1) *Fomes fomentarius* vs. *F. inzengae*

Description: *Fomes fomentarius*, iconic polypore, in fact represents two cryptic species - *F. fomentarius* sensu stricto and *F. inzengae*. So far, no reliable morphological features have been found to distinguish between them. However, there is a hypothesis about partial ecological difference.
F. fomentarius - on *Fagus* and *Betula*, mainly in higher elevations, in more natural forests.
F. inzengae - on broad spectrum of broadleaved trees (incl. *Fagus* and *Betula*) in lower elevations, more synanthropic.
Verify this hypothesis by your samples from summer school. Confirm the identity of your samples by ITS barcode and study the micromorphology of the collected specimens.

Important features to observe: host tree species, morphology

Markers: ITS

Literature:
Tomšovský M., Kaeochulsri S., Kudláček T., Dálya L.B. (2023). Ecological, morphological and phylogenetic survey of *Fomes fomentarius* and *F. inzengae* (Agaricomycetes, Polyporaceae) co-occurring in the same geographic area in Central Europe. Mycological Progress 22:79.

Fomes fomentarius s.l. (Troudnatec kopytovitý) (mykologie.net)

2) Fast versus reliable - what is the best method for DNA sequencing from fungi?

Description: Identification of fungi based on molecular data gains more attention with the decreasing cost for sequencing and various protocols allowing fast DNA extraction from freshly collected fruit bodies. One approach is also “direct PCR” that omits completely the lengthy extraction.

Important features to observe: select 2-3 fungal species differing in i.e. consistency of fruit body (hard vs. fleshy) or color (some secondary metabolites may inhibit PCR reaction) and test several approaches to obtain ITS rDNA sequences. Compare the time necessary and success ratio. These approaches will used DNA extraction, fast extraction using Bento dipsticks and no extraction followed by direct PCR.

Markers: ITS rDNA.

Literature: Hofstetter, V., Buyck, B., Eyssartier, G. *et al.* 2019: The unbearable lightness of sequenced-based identification. Fungal Diversity 96, 243–284

3) Stromatic pyrenomycetes - *Hypoxylon* s.l.

Description: *Hypoxylon* s.l. is genus which includes members with resupinate or more or less globose stromata. Apart from classical morphology, pigments extractable from the stroma in KOH solution are also important for species identification Collect samples of various *Hypoxylon* species on dead wood of deciduous trees and test success rate of sequence obtaining by extracting DNA from different parts of stroma - perithecial content, surface with conidia, interior of stroma etc.

Important features to observe: host tree; colour, shape and size of stroma and spores; KOH extractable pigments

Markers: ITS

Note: cooperate with project 5

Literature:
Fungi of Temperate Europe
Lambert et al. (2021): Resolution of the *Hypoxylon fuscum* Complex (Hypoxylaceae, Xylariales) and Discovery and Biological Characterization of Two of Its Prominent Secondary Metabolites. Journal of Fungi 7:131.
Yu-Ming Ju and Jack D. Rogers (1996): A Revision of the Genus Hypoxylon (Mycologia Memoir)

https://www.mykologie.net/index.php/houby/podle-morfologie/perithecia/item/1327:hypoxylon-rubiginosum

4) Stromatic pyrenomycetes - other genera (*Biscogniauxia*, *Camarops*, *Daldinia*, *Nemania*, *Xylaria* etc.)

Description: Members of *Xylariaceae* include genera with typical stromata which are usually dark by melanin - possible inhibitor of PCR. Collect samples of various stromatic genera on dead wood of deciduous trees and test success rate of sequence obtaining by extracting DNA from different parts of stroma - perithecial content, surface with conidia, interior of stroma etc.

Important features to observe: host tree; shape and size of stroma and spores

Markers: ITS

Note: cooperate with project 4

Literature:

Fungi of Temperate Europe
Derek Peršoh, Martina Melcher, Katrin Graf, Jacques Fournier, Marc Stadler & Gerhard Rambold (2009) Molecular and morphological evidence for the delimitation of *Xylaria hypoxylon*, Mycologia, 101:2, 256-268, DOI: 10.3852/08-108

Dřevnatka dlouhonohá Xylaria longipes (hlasek.com)

5) Cute (hairy) Helotiales

Description: Minute apothecia of various members of Helotiales are rather common on dead wood, cones and also needles in the litter. They can be recognized on the substrate already in the field, but identification to genus (eventually also species) can be done only after detailed study of micromorphological characteristics. Though the apothecia are rather small, they may grow in abundant groups and thus enough material may be obtained also for DNA extraction. Collect various samples among different habitats and confront morphological identification with molecular data.

Important features to observe: host tree; color, shape and size of hairs, shape and size of ascospores, reaction of asci in iodine

Markers: ITS

Literature:
Dennis (1968): British Ascomycetes
Fungi of Temperate Europe
Raitviir A. (2004): Revised Synopsis of the Hyaloscyphaceae. 133 pp., Tartu.

6) MinION in the field - is barcoding in the field feasible? *

Description: Traditionally, fungal specimens are barcoded in the laboratory. In the field fungal specimens are collected and vauchered and all needed molecular steps are performed later on. Sometimes preliminary steps (i.e. DNA extraction and PCR) of molecular identification can be performed in the field but for sequencing samples are sent to external laboratories. Rapid development of nanopore sequencing technology seems to enable fungal barcoding in the field. Your task is to sample 24 fungal specimens (at least half of them should be samples from specimens on which other groups work) and try to sequence their ITS using **Native Barcoding Kit 24** V14 (SQK-NBD114.24) and MinION device. Basecalling should be performed using guppy software on servers of the Institute of Evolutionary Biology.

Markers: ITS

Literature: https://nanoporetech.com/support

Note: For project presentation we would like you to present the pipeline and compare your results with results of traditional Sanger sequencing performed by other groups. If you are not successful, you should prepare a detailed presentation of the pipeline with discussion problems.

7) Plant parasites (rusts, smuts, powdery mildews)

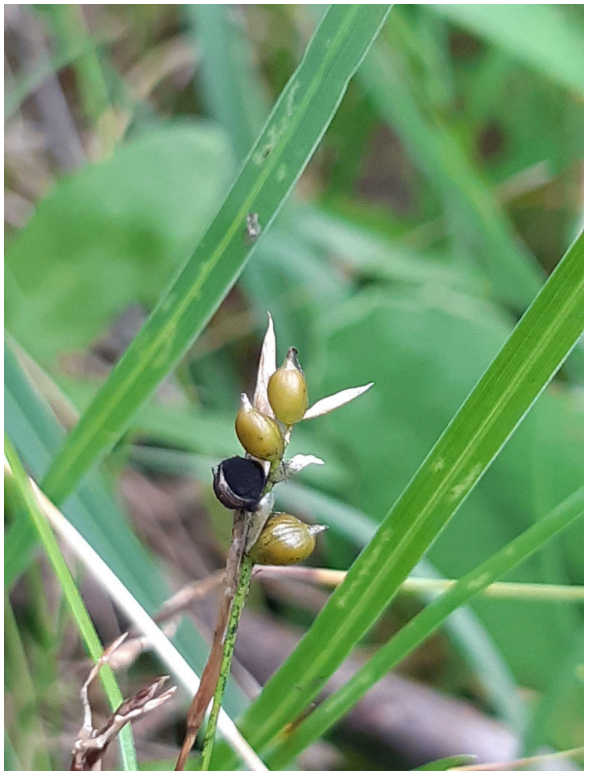
Description: Thousands of fungi associate with the above part of plants, engaging in a variety of biotrophic interactions. Many are so-called endophytes with a poorly understood but partly mutualistic ecology, while others are well known parasites, e.g. rusts, smuts and powdery mildews. In many cases host specialization is prominent, and hence plant identification is crucial for species identification. You need to select a number of plant hosts where you should aim to find and identify as many fungal species as possible, exploring their ecological strategies if possible.

Important features to observe: plant host species, plant part and stage (living, senescent, dead)

Markers: ITS

Literature:

Ellis, M. B., & Ellis, J. P. (1985). Microfungi on land plants. An identification handbook. Croom Helm Ltd.
Plant Parasites of Europe – leafminers, galls and fungi (bladmineerders.nl)
(Obligat) Phytoparasitische Kleinpilze Mitteleuropa (www.phytoparasiten.de/)
Klenke, F. & Scholler, M. (2015) Pflanzenparasitische Kleinpilze. Bestimmungsbuch für Brand-, Rost-, Mehltau-, Flagellatenpilze und Wucherlingsverwandte in Deutschland, Österreich, der Schweiz und Südtirol. Berlin-Heidelberg, Germany: Springer.



Anthracoidea caricis-albae (smut) on *Carex alba* (fot. Marcin Piątek)

8) Corticioid fungi

Description: Corticioid fungi are basidiomycetes that form crust-like fruit bodies on wood. They are characterized by the presence of holobasidia. They can be saprotrophs, parasites or mycorrhizal fungi. Usually they are underrepresented in collections because of the simple macromorphology and difficulties in their identification. Collect various samples from different habitats and confront morphological identification with molecular data.

Important features to observe: plant host species; character of fruit body surface and colour; microscopic features (basidiospores, cystidia etc.)

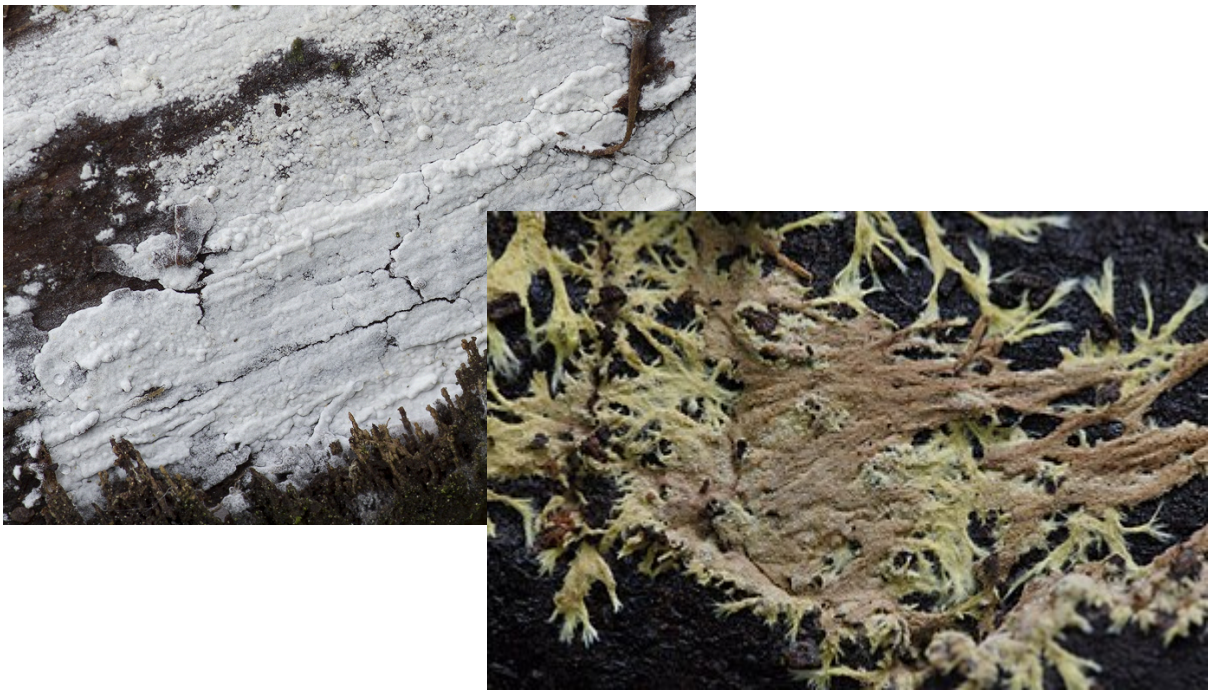
Markers: ITS

Literature:

A. Bernicchia & S. P. Gorjón (2010): Fungi Europaei 12 Corticiaceae s.l.

K.H. Larsson, L. Ryvarden (2021): Synopsis Fungorum 43: Corticioid fungi of Europe 1.volume

K.H. Larsson (2007): Re-thinking the classification of corticioid fungi. Mycological Research 11(9): 140-1063.



https://pl.wikipedia.org/wiki/Xenasmatella_vaga
https://www.mykologie.net/index.php/houby/podle-morfologie/korticie/item/130:lyomyces-sambuci

9) Microfungi in different environments

Description: Microfungi are ubiquitous in the environment due to the huge amount of spores that spread mostly with the wind. Culturable airborne fungi may be easily observed, counted and identified using various types of spore traps. The most simple ones include Petri dishes with nutrition media that is opened and exposed for a particular time in a given environment.

Important features to observe: Fast growing microfungi should emerge on the agar plates already after 24 hours of incubation. Count the colonies, distinguish between members of Mucoromycota and Ascomycota and isolate selected fungi into pure cultures. Identify the most frequent (the most beautiful 🙂) using morphology and molecular data.

Markers: ITS

Literature:
Kubátová A.: Atlas of microscopic fungi (on-line, in Czech only)
Seifert et al.: The Genera of Hyphomycetes

https://www.sciencephoto.com/media/13593/view/petri-dishes-with-cultures-of-fungi

10) Hyphomycetes - they grow everywhere!

Description: Asexual (anamorphic) ascomycetes forming colonies composed of dark melanized hyphae and various types of asexual spores (conidia) are traditionally called hyphomycetes. They frequently grow as saprotrophs on decaying plant material and though generally overlooked, they represent a highly diverse group of fungi.

Important features to observe: Saprotrophic hyphomycetes require humidity as other fungi, but due to their small size they may be found also in dry conditions, growing in humid microhabitats, such as twigs, branches and other decaying plant material in the litter. Collect plant material with indication of hyphomycete colonies and check their presence in the stereomicroscope. Revive the colonies after 1-2 days in a damp chamber. Identify the species based on phenotype and try also extract DNA and obtain molecular barcode.

Markers: ITS

Literature: Ellis, M.B. (1971) Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England, 608.
Ellis, M.B. (1976) More dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England, 507.
Seifert et al.: The Genera of Hyphomycetes

Menispora glauca (iNaturalist and Researchgate)

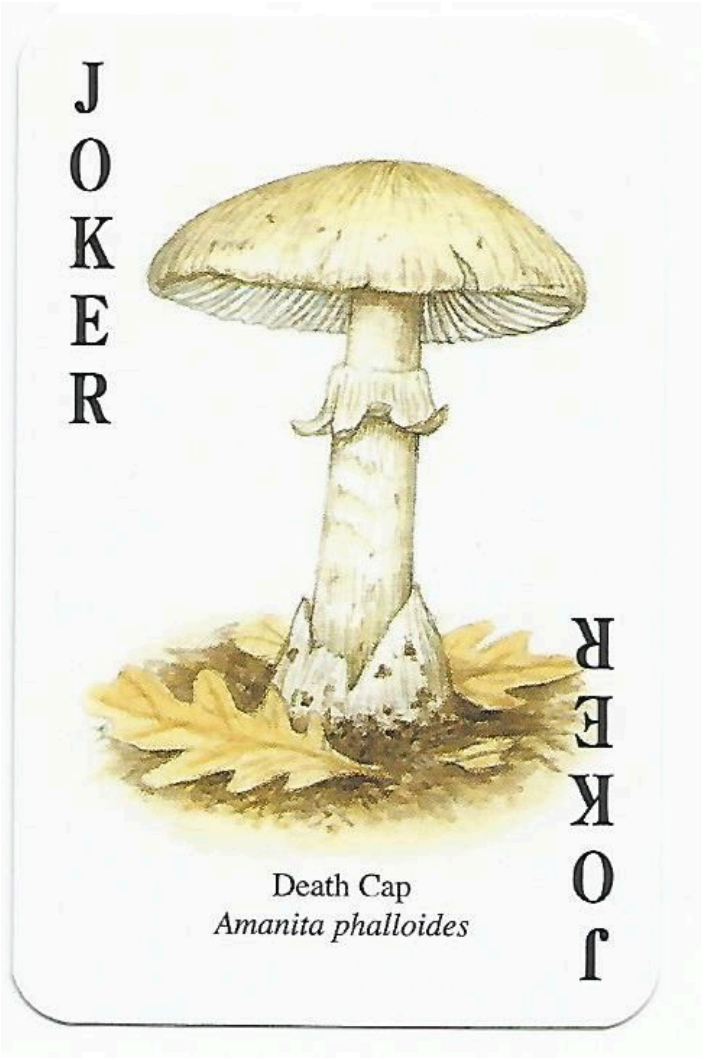
11) JOCKER (you can propose your own project)

Description: Do you have your own idea for a project? Your favorite fungal species is not included in the other topics? Go on and suggest your own project!

Important features to observe: we will help you to find the most important phenotype characteristics to observe and compare with molecular markers.

Markers: ITS should work in most cases, but feel free to select another common one

Literature: hopefully, our selection of books will cover your needs.



12) One piece of wood - how many fungal species?

Description: Dead wood may be inhabited by an immense number of various fungi. Find a fallen trunk in the middle stage of decay and try to describe actual diversity on it based on present fruitbodies. Select several interesting or difficult-to-determine samples for sequencing.

Important features to observe: host tree, macro- and micromorphology

Markers: ITS

Literature:
depends on group of Fungi - ask the teachers

Heilmann-Clausen, J. (2001). A gradient analysis of communities of macrofungi and slime moulds on decaying beech logs. *Mycological research*, *105*(5), 575-596.

Holec J. et Kučera T. (2020). Richness and composition of macrofungi on large decaying trees in a Central European old-growth forest: a case study on silver fir (Abies alba). *Mycological progress 19: 1429-1443*.

https://www.freepik.com/premium-photo/fungi-old-tree-trunk-fallen-tree-with-mushroom-formations-growing-it-magical-green-forest_14516181.htm

13) Fungal succession on deadwood

Description: Fungi are crucial for decomposition of deadwood, which hosts a very diverse fungal community. The species composition is affected by many factors, including tree species, dead wood dimensions and position, and shows marked turnover from fresh to strongly decayed deadwood. You have to collect and identify sporocarps from dead wood in diverse decay stages, of a selected tree species, to describe the successional turnover and the various ecological strategies and morphological traits expressed over the decay process.

Important features to observe: tree species, dead wood dimension, decay stage, microhabitat

Markers: ITS (to sequence interesting collections)

Literature:

Boddy, L., & Heilmann-Clausen, J. 2008. Basidiomycete community development in temperate angiosperm wood. In *British Mycological Society Symposia Series* (Vol. 28, pp. 211-237). Academic Press.

Heilmann-Clausen, J. (2001). A gradient analysis of communities of macrofungi and slime moulds on decaying beech logs. *Mycological research*, *105*(5), 575-596.

Holec J. et Kučera T. (2020). Richness and composition of macrofungi on large decaying trees in a Central European old-growth forest: a case study on silver fir (Abies alba). *Mycological progress 19: 1429-1443*.

Hericium coralloides - Photo: Jacob Hheilmann-Clausen, Bjurkärr, Sweden