merkli* Korotyaev, 1994.

Example of a non-destructive method: µCT-scanning

Photo: Pasi Sihvonen



µCT-scan of pinned moth

Data used in taxonomic research: Molecular characters

- In molecular taxonomy/systematics scientists compare molecules to gain information on an organism's evolutionary relationships
  - Micromolecules, e.g., small molecules responsible for colors, scents, and chemical defenses
  - Macromolecules, e.g., proteins and nucleic acids (DNA, RNA)
- Closely related organisms have a high degree of similarity in the molecular structure, while molecules of distantly related organisms often show a pattern of dissimilarity
- Comparison of several hundred to hundreds of millions of traits depending on the technique

Storage and preservation of samples

- DNA degradation
  - natural postmortem processes
  - preservation methods
- Important to document treatment practices to facilitate future analyses enabled by as yet undiscovered technologies

Frozen tissue and DNA collections

- Collections of tissue and DNA from laboratories, zoos, aquariums and museums
- Also samples from wild populations and organisms that are now extinct

Development of new methods to extract DNA from museum specimens

- e.g. from very old samples or specimens in formaldehyde
- Non-destructive methods

Summary

- Biological collections contain specimens collected over centuries of field exploration, including rare and extinct species
- You do not have to go in the field to collect everything all over again
- Modern technology is helping us examine morphological structures without having to dissect or damage the specimens
- Modern methods are helping us extract DNA from museum specimens
- New methods to preserve collection specimens and samples are sought

Musical break

- https://m.youtube.com/watch?v=gfQL7bXwzvM

Use of biological collections in taxonomic research with a focus on genetic data

Annina Kantelinen

25.8.2020

Contents

- Barcoding
- Metabarcoding
- Genomics
- Metagenomics
- AncientDNA

Lichens are small ecosystems:
mycobiont & photobiont, but also
other fungi, algae, secondary photobionts,
protozoa and non-photosynthetic bacteria.

You will learn:

1.) To explain what kind of DNA methods are used in collection based research.

2.) To explain what kind of progress has happend within the last decade and what kind of possibilities these new DNA technologies are opening.

3.) To create research questions as part of today´s group excercise.

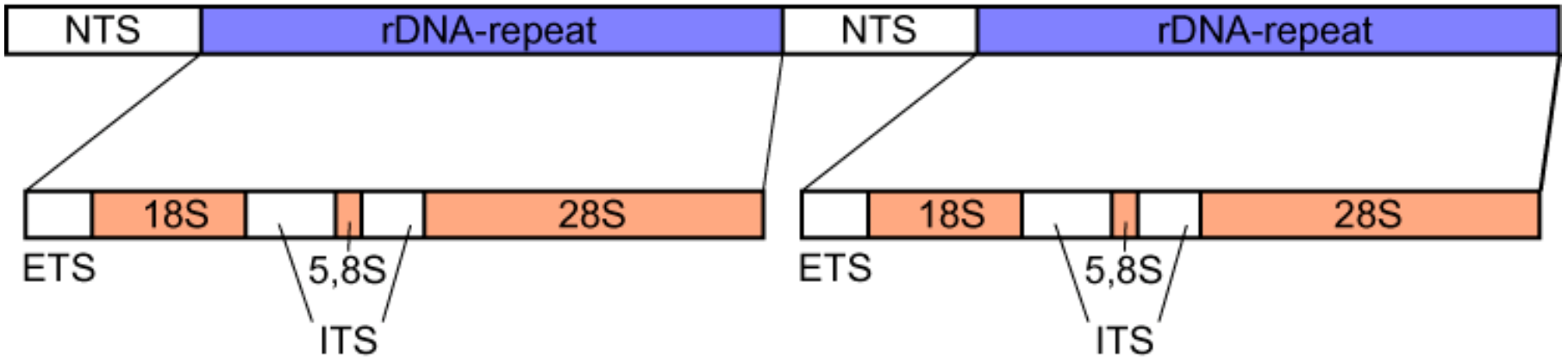
4.) To analyze and reflect all this in your learning diary.

Collections

- Natural history museums are a diverse biobank of biodiversity, including extant, rare and extinct taxa
- Many species are more accessible in collections than in their original habitats
  - remote geographical areas
  - rare or endangered taxa
  - taxa that have gone extinct
  - taxa that have not been seen since their initial collection

DNA barcoding

- DNA barcode: a sequence that can be used to uniquely identify an organism to species
- Standardized DNA area (500-1000 bp.)
  - ITS, COI



Picture: Norbert Holstein 2001

Barcoding

- ”Deficiently known forest lichens – identification through DNA-barcoding” 2011-2012
- Specimens were collected, morphologically identified, sequenced, and deposited in the herbarium and DNA databases
- DNA barcode was created for 108 lichen species
  - Also scientifically new species

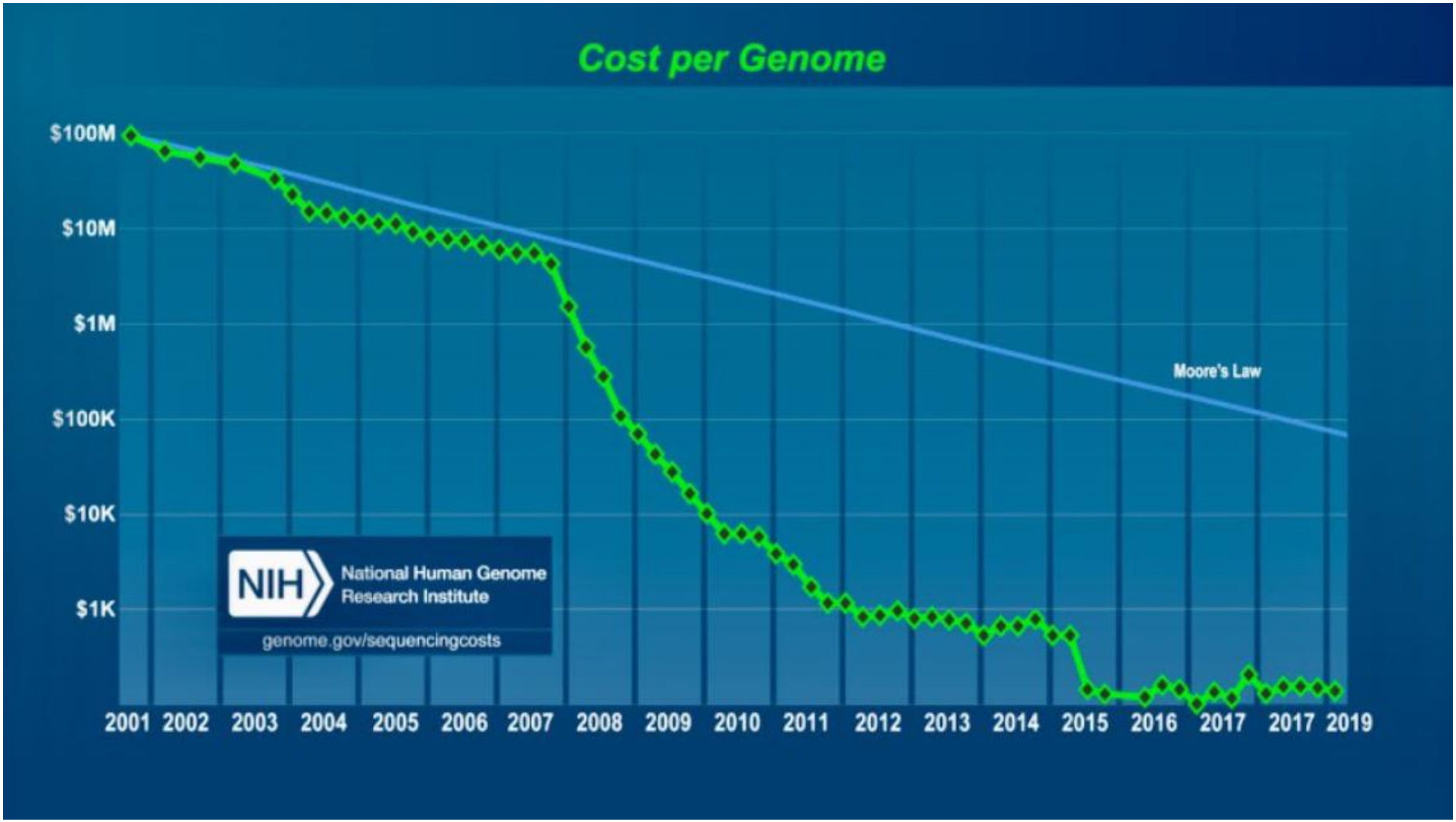
PUTTE

Puutteellisesti tunnettujen ja uhanalaisten metsälajien tutkimus-ohjelma

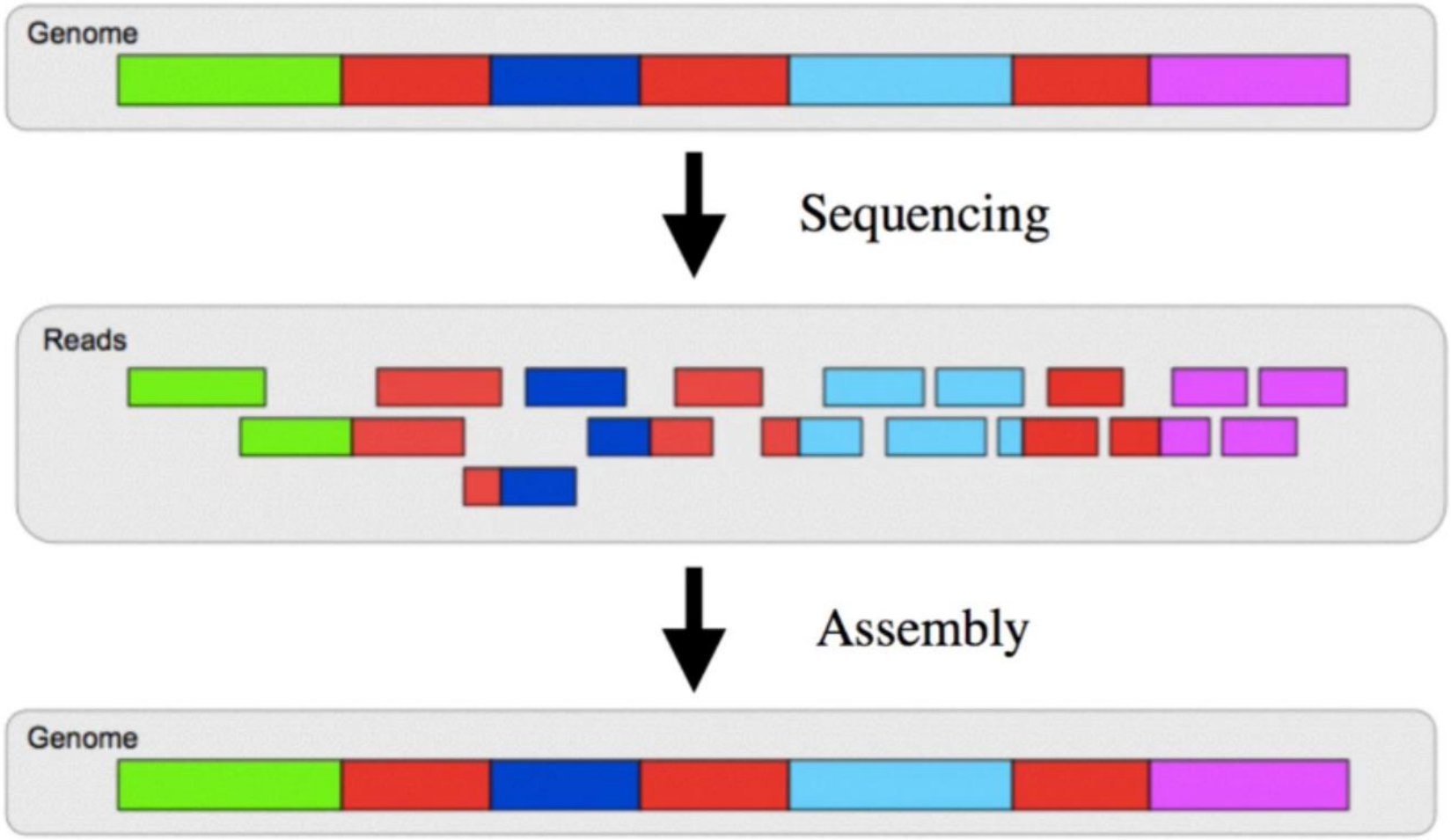
High-throughput sequencing (HTS)

- Metabarcoding, genomics, metagenomics and ancientDNA.
  - Also known as next-generation sequencing (NGS)
- Rapid and cost-effective
- HTS techs enable hundreds of millions of DNA molecules to be sequenced at a time
- Enable more reliable phylogenetic and evolutionary analyses

High-throughput sequencing



What is Genome Assembly?



Museomics

- High-throughput sequencing (HTS) technologies offers a promise of efficient ways of sequencing DNA from museum specimens
  - HTS involves sequencing of short fragments of DNA, which is characteristic of DNA extracted from old museum specimens, eg. type specimens
  - Large volumes of sequence data from relatively small amounts of starting material

Museomics

- The ability to sequence genomes from old specimens has expanded the variety of interesting taxa available to study AND the scope of questions that can be investigated in order to further knowledge about biodiversity

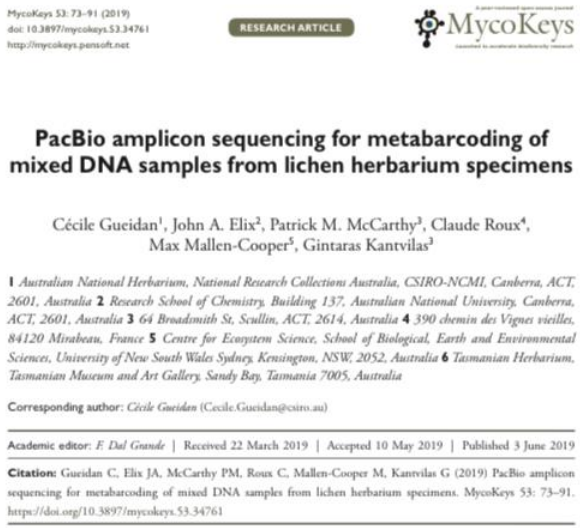
New article: Museomics of a rare taxon: placing Whalleyanidae in the Lepidoptera Tree of Life. 2020. Twort, V. et al.

  - Genome-wide data was recovered and analyzed from insect specimens collected in 1960s

Metabarcoding

- DNA-based identification meets HTS
  - 500-1000 bp, 16S rDNA
- Great for mixed species samples
  - environmental samples, eg. dead wood
  - community ecology

New article: PacBio amplicon sequencing for metabarcoding of mixed DNA samples from lichen herbarium specimens. 2019. Gueidan, C. et al.



MycoKeys 53: 73–91 (2019)
doi: 10.3897/mycokeys.53.34761
http://mycokeys.pensoft.net

RESEARCH ARTICLE

MycoKeys

PacBio amplicon sequencing for metabarcoding of mixed DNA samples from lichen herbarium specimens

Cécile Gueidan[1], John A. Elix[2], Patrick M. McCarthy[3], Claude Roux[4], Max Mallen-Cooper[5], Gintaras Kantvilas[3]

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Corresponding author: *Cécile Gueidan* (Cecile.Gueidan@csiro.au)

Academic editor: *F. Dal Grande* | Received 22 March 2019 | Accepted 10 May 2019 | Published 3 June 2019

Citation: Gueidan C, Elix JA, McCarthy PM, Roux C, Mallen-Cooper M, Kantvilas G (2019) PacBio amplicon sequencing for metabarcoding of mixed DNA samples from lichen herbarium specimens. MycoKeys 53: 73–91. https://doi.org/10.3897/mycokeys.53.34761

Whole genome sequencing

- More data, wide range of research questions
  - Evolution, adaptation, metabolism, genetics…

New article: The lichen symbiosis re-viewed through the genomes of *Cladonia grayi* and its algal partner *Asterochloris glomerata.* Armaleo, D. et al. 2019.

-The first parallel genomic analysis of lichen symbionts

-From cultures

Metagenomics

- Study of ALL genomes from a mixed community of organisms
  - Environmental samples, eg. microbes
  - Symbiotic organisms, eg. lichens

"Because of its ability to reveal the previously hidden diversity of microscopic life, metagenomics offers a powerful lens for viewing the microbial world that has revolutionized understanding of the entire living world"

Marco, D. 2011

Metagenomics & lichens

- Genomic studies in lichenology are considerable delayed due to the symbiotic nature of lichens
- Their symbiotic nature makes it really difficult to obtain mycobiont genomes by techniques widely used in other groups of organisms
  - Researchers have tried to culture the mycobiont, but obtaining and maintaining such cultures is difficult and unpredictable
- In metagenomics the DNA present in the entire lichen symbiosis is massively sequenced, and the mycobiont part is recovered using different computational tools

Metagenomics & lichens

New(ish) articles:

Fungal Diversity
https://doi.org/10.1007/s13225-018-0407-7

Phylogenomic analysis of 2556 single-copy protein-coding genes resolves most evolutionary relationships for the major clades in the most diverse group of lichen-forming fungi

David Pizarro[1] · Pradeep K. Divakar[1] · Felix Grewe[2] · Steven D. Leavitt[3] · Jen-Pan Huang[2] · Francesco Dal Grande[1,4] · Imke Schmitt[4,5] · Mats Wedin[6] · Ana Crespo[1] · H. Thorsten Lumbsch[2]

Received: 7 December 2017 / Accepted: 1 August 2018

Abstract
Phylogenomic datasets continue to enhance our understanding of evolutionary relationships in many lineages of organisms. However, genome-scale data have not been widely implemented in reconstructing relationships in lichenized fungi. Here we generate a data set comprised of 2556 single-copy protein-coding genes to reconstruct previously unresolved relationships in the most diverse family of lichen-forming fungi, Parmeliaceae. Our sampling included 51 taxa, mainly from the subfamily Parmelioideae, and represented six of the seven previously identified major clades within the family. Our results provided strong support for the monophyly of each of these major clades and most backbone relationships in the topology were recovered with high nodal support based on concatenated dataset and species tree analyses. The alectorioid clade was strongly supported as sister-group to all remaining clades, which were divided into two major sister-groups. In the first major clade the anzioid and usneoid clades formed a strongly supported sister-group relationship with the cetrarioid + hypogymnioid group. The sister-group relationship of *Evernia* with the cetrarioid clade was also strongly supported, whereas that between the anzioid and usneoid clades needs further investigation. In the second major clade *Oropogon* and *Platismatia* were sister to the parmelioid group, while the position of *Omphalora* was not fully resolved. This study demonstrates the power of genome-scale data sets to resolve long-standing, ambiguous phylogenetic relationships of lichen-forming fungi. Furthermore, the topology inferred in this study will provide a valuable framework for better understanding diversification in the most diverse lineage of lichen-forming fungi, Parmeliaceae.

Keywords Fungi · Lecanorales · Lichenized fungi · Parmeliaceae · Parmelioideae · Phylogeny · Systematics

SCIENTIFIC REPORTS

OPEN

Received: 15 June 2017
Accepted: 12 October 2017
Published online: 02 November 2017

Sequencing genomes from mixed DNA samples - evaluating the metagenome skimming approach in lichenized fungi

Anjuli Meiser[1,2], Jürgen Otte[2], Imke Schmitt[1,2] & Francesco Dal Grande[2]

The metagenome skimming approach, i.e. low coverage shotgun sequencing of multi-species assemblages and subsequent reconstruction of individual genomes, is increasingly used for in-depth genomic characterization of ecological communities. This approach is a promising tool for reconstructing genomes of facultative symbionts, such as lichen-forming fungi, from metagenomic reads. However, no study has so far tested accuracy and completeness of assemblies based on metagenomic sequences compared to assemblies based on pure culture strains of lichenized fungi. Here we assembled the genomes of *Evernia prunastri* and *Pseudevernia furfuracea* based on metagenomic sequences derived from whole lichen thalli. We extracted fungal contigs using two different taxonomic binning methods, and performed gene prediction on the fungal contig subsets. We then assessed quality and completeness of the metagenome-based assemblies using genome assemblies as reference which are based on pure culture strains of the two fungal species. Our comparison showed that we were able to reconstruct fungal genomes from uncultured lichen thalli, and also cover most of the gene space (86–90%). Metagenome skimming will facilitate genome mining, comparative (phylo)genomics, and population genetics of lichen-forming fungi by circumventing the time-consuming, sometimes unfeasible, step of aposymbiotic cultivation.

In recent years, the decreasing costs and higher accessibility of high-throughput DNA sequencing technologies have revolutionized microbial ecology research. Direct sequencing of genomic material from the environment, commonly referred to as metagenomics, can provide a cultivation-independent assessment of the largely untapped genetic diversity and functional aspects of microbial communities. Whole-metagenome shotgun sequencing has been applied to study diverse microbiomes, spanning a range of natural environments, including the human body[1–3]. Metagenomics has not only been used to catalogue diversity, but it has also provided a fresh perspective on our understanding of the intricate, multi-species interactions driving symbiotic communities, and how these interactions influence ecosystems[4]. On the other hand, the conversion of these large volumes of sequencing data to biologically useful information remains a major challenge[5].

With the improvement of bioinformatics tools, it is increasingly possible to assemble whole genomes from environmental communities of both prokaryotes and eukaryotes, and analyse their strain-level variation[4]. Although research on metagenomic assembly is still in its infancy, valuable insights have already been derived[7]. The annotation of metagenomic contigs from multi-species communities has proven useful to study evolutionary patterns, metabolic complementation, genetic exchange and/or modification between symbionts and their hosts in several symbiotic systems. The reconstruction of individual genomes from multi-species communities has also been used to isolate genes associated with the biosynthesis of novel biomolecules[8]. Assembly and annotation of sequencing data, however, pose several analytical challenges[9]. In particular, the co-occurrence of multiple strains or similar species – sometimes present at highly uneven ratios – may drastically reduce the quality of the reconstructed genomes[10].

[1]Institute of Ecology, Evolution and Diversity, Goethe University Frankfurt, Max-von-Laue Str. 13, D-60438, Frankfurt, Germany. [2]Senckenberg Biodiversity and Climate Research Centre (SBiK-F), Senckenberganlage 25, D-60486, Frankfurt, Germany. Correspondence and requests for materials should be addressed to I.S. (email: imke.schmitt@senckenberg.de) or F.D.G. (email: francesco.dalgrande@senckenberg.de)

Ancient DNA

- Collections are increasingly used in biogeographical, environmental and taxonomic studies.
- Correct species identification and genetic information is crucial!
- Type specimens are often old and their DNA is degraded
  - Their successful sequencing is of high priority

New article: DNA sequencing historical lichen specimens. 2019. Kistenich et al.

-Target sequences (mtSSU)

-Samples from every 25 years from present to 150 years back in time.

-Received satisfactory DNA sequence information for 54 of 56 specimens

-Recovered full-length sequences for several more than 100-years-old specimens!

Challenges and future aspects

- HTS challenges:
  - De novo assembly, because no reference data available
  - Short reads are bioinformatically demanding
  - Low recovery
- HTS future:
  - Short reads are becoming longer (Illumina 200 bp -> PacBio 1500 bp)
- Not just what you CAN do, but what you WANT to do
  - What is your research question?
  - What is your research question 5 years from now?

More info about the methods

- A lecture “Fundamentals of Genome Assembly” by Jared Simpson (Ontario Institute for Cancer Research) https://www.youtube.com/watch?v=5wvGapmA5zM

- Workshop on Genomics, Cesky Krumlov (www.evomics.org)

References

1. Victoria G. Twort, Joël Minet, Christopher W. Wheat and Niklas Wahlberg. (2020). Museomics of a rare taxon: placing Whalleyanidae in the Lepidoptera Tree of Life bioRxiv preprint doi: https://doi.org/10.1101/2020.08.18.255182.

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Today´s group work

- Form groups of 2-3 persons

- Examine a specimen and discuss how it could be used in research
  - Are DNA-studies possible?
  - What might be the challenges?
  - What kind of research questions would you like to ask?