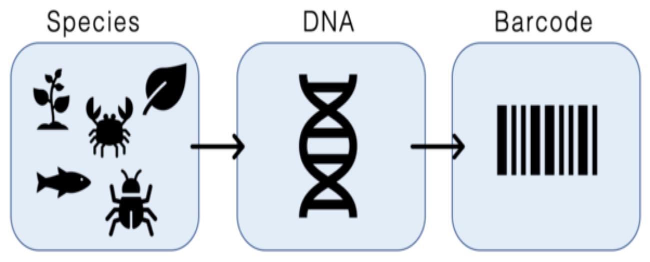
laboratórium
Molekulárnej
Ekológie a
Metagenomiky

Slavomír Adamčík

Postavenie a význam pôdnych mikroorganizmov v OP

Centrum biológie rastlín a
biodiverzity SAV

Barcoding



- DNA barcoding – identifikácia druhov na základe analýzy krátkych úsekov sekvencií (ca 800-1200 bp)
- cieľom je identifikovať neznáme vzorky celých organizmov alebo ich častí
- je potrebné stanoviť hranice druhov v barcode
- termín zavedený Hebert et al. (2003): standardized species identification as well as assingment of the unidentified sequences to higher taxonomic ranks (orders, phyla...)

- pravidlá Barcode
  - vysoká medzidruhová variabilita v porovnaní s nízkou vnútrodruhovou (barcode gap)
  - ohraničenie vysoko konzervatívnymi úsekmi vhodnými na dizajn efektívnych primerov
  - dĺžka barcode regiónu umožňuje PCR amplifikáciu a sekvenáciu bez odchýlok
  - cieľový barcode región má vysokú úspešnosť PCR amplifikácie

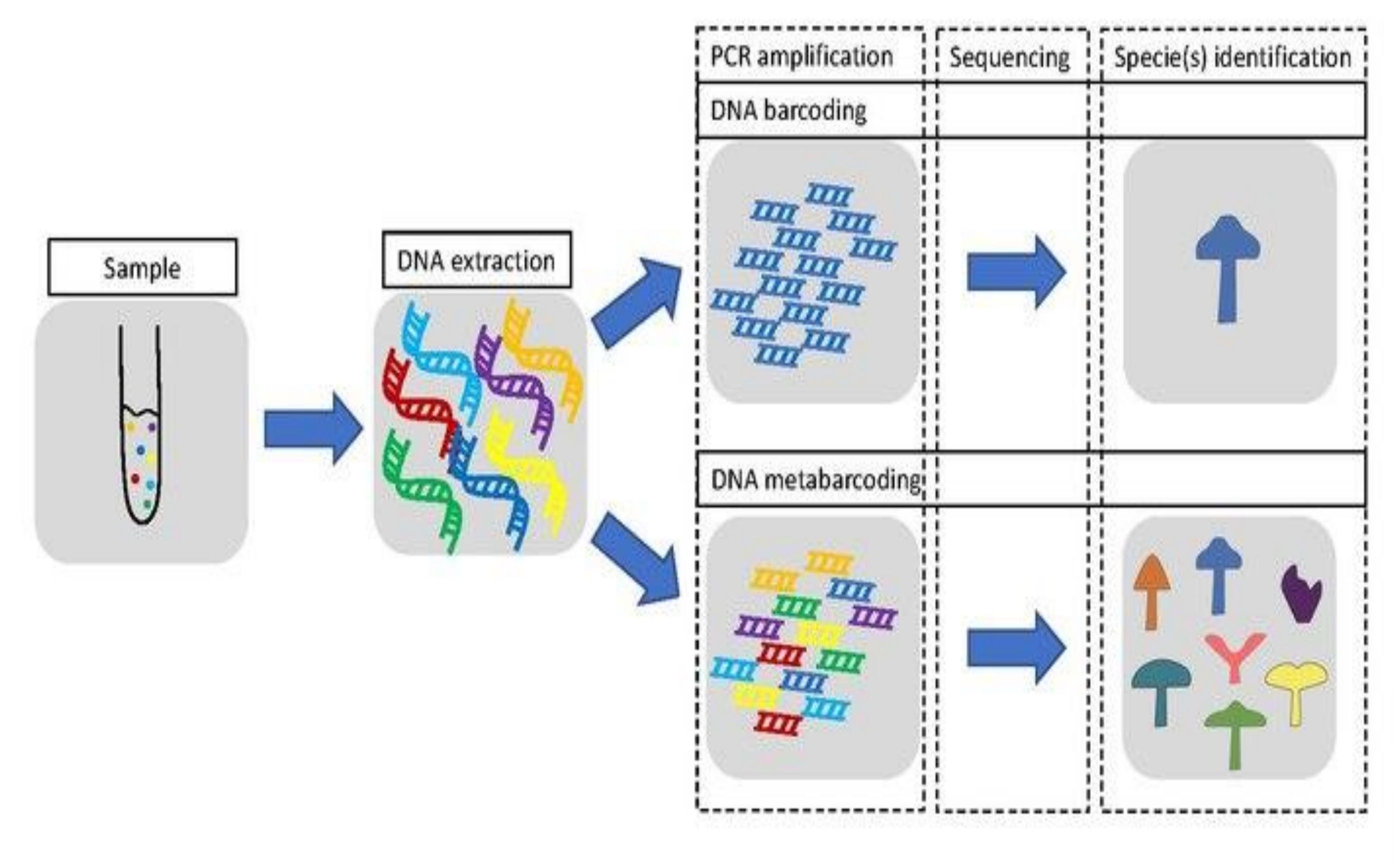
Barcoding

plasmid gény (e.g. rbcL, matK and non coding region of trnH-psbAa tiež ribosomal nuclear DNA (internal transcribed spacer nrITS)

mitochondrial cytochrome *c* oxidase 1 gene ("CO1")

ribosomal nuclear DNA (internal transcribed spacer nrITS)

DNA barcoding vs DNA metabarcoding

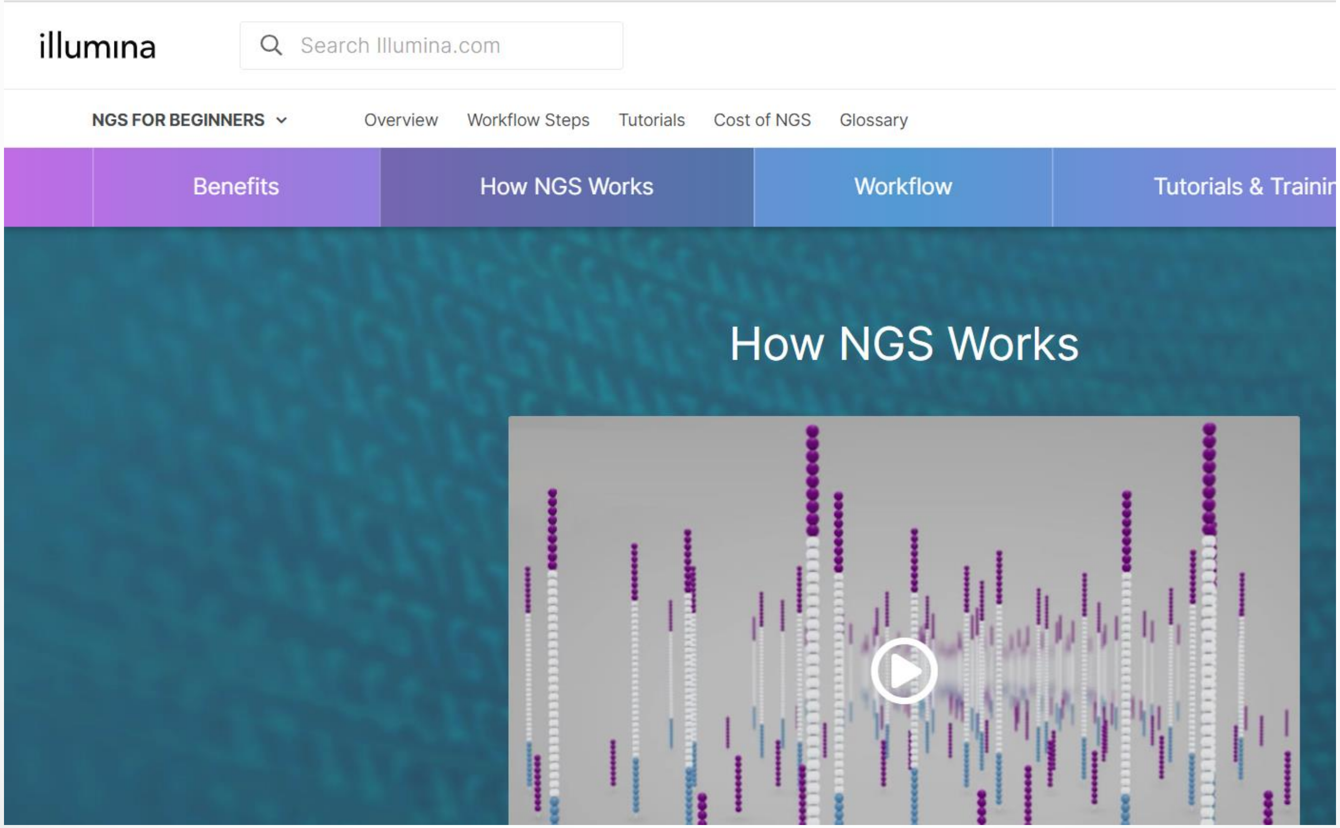


Metabarcoding

- Taberlet et al. (2012): zaviedol termín **DNA metabarcoding** na pomenovanie identifikácie širokého spektra druhov pomocou high-throughput sequencing (NGS) vzoriek environmentálnej DNA (eDNA)
- pôvodne bola identifikácia širokého spektra mikroorganizmov mikrobiálnych komunít (microbiome) z environmentálnej DNA označená termínom metagenomic
- v súčasnosti sú tieto termíny rozlišované
    - Metabarcoding: štúdium **vybraných regiónov** v eDNA za použitia amplicon sekvenácie, štúdie zamerané na **diverzitu organizmov**
    - Metagenomics: štúdium **celého genómu alebo jeho veľkých častí** v eDNA za použitia "shotgun" sekvenácie, štúdie zamerané na **funkčnú diverzitu** mikrobiálnych spoločenstiev

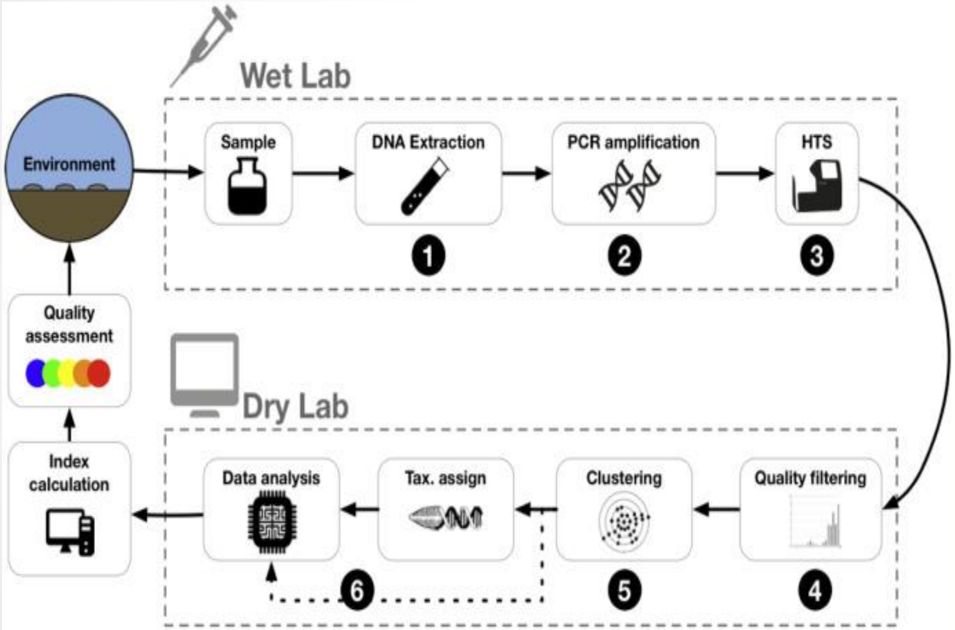
Illumina sekvenovanie, príklad

https://www.illumina.com/science/technology/next-generation-sequencing/beginners.html



How NGS Works

amplikókové sekvenovanie



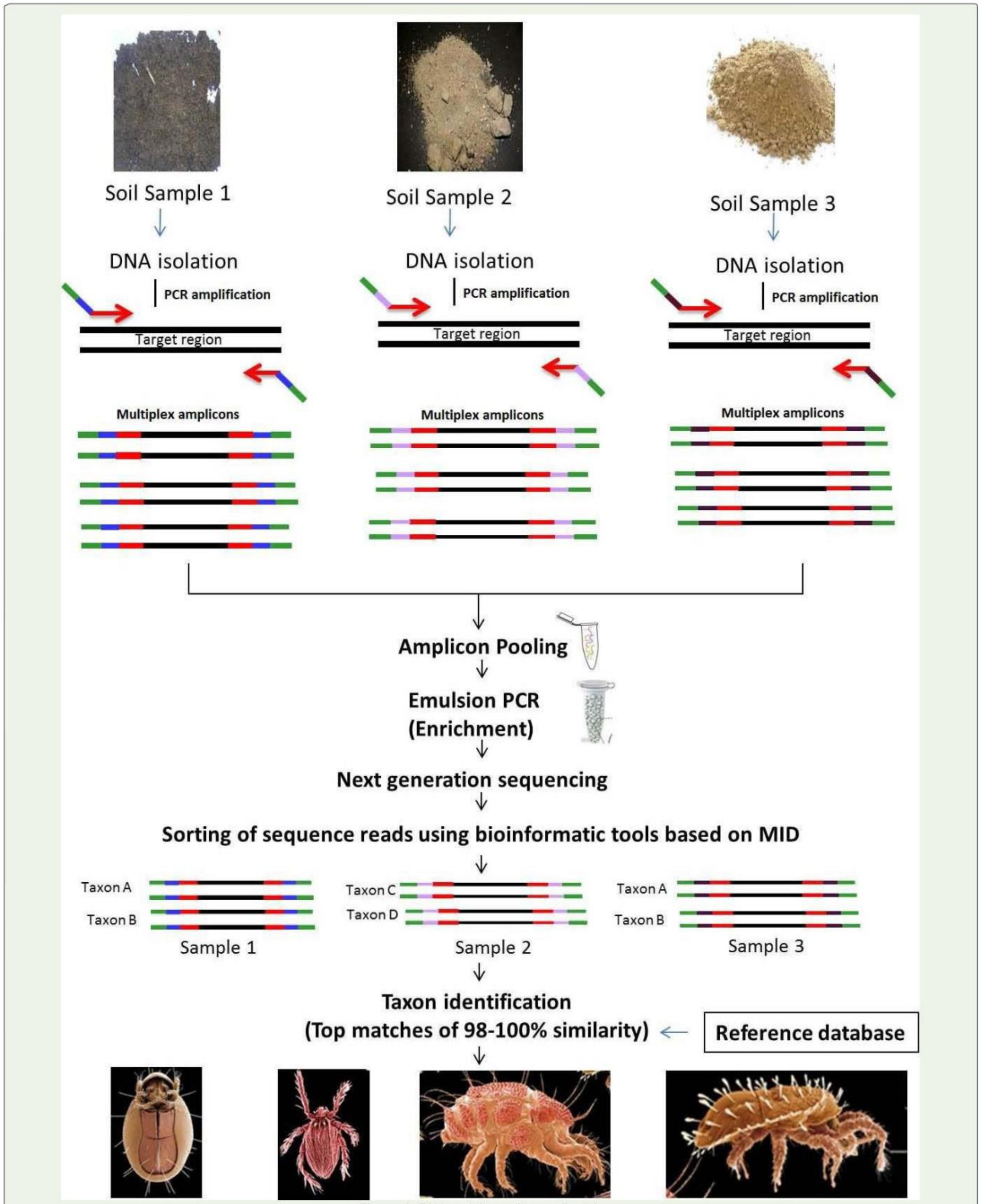
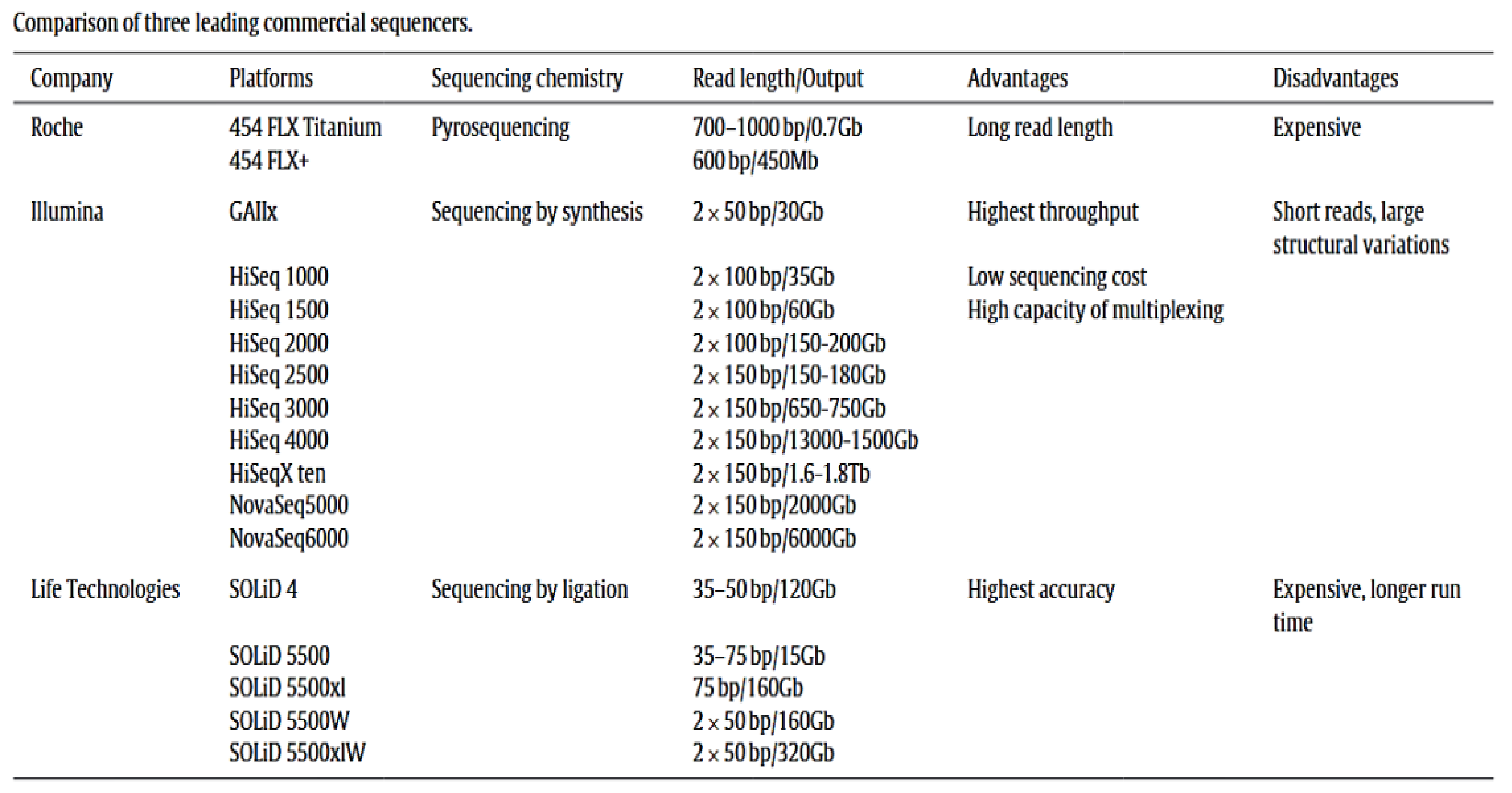


Figure 1: DNA metabarcoding methodology.

Citation: Pavan-Kumar A, Gireesh-Babu P, Lakra WS. DNA Metabarcoding: A New Approach for Rapid Biodiversity Assessment. J Cell Sci Molecul Biol. 2015;2(1): 111.

porovnanie sekvenačných platform



Comparison of three leading commercial sequencers.

Company	Platforms	Sequencing chemistry	Read length/Output	Advantages	Disadvantages
Roche	454 FLX Titanium	Pyrosequencing	700–1000 bp/0.7Gb	Long read length	Expensive
	454 FLX+		600 bp/450Mb		
Illumina	GAIIx	Sequencing by synthesis	2 × 50 bp/30Gb	Highest throughput	Short reads, large structural variations
	HiSeq 1000		2 × 100 bp/35Gb	Low sequencing cost	
	HiSeq 1500		2 × 100 bp/60Gb	High capacity of multiplexing	
	HiSeq 2000		2 × 100 bp/150-200Gb		
	HiSeq 2500		2 × 150 bp/150-180Gb		
	HiSeq 3000		2 × 150 bp/650-750Gb		
	HiSeq 4000		2 × 150 bp/13000-1500Gb		
	HiSeqX ten		2 × 150 bp/1.6-1.8Tb		
	NovaSeq5000		2 × 150 bp/2000Gb		
	NovaSeq6000		2 × 150 bp/6000Gb		
Life Technologies	SOLiD 4	Sequencing by ligation	35–50 bp/120Gb	Highest accuracy	Expensive, longer run time
	SOLiD 5500		35–75 bp/15Gb		
	SOLiD 5500xl		75 bp/160Gb		
	SOLiD 5500W		2 × 50 bp/160Gb		
	SOLiD 5500xlW		2 × 50 bp/320Gb		

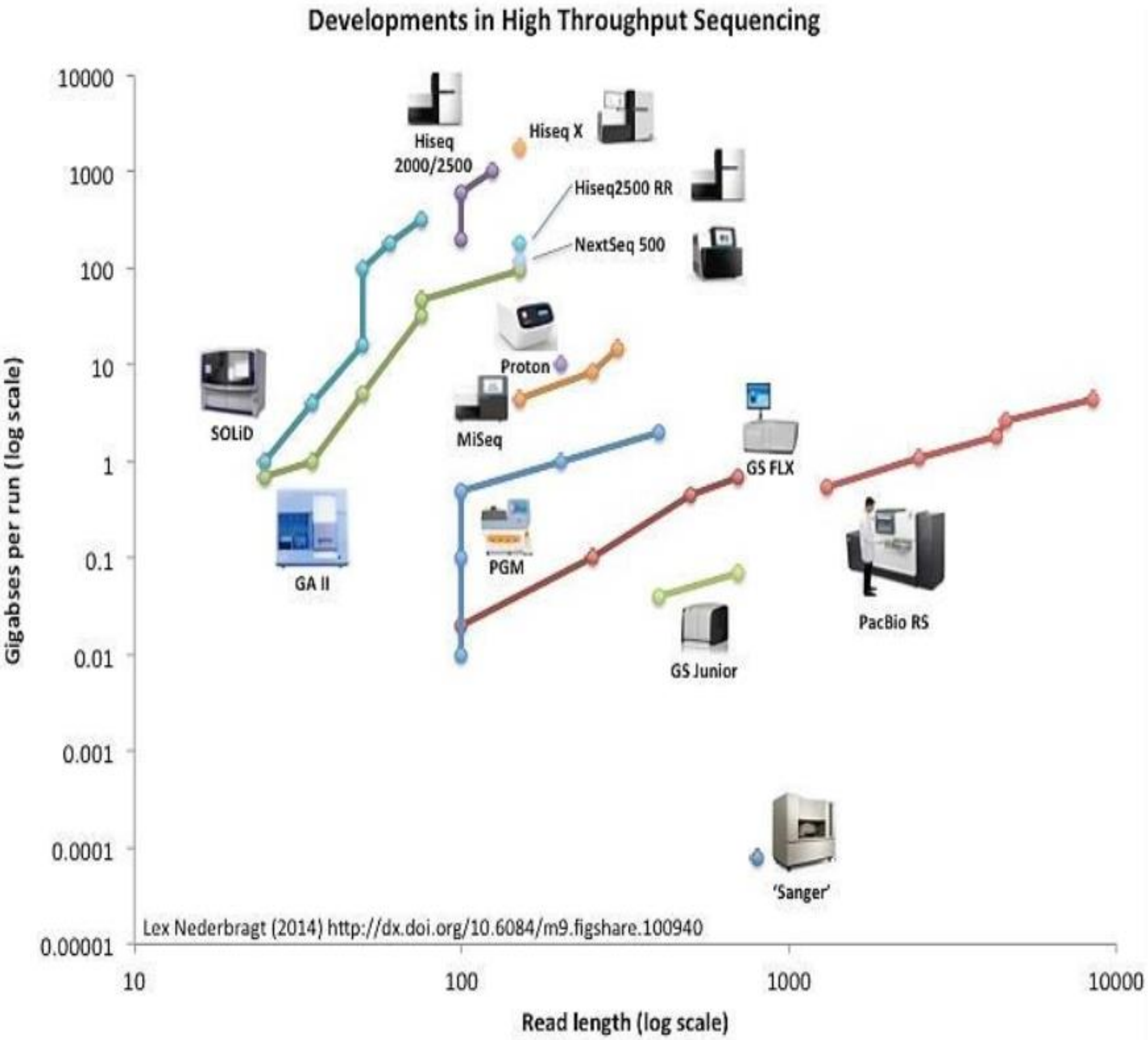
- Pillai, S.; Gopalan, V.; Lam, A.K. (2017) Review of sequencing platforms and their applications in phaeochromocytoma and paragangliomas. *Critical Reviews in Oncology/Hematology* **116:** 58–67.

Summarising:

Roche 454:
GS Junior,
GS FLX

Illumina:
GA II,
HiSeq series,
MiSeq,
NextSeq

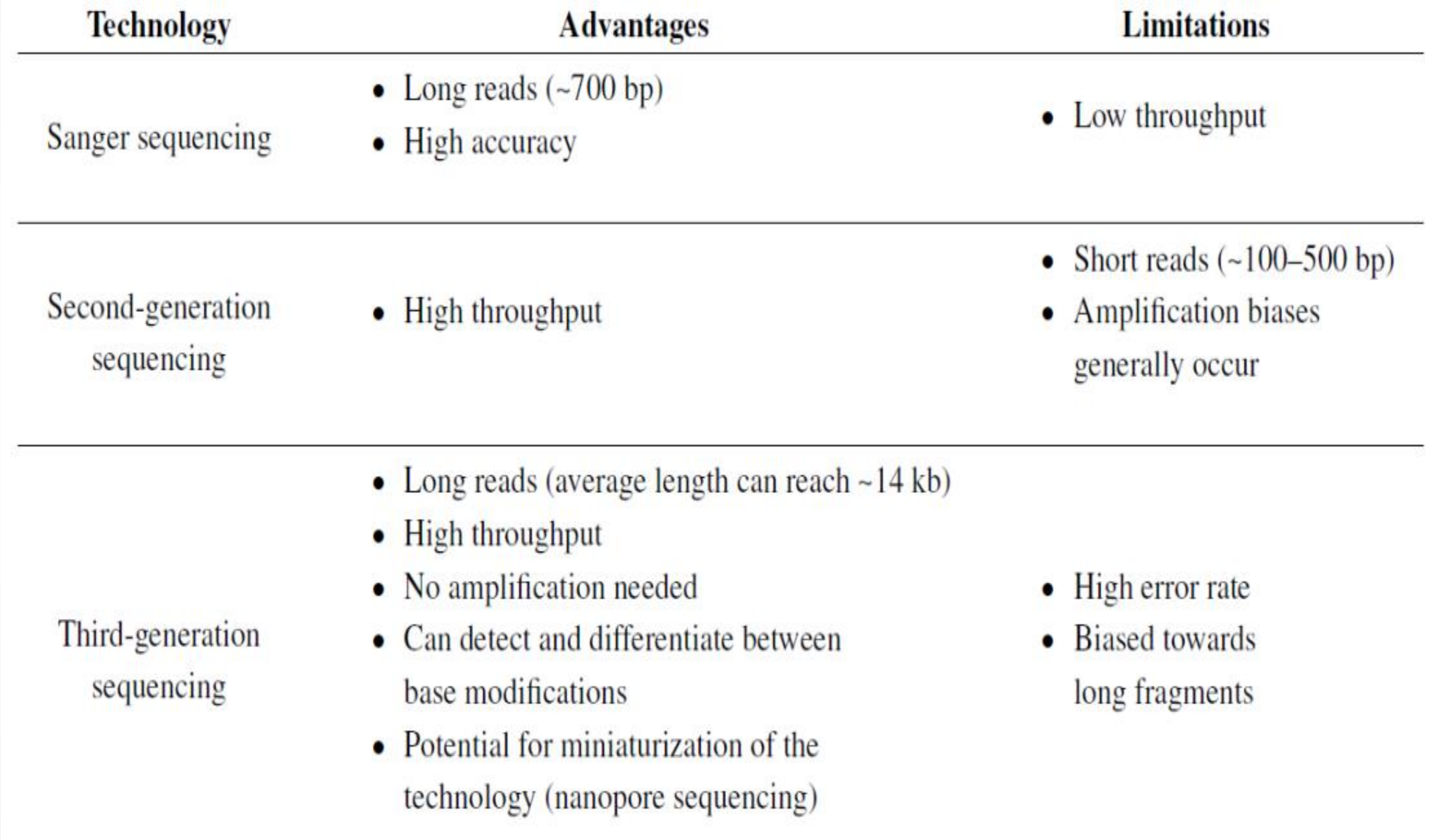
Thermo
Fisher -
Ion Torrent:
PGM,
Proton



Developments in High Throughput Sequencing

Lex Nederbragt (2014) http://dx.doi.org/10.6084/m9.figshare.100940

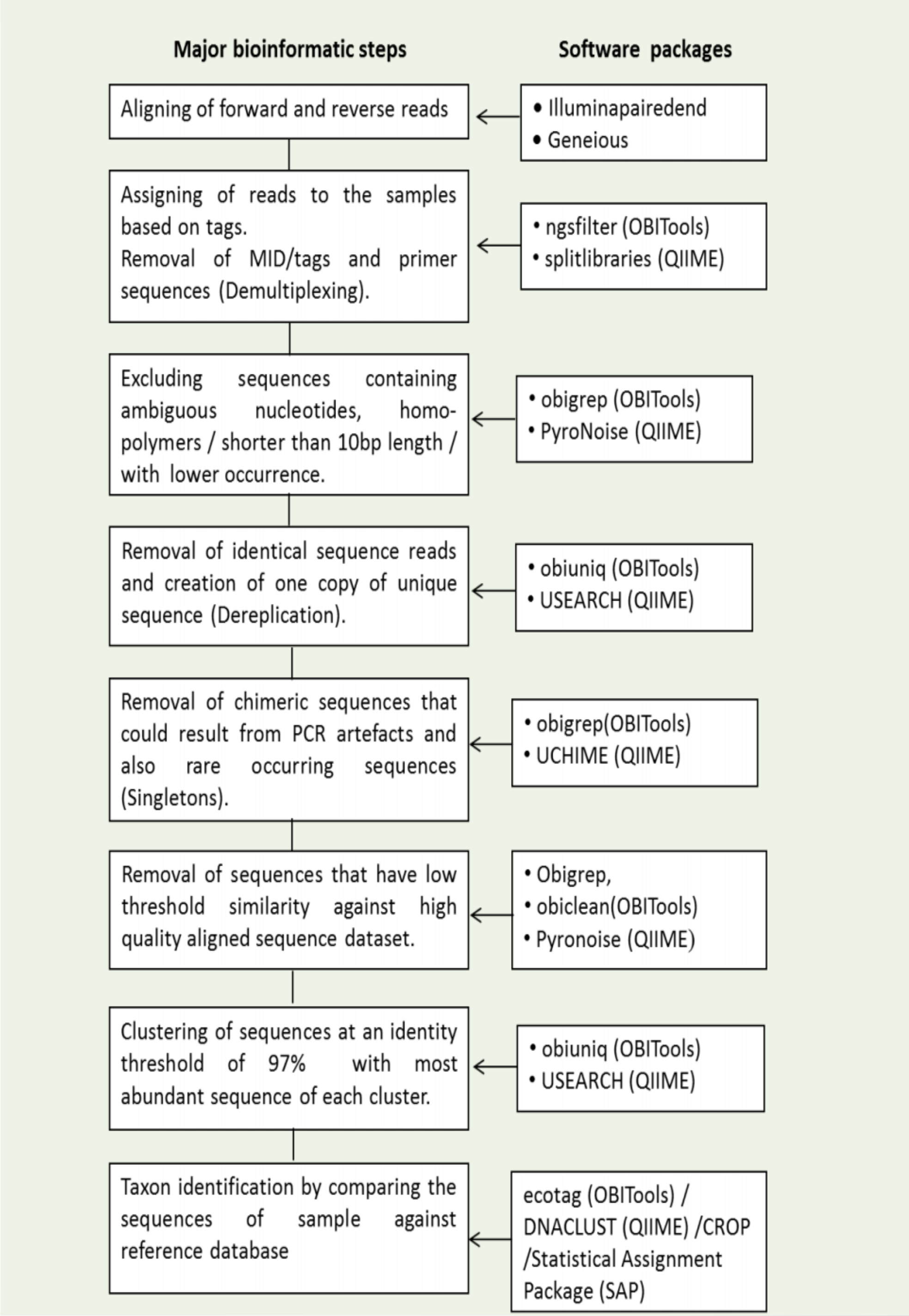
porovnanie sekvenačných postupov



Technology	Advantages	Limitations
Sanger sequencing	• Long reads (~700 bp) • High accuracy	• Low throughput
Second-generation sequencing	• High throughput	• Short reads (~100–500 bp) • Amplification biases generally occur
Third-generation sequencing	• Long reads (average length can reach ~14 kb) • High throughput • No amplification needed • Can detect and differentiate between base modifications • Potential for miniaturization of the technology (nanopore sequencing)	• High error rate • Biased towards long fragments

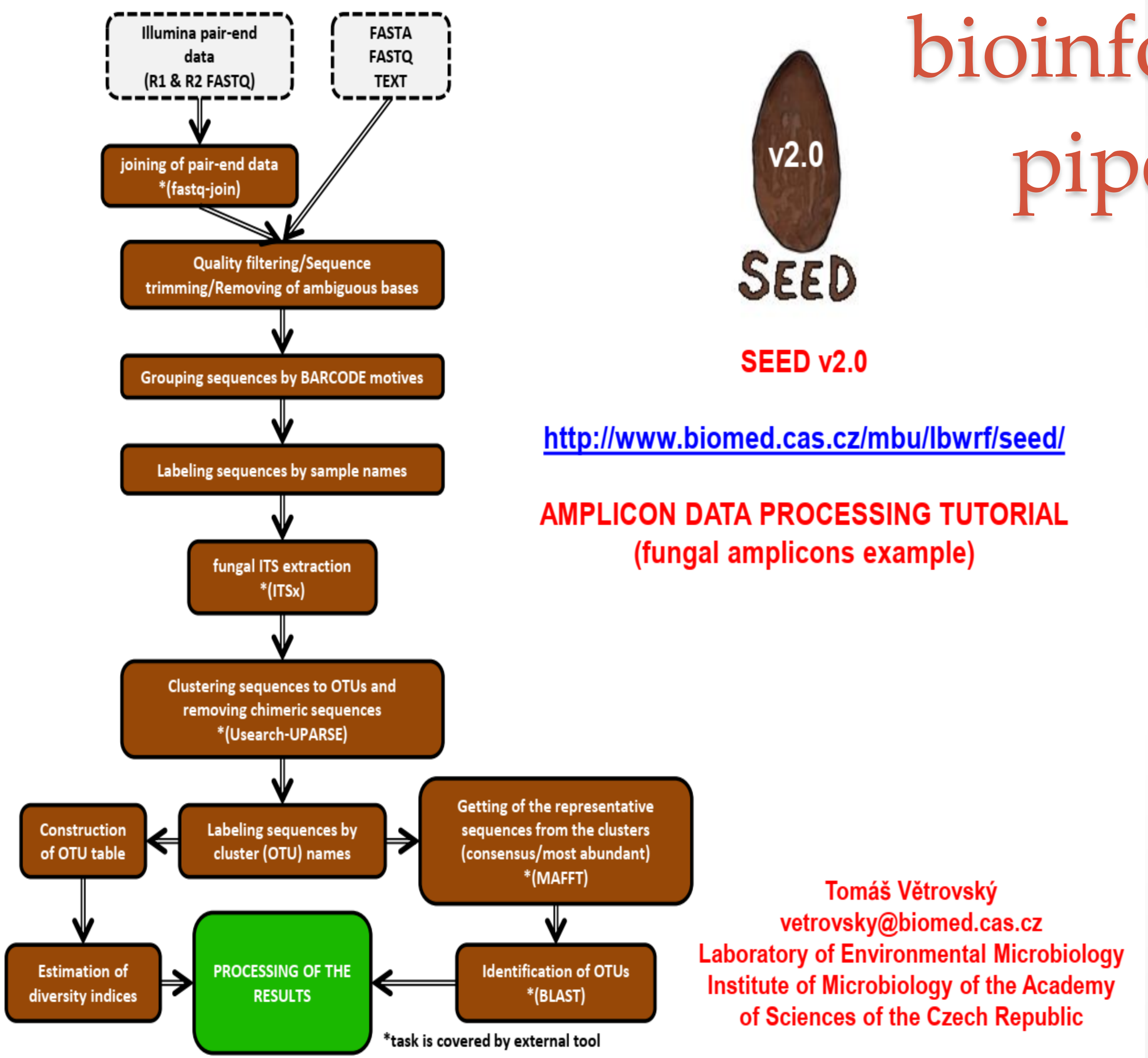
LeBlanc, V.G. & Marra, M.A. (2015) Next-Generation Sequencing Approaches in Cancer: Where Have They Brought Us and Where Will They Take Us? *Cancers* **7(3):** 1925-1958.

bionformatická analýza



Major bioinformatic steps	Software packages
Aligning of forward and reverse reads	• Illuminapairedend • Geneious
Assigning of reads to the samples based on tags. Removal of MID/tags and primer sequences (Demultiplexing).	• ngsfilter (OBITools) • splitlibraries (QIIME)
Excluding sequences containing ambiguous nucleotides, homo-polymers / shorter than 10bp length / with lower occurrence.	• obigrep (OBITools) • PyroNoise (QIIME)
Removal of identical sequence reads and creation of one copy of unique sequence (Dereplication).	• obiuniq (OBITools) • USEARCH (QIIME)
Removal of chimeric sequences that could result from PCR artefacts and also rare occurring sequences (Singletons).	• obigrep(OBITools) • UCHIME (QIIME)
Removal of sequences that have low threshold similarity against high quality aligned sequence dataset.	• Obigrep, • obiclean(OBITools) • Pyronoise (QIIME)
Clustering of sequences at an identity threshold of 97% with most abundant sequence of each cluster.	• obiuniq (OBITools) • USEARCH (QIIME)
Taxon identification by comparing the sequences of sample against reference database	ecotag (OBITools) / DNACLUST (QIIME) /CROP /Statistical Assignment Package (SAP)

bioinformatic pipeline



Illumina pair-end data (R1 & R2 FASTQ)

FASTA
FASTQ
TEXT

joining of pair-end data *(fastq-join)

Quality filtering/Sequence trimming/Removing of ambiguous bases

Grouping sequences by BARCODE motives

Labeling sequences by sample names

fungal ITS extraction *(ITSx)

Clustering sequences to OTUs and removing chimeric sequences *(Usearch-UPARSE)

Labeling sequences by cluster (OTU) names

Construction of OTU table

Getting of the representative sequences from the clusters (consensus/most abundant) *(MAFFT)

Estimation of diversity indices

Identification of OTUs *(BLAST)

PROCESSING OF THE RESULTS

*task is covered by external tool

v2.0
SEED

SEED v2.0

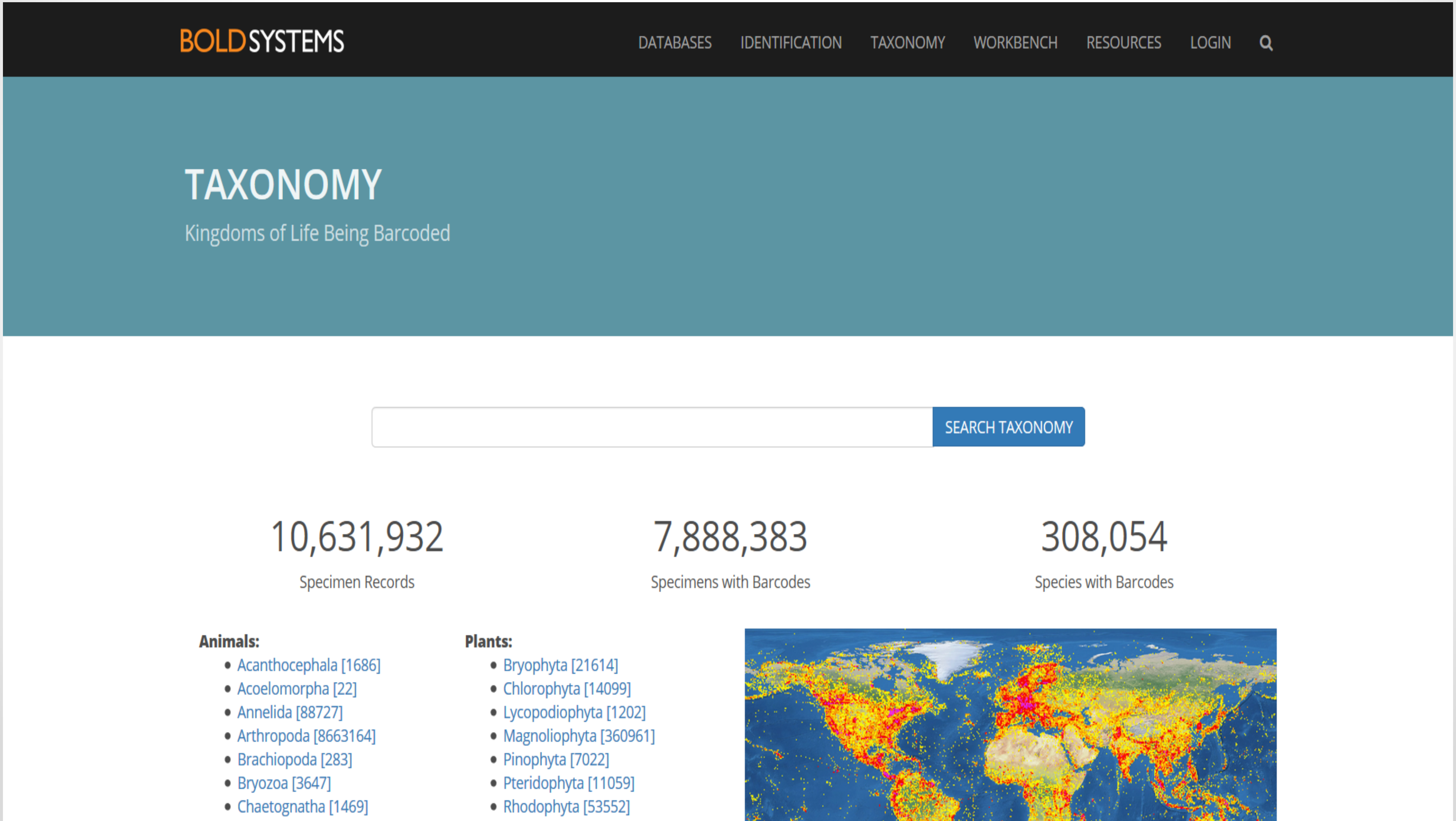
http://www.biomed.cas.cz/mbu/lbwrf/seed/

AMPLICON DATA PROCESSING TUTORIAL
(fungal amplicons example)

Tomáš Větrovský
vetrovsky@biomed.cas.cz
Laboratory of Environmental Microbiology
Institute of Microbiology of the Academy
of Sciences of the Czech Republic

barcoding databáza všeobecná

Barcode of Life Data System



TAXONOMY

Kingdoms of Life Being Barcoded

10,631,932 Specimen Records

7,888,383 Specimens with Barcodes

308,054 Species with Barcodes

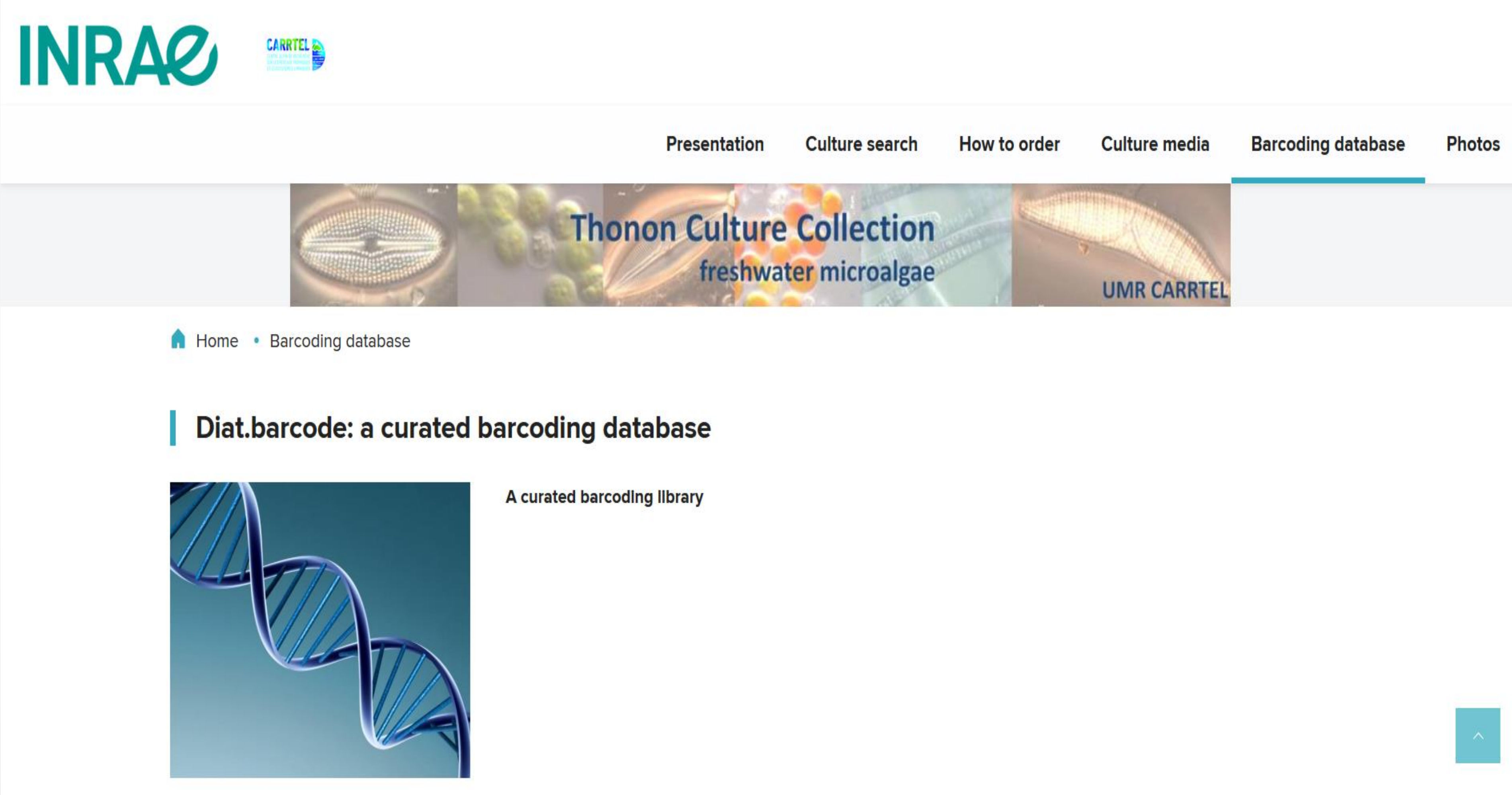
Animals:

- Acanthocephala [1686]
- Acoelomorpha [22]
- Annelida [88727]
- Arthropoda [8663164]
- Brachiopoda [283]
- Bryozoa [3647]
- Chaetognatha [1469]

Plants:

- Bryophyta [21614]
- Chlorophyta [14099]
- Lycopodiophyta [1202]
- Magnoliophyta [360961]
- Pinophyta [7022]
- Pteridophyta [11059]
- Rhodophyta [53552]

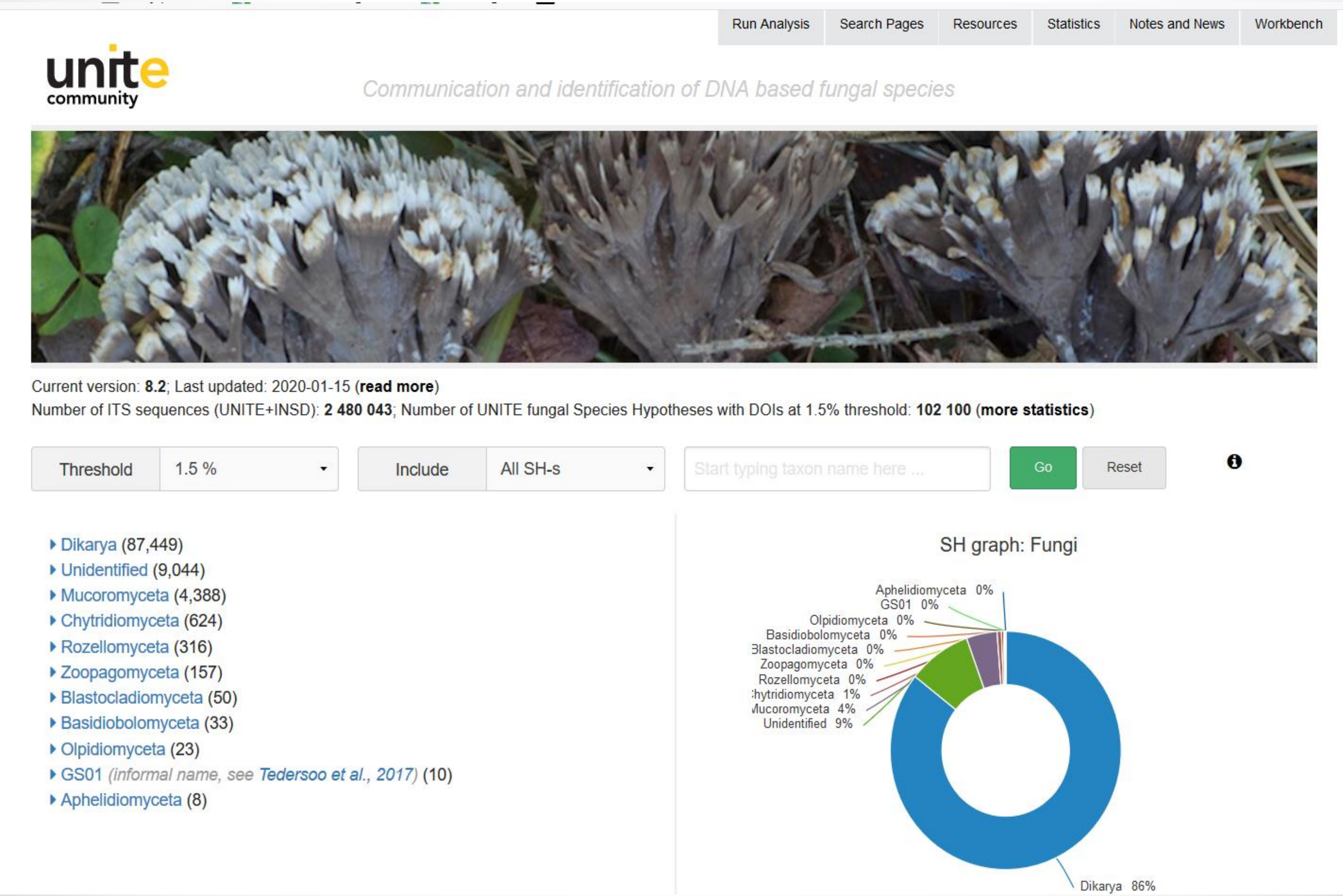
špecializovaná barcoding databáza pre rozsievky: Diat.barcode



Diat.barcode: a curated barcoding database

A curated barcoding library

barcode huby: UNITE database



Communication and identification of DNA based fungal species

Current version: **8.2**; Last updated: 2020-01-15 (**read more**)

Number of ITS sequences (UNITE+INSD): **2 480 043**; Number of UNITE fungal Species Hypotheses with DOIs at 1.5% threshold: **102 100** (**more statistics**)

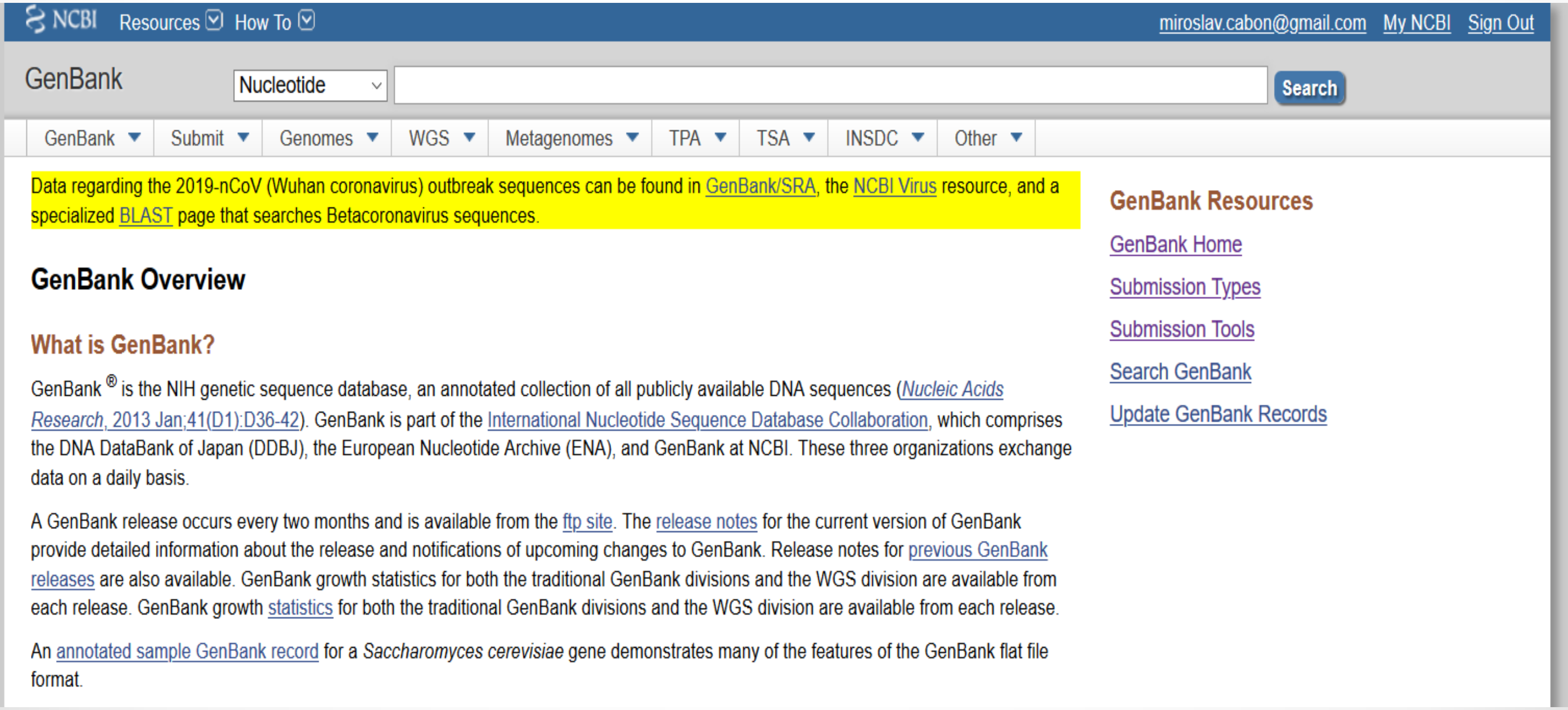
Threshold: 1.5 %

Include: All SH-s

- Dikarya (87,449)
- Unidentified (9,044)
- Mucoromyceta (4,388)
- Chytridiomyceta (624)
- Rozellomyceta (316)
- Zoopagomyceta (157)
- Blastocladiomyceta (50)
- Basidiobolomyceta (33)
- Olpidiomyceta (23)
- GS01 *(informal name, see Tedersoo et al., 2017)* (10)
- Aphelidiomyceta (8)

SH graph: Fungi

GenBank všeobecná databáza sekvencií používaná ako barcode



Data regarding the 2019-nCoV (Wuhan coronavirus) outbreak sequences can be found in GenBank/SRA, the NCBI Virus resource, and a specialized BLAST page that searches Betacoronavirus sequences.

GenBank Overview

What is GenBank?

GenBank ® is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences (*Nucleic Acids Research*, 2013 Jan;41(D1):D36-42). GenBank is part of the International Nucleotide Sequence Database Collaboration, which comprises the DNA DataBank of Japan (DDBJ), the European Nucleotide Archive (ENA), and GenBank at NCBI. These three organizations exchange data on a daily basis.

A GenBank release occurs every two months and is available from the ftp site. The release notes for the current version of GenBank provide detailed information about the release and notifications of upcoming changes to GenBank. Release notes for previous GenBank releases are also available. GenBank growth statistics for both the traditional GenBank divisions and the WGS division are available from each release. GenBank growth statistics for both the traditional GenBank divisions and the WGS division are available from each release.

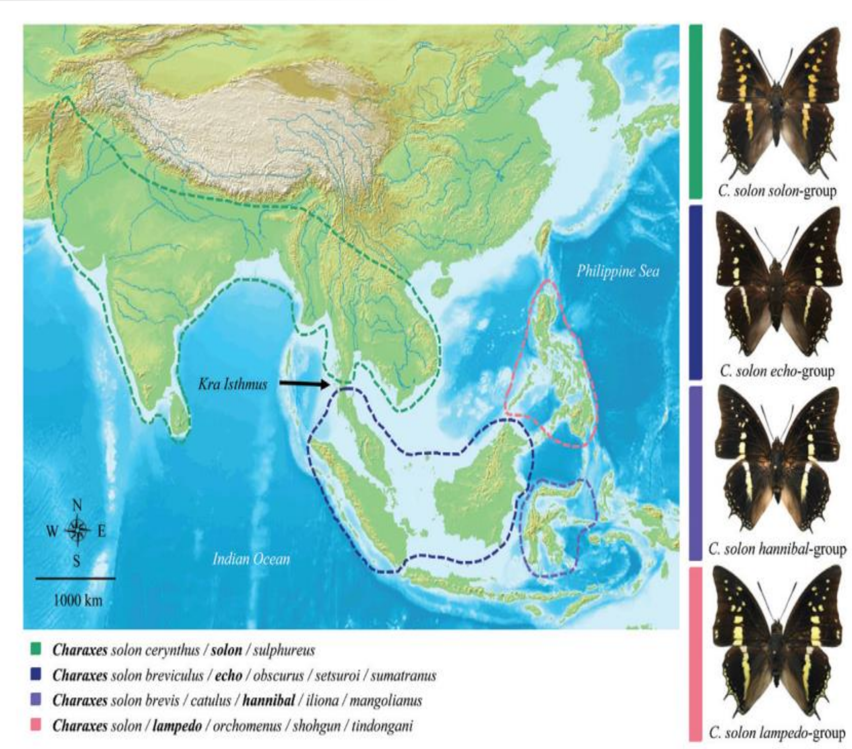
An annotated sample GenBank record for a *Saccharomyces cerevisiae* gene demonstrates many of the features of the GenBank flat file format.

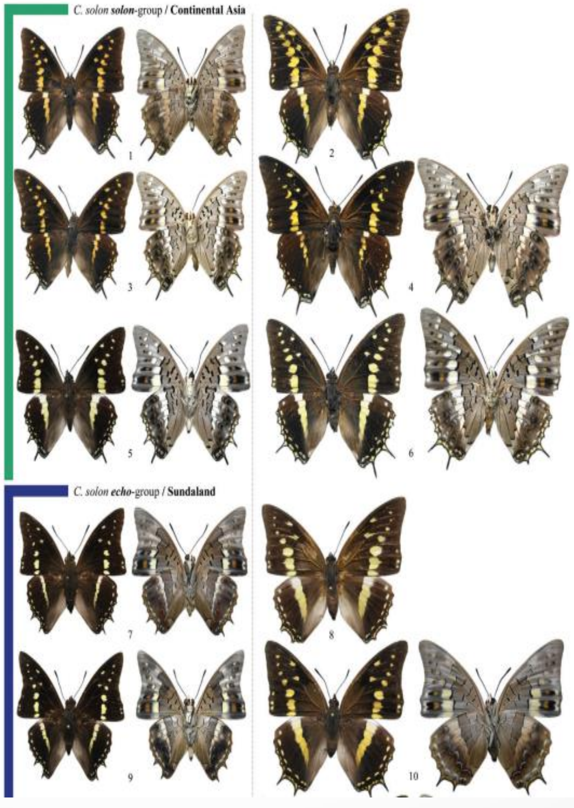
GenBank Resources

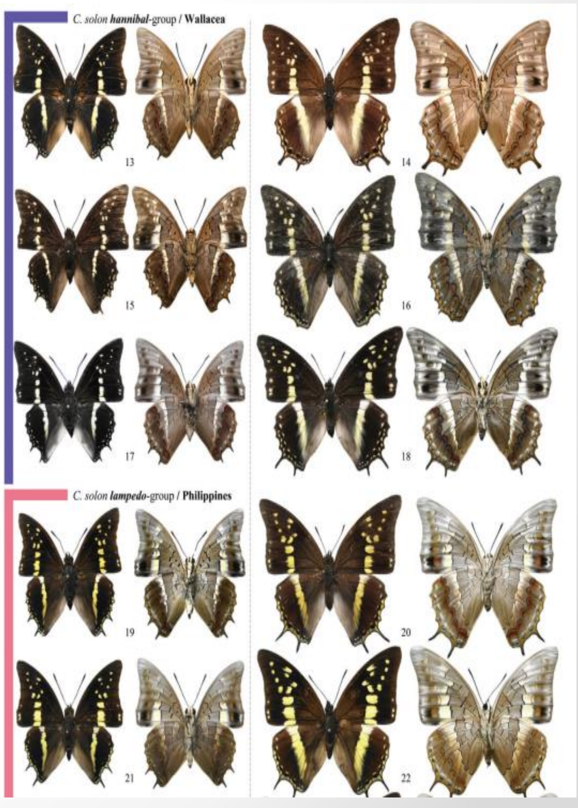
- GenBank Home
- Submission Types
- Submission Tools
- Search GenBank
- Update GenBank Records

výhody metabarcoding

- jednoduchá identifikácia skrytých (cryptic) druhov aj v zmesných vzorkách environmentálnej DNA
- Identifikácia je možná aj v štádiách životných cyklov kde nie sú dostupné diagnostické znaky
- Toussaint et al. 2019







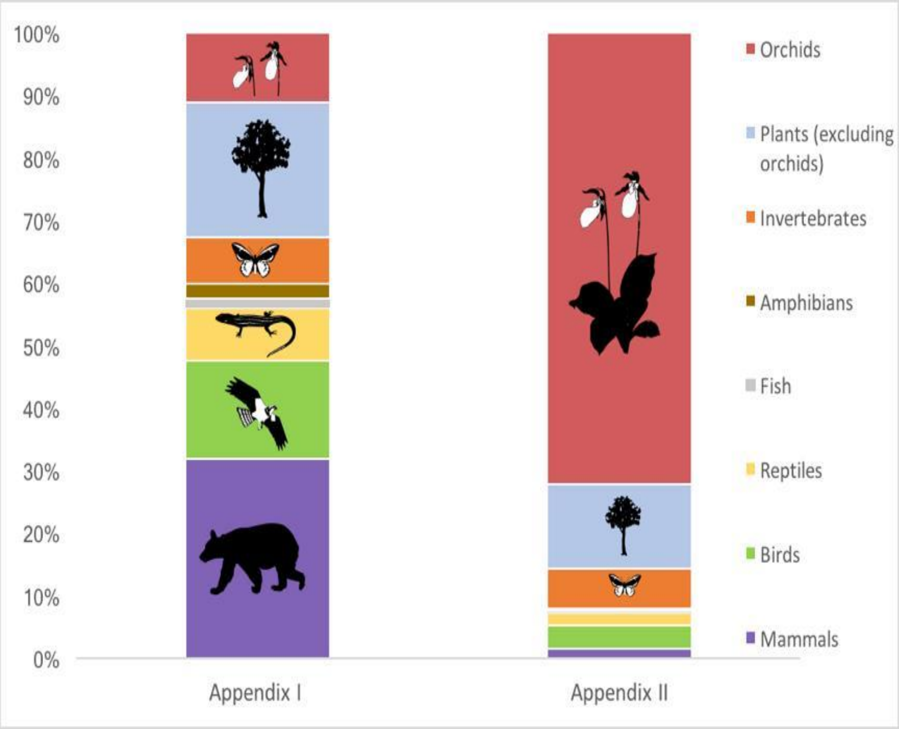
možné využitie v ochrane prírody

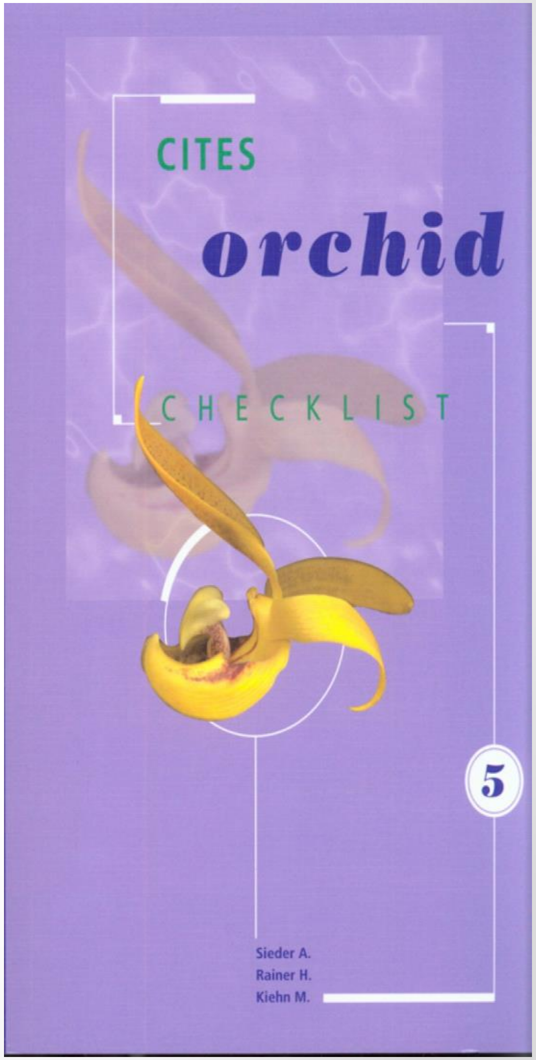
- identifikácia of CITES druhov

- Cooper et al. 2019

Identification of CITES-listed Tarantulas

Aphonopelma, Brachypelma and Sericopelma species





CITES
orchid
CHECKLIST
5

Sieder A.
Rainer H.
Kiehn M.

možné využitie v ochrane prírody

detekcia inváznych druhov


823

Detection of invasive freshwater fish in British Columbia lakes, using eDNA metabarcoding

Davon Callander, Matthias Herborg, Cathryn Abbott
Fisheries and Oceans Canada, Pacific Biological Station, Nanaimo B.C.

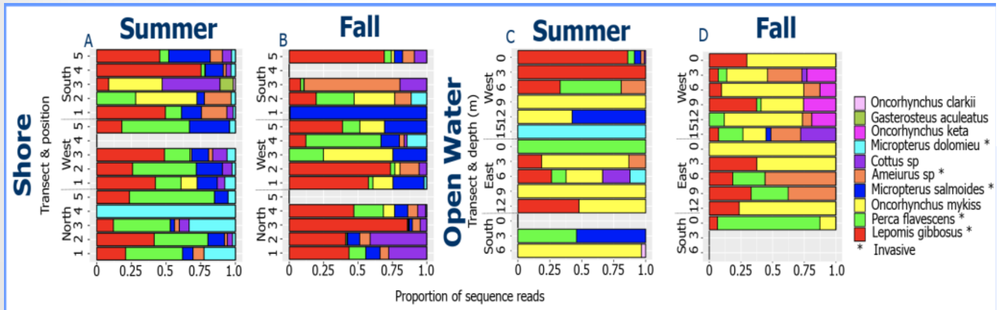
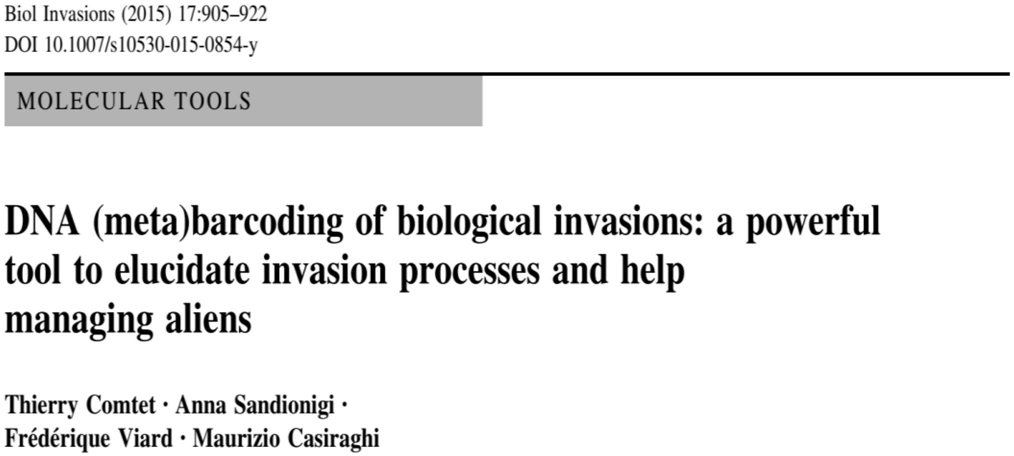


Figure 4. Proportion of metabarcoding sequence reads per taxon in eDNA samples analyzed.


Biol Invasions (2015) 17:905–922
DOI 10.1007/s10530-015-0854-y

MOLECULAR TOOLS

DNA (meta)barcoding of biological invasions: a powerful tool to elucidate invasion processes and help managing aliens

Thierry Comtet · Anna Sandionigi ·
Frédérique Viard · Maurizio Casiraghi


Micropterus dolomieau

Ameiurus


Micropterus salmoides

Perca flavescens


Lepomis gibbosus

možné využitie v ochrane prírody

rekonštrukcie potravinových reťazcov

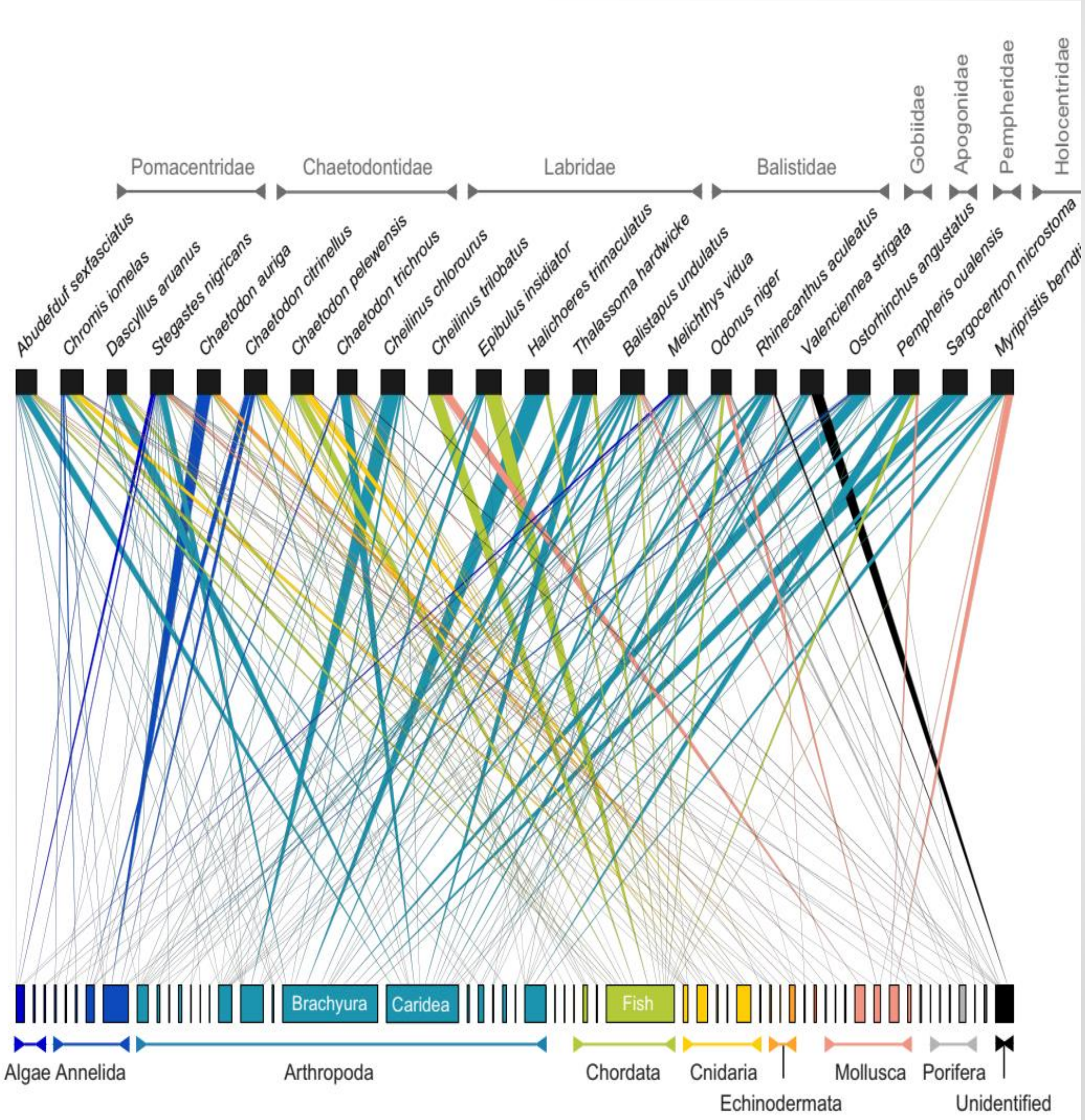
zdroj eDNA: obsah žalúdka koralov

DOI: 10.1111/2041-210X.13206

RESEARCH ARTICLE

Reconstructing hyperdiverse food webs: Gut content metabarcoding as a tool to disentangle trophic interactions on coral reefs

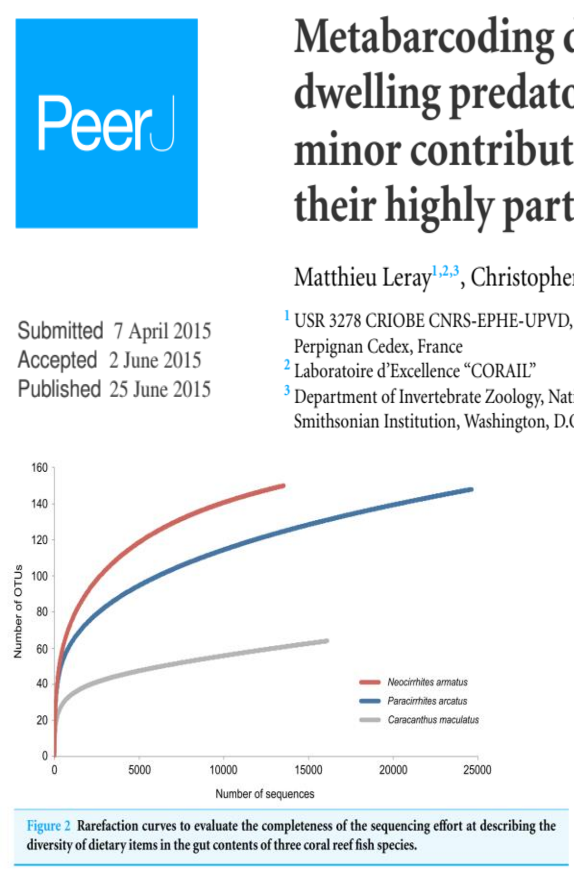
Jordan M. Casey[1,2,3] | Christopher P. Meyer[3] | Fabien Morat[1,2] | Simon J. Brandl[4] | Serge Planes[1,2] | Valeriano Parravicini[1,2]



možné využitie v ochrane prírody

rekonštrukcie potravinových reťazcov

zdroj eDNA: obsah žalúdka morských rýb



PeerJ

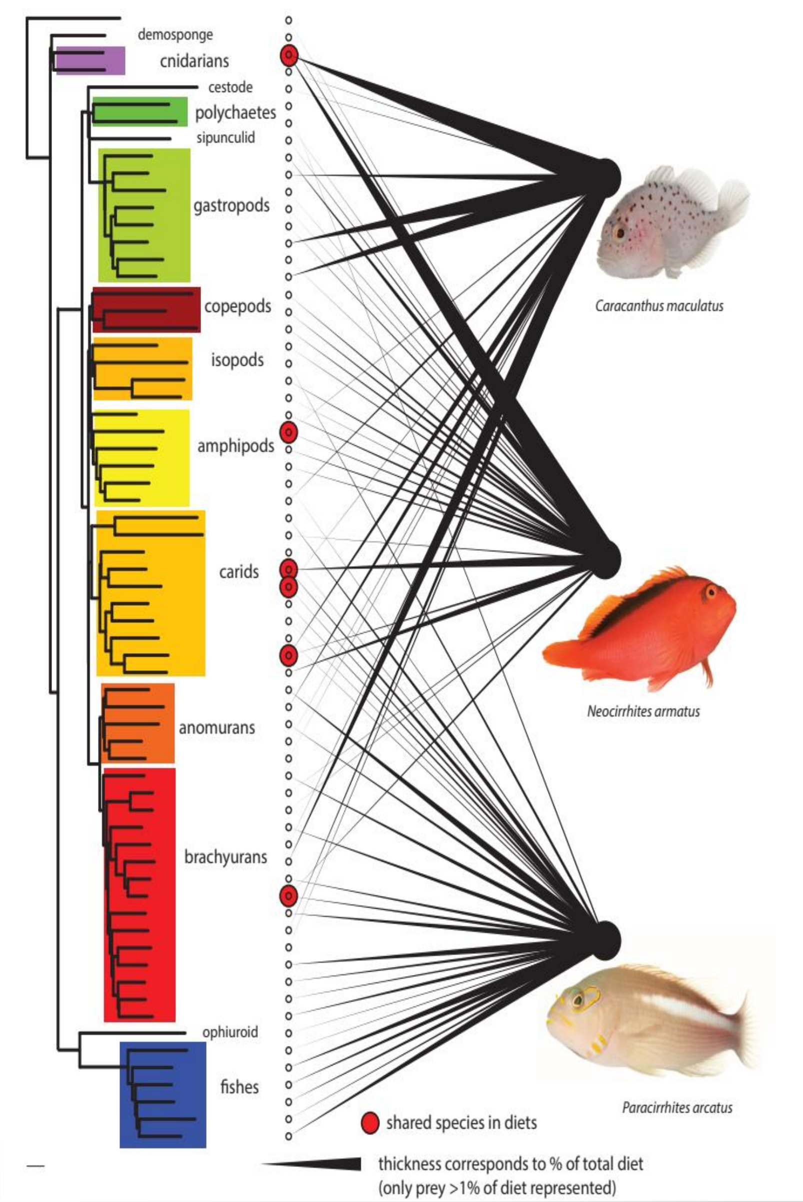
Metabarcoding dietary analysis of coral dwelling predatory fish demonstrates the minor contribution of coral mutualists to their highly partitioned, generalist diet

Matthieu Leray[1,2,3], Christopher P. Meyer[3] and Suzanne C. Mills[1,2]

[1] USR 3278 CRIOBE CNRS-EPHE-UPVD, CBETM de l'Université de Perpignan, Perpignan Cedex, France
[2] Laboratoire d'Excellence "CORAIL"
[3] Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA

Submitted 7 April 2015
Accepted 2 June 2015
Published 25 June 2015

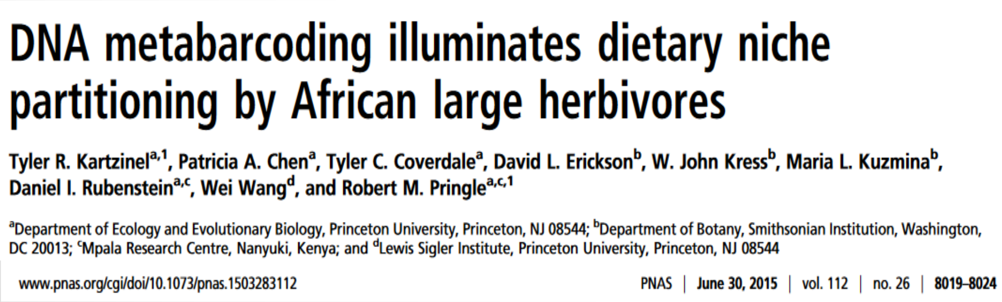
Figure 2 Rarefaction curves to evaluate the completeness of the sequencing effort at describing the diversity of dietary items in the gut contents of three coral reef fish species.



možné využitie v ochrane prírody

rekonštrukcie potravinových reťazcov

zdroj eDNA: výkaly

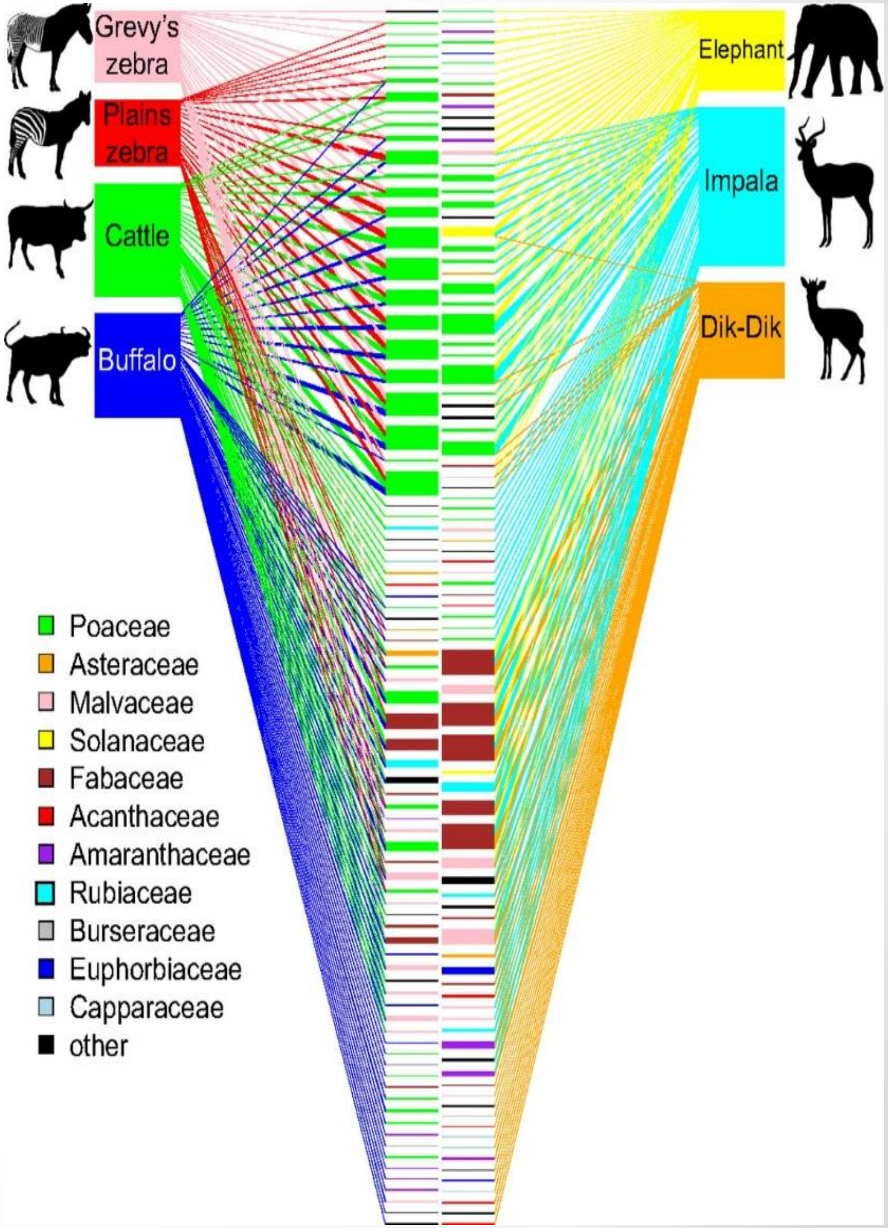


DNA metabarcoding illuminates dietary niche partitioning by African large herbivores

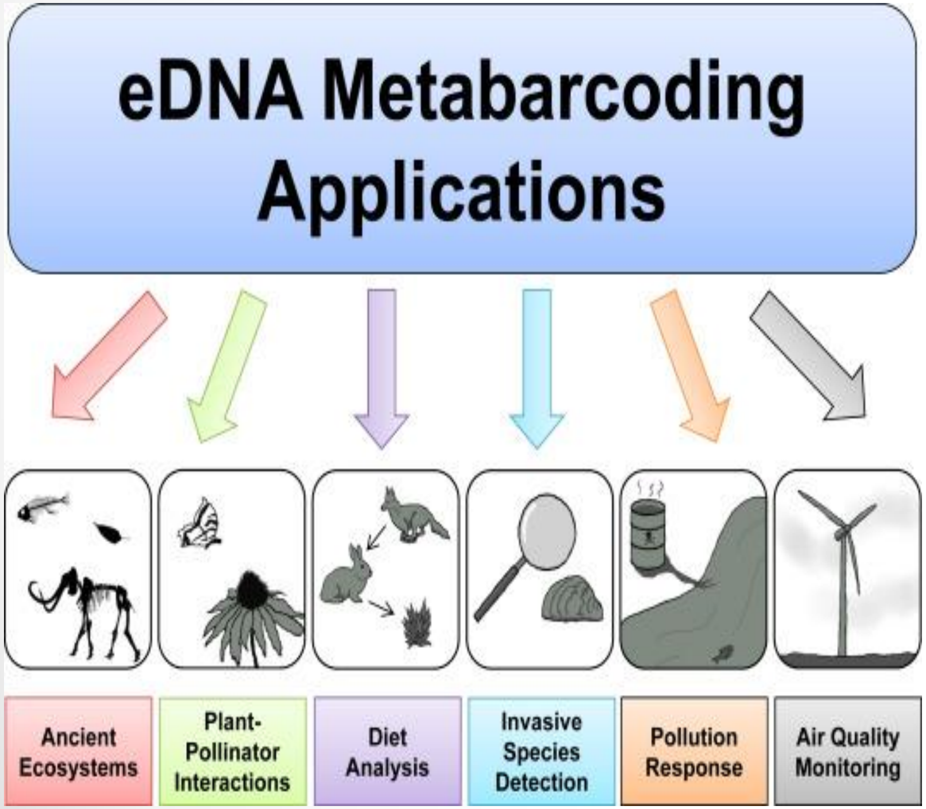
Tyler R. Kartzinel[a,1], Patricia A. Chen[a], Tyler C. Coverdale[a], David L. Erickson[b], W. John Kress[b], Maria L. Kuzmina[b], Daniel I. Rubenstein[a,c], Wei Wang[d], and Robert M. Pringle[a,c,1]

[a]Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544; [b]Department of Botany, Smithsonian Institution, Washington, DC 20013; [c]Mpala Research Centre, Nanyuki, Kenya; and [d]Lewis Sigler Institute, Princeton University, Princeton, NJ 08544

www.pnas.org/cgi/doi/10.1073/pnas.1503283112 PNAS | June 30, 2015 | vol. 112 | no. 26 | 8019–8024



možné využitie v ochrane prírody



eDNA Metabarcoding Applications

Ancient Ecosystems

Plant-Pollinator Interactions

Diet Analysis

Invasive Species Detection

Pollution Response

Air Quality Monitoring

Global Ecology and Conservation 17 (2019) e00547

Contents lists available at ScienceDirect

Global Ecology and Conservation

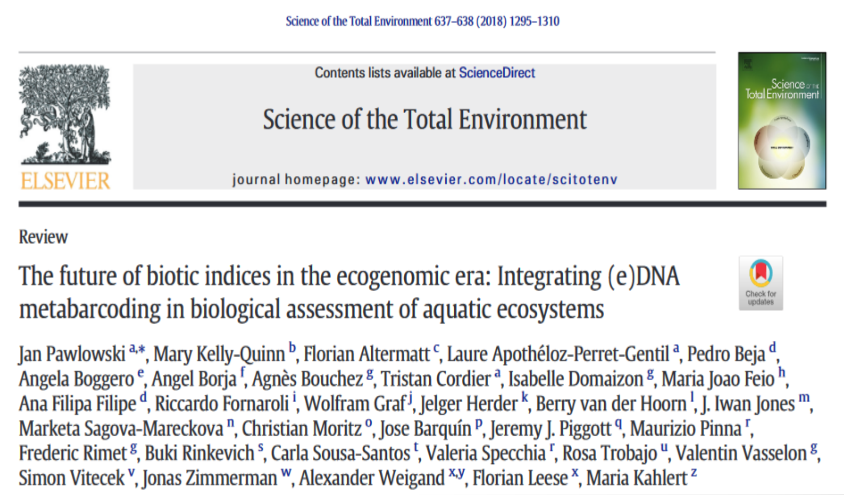
journal homepage: http://www.elsevier.com/locate/gecco

Review Paper

Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA

Krista M. Ruppert [a, *], Richard J. Kline [a, b, **], Md Saydur Rahman [a, b, ***]

[a] School of Earth, Environmental, and Marine Sciences, University of Texas Rio Grande Valley, Brownsville, TX, 78520, USA
[b] Department of Biology, University of Texas Rio Grande Valley, Brownsville, TX, 78520, USA



Science of the Total Environment 637–638 (2018) 1295–1310

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Review

The future of biotic indices in the ecogenomic era: Integrating (e)DNA metabarcoding in biological assessment of aquatic ecosystems

Jan Pawlowski [a,*], Mary Kelly-Quinn [b], Florian Altermatt [c], Laure Apothéloz-Perret-Gentil [a], Pedro Beja [d], Angela Boggero [e], Angel Borja [f], Agnès Bouchez [g], Tristan Cordier [a], Isabelle Domaizon [g], Maria Joao Feio [h], Ana Filipa Filipe [d], Riccardo Fornaroli [i], Wolfram Graf [j], Jelger Herder [k], Berry van der Hoorn [l], J. Iwan Jones [m], Marketa Sagova-Mareckova [n], Christian Moritz [o], Jose Barquín [p], Jeremy J. Piggott [q], Maurizio Pinna [r], Frederic Rimet [g], Buki Rinkevich [s], Carla Sousa-Santos [t], Valeria Specchia [r], Rosa Trobajo [u], Valentin Vasselon [g], Simon Vitecek [v], Jonas Zimmerman [w], Alexander Weigand [x,y], Florian Leese [x], Maria Kahlert [z]

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Water Research
Volume 138, 1 July 2018, Pages 192-205

Review

Implementation options for DNA-based identification into ecological status assessment under the European Water Framework Directive

Daniel Hering, Angel Borja, J.Iwan Jones, Didier Pont, Pieter Boets, Agnes Bouchez, Kat Bruce, Stina Drakare, Bernd Hänfling, Maria Kahlert, Florian Leese, Kristian Meissner, Patricia Mergen, Yorick Reyjol, Pedro Segurado, Alfried Vogler, Martyn Kelly

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https://doi.org/10.1016/j.watres.2018.03.003

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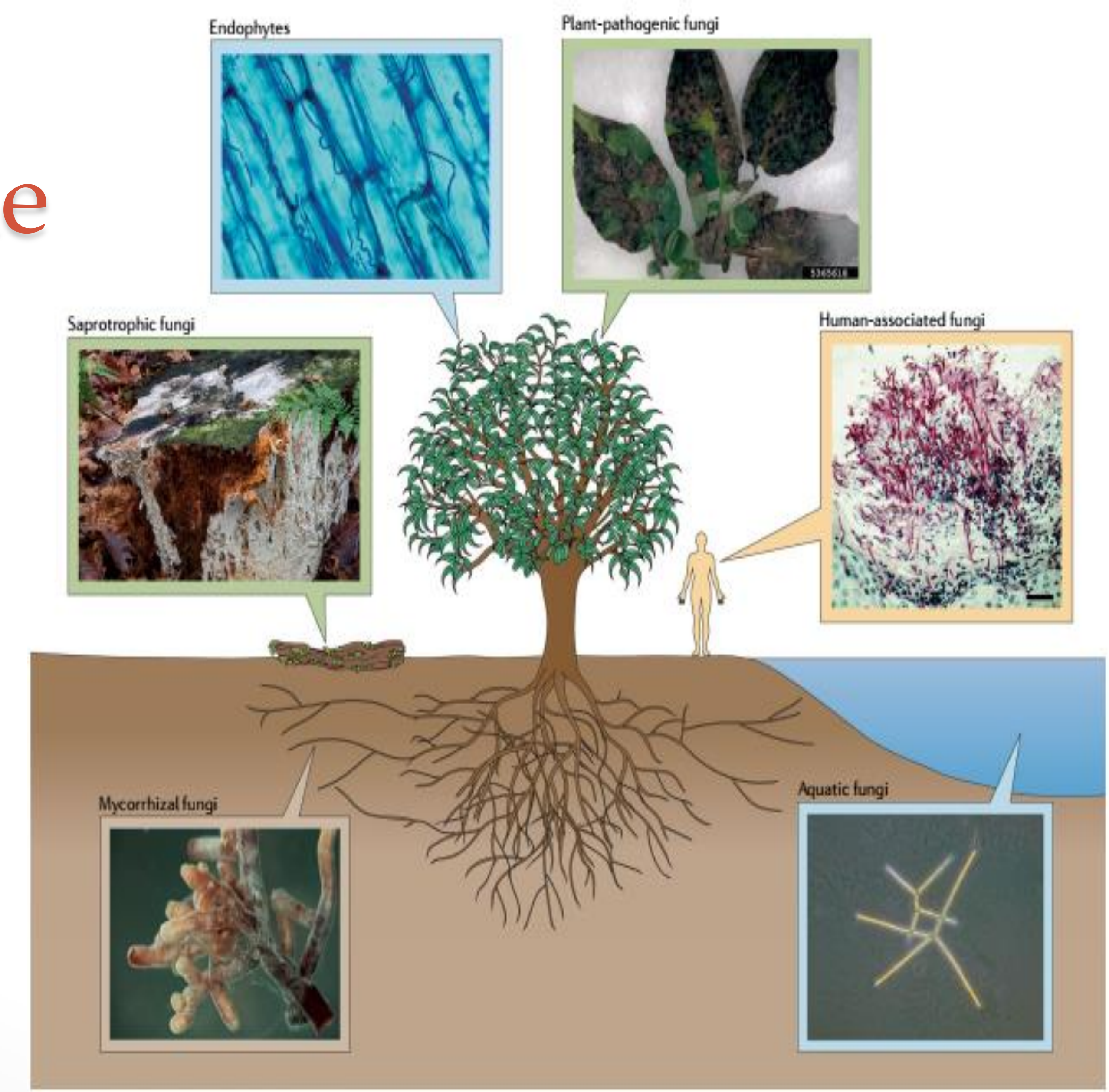
problémy a výzvy metabarcoding

- neumožňuje získať dáta o pohlaví, veku, zdravotnom stave jedincov v študovaných spoločenstvách
- DNA v niektorých typoch environmentálnych vzoriek (napr. vývžky a exkrementy)degraduje
- použitím všeobecných primerov, DNA sa môže amplifikovať DNA rôznych skupín organizmov dominantne či okrajovo (disproporčne)
- nemožná alebo zložitá kvantifikácia DNA

huby v ekosystéme

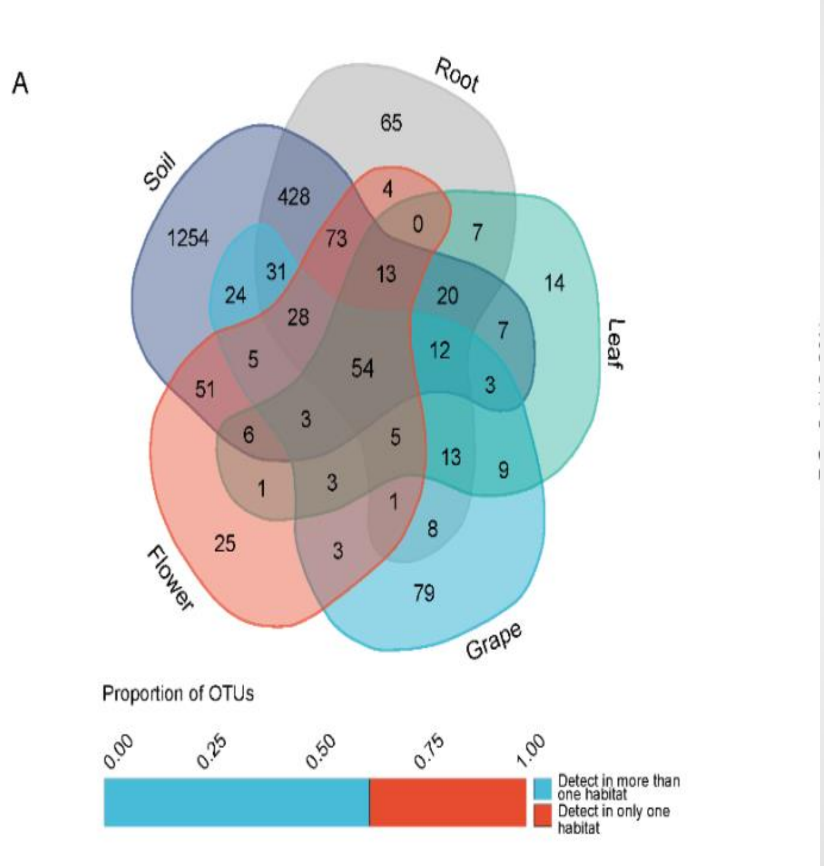


kritické kroky v príprave štúdií založených na HTS sekvenovaní húb

sampling a príprava vzoriek

niektoré huby (napríklad enkapsulované kvasinky) sú zvlášť bohaté na polysacharidy a príprava vzoriek môže vyžadovať špeciálne postupy (Huang et al. 2018)

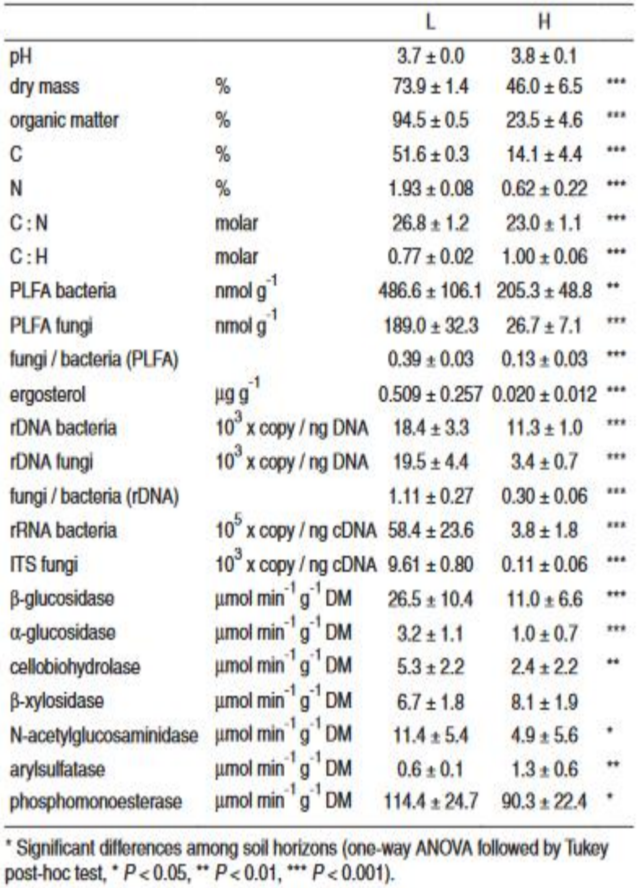
optimalizácia prípravy vzoriek pre určité substráty akými sú pôda, rastlinné pletivá, alebo voda je často potrebná (Nilsson et al. 2018)



Liu & Howell 2021

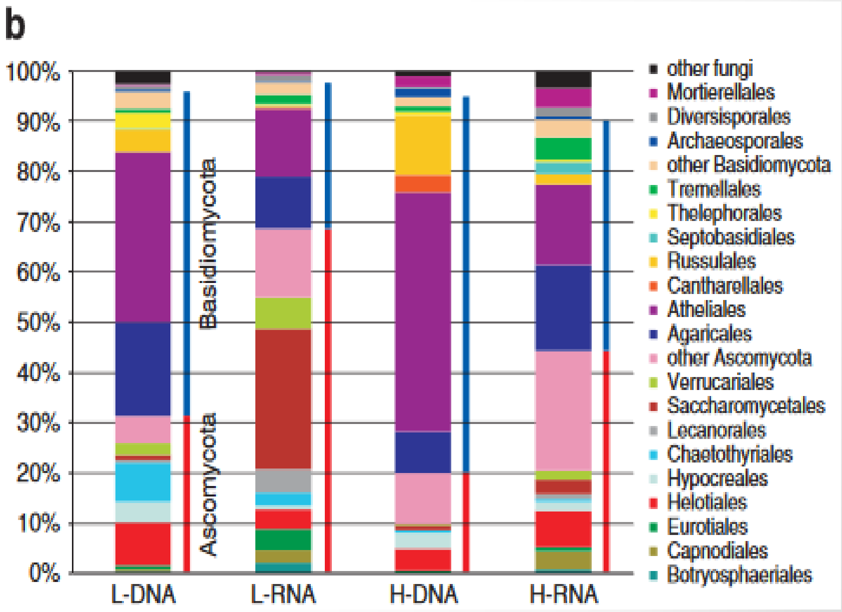
príprava vzoriek pôdných húb

stratifikácia pôdy a s tým súvisiaca zmena spoločenstva húb a jeho funkcie



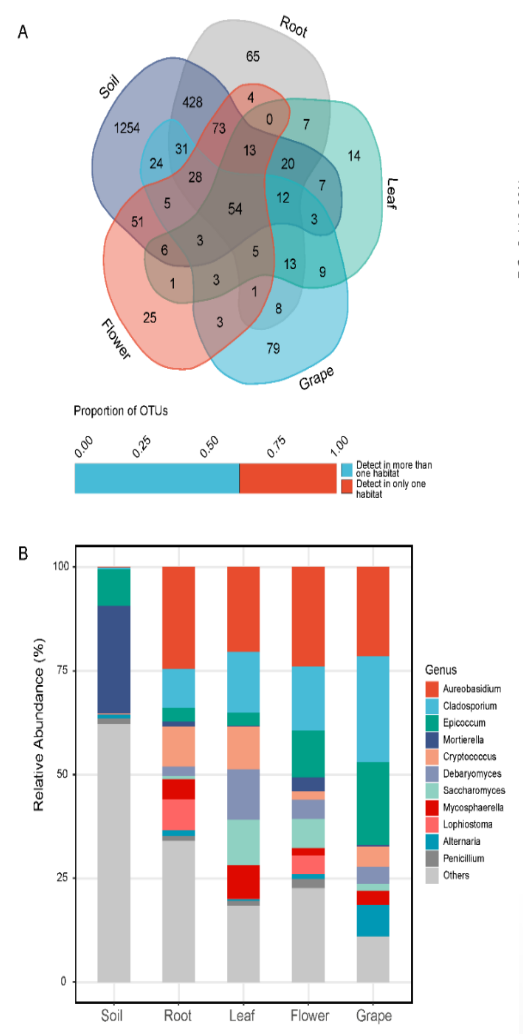
		L	H	
pH		3.7 ± 0.0	3.8 ± 0.1	
dry mass	%	73.9 ± 1.4	46.0 ± 6.5	***
organic matter	%	94.5 ± 0.5	23.5 ± 4.6	***
C	%	51.6 ± 0.3	14.1 ± 4.4	***
N	%	1.93 ± 0.08	0.62 ± 0.22	***
C : N	molar	26.8 ± 1.2	23.0 ± 1.1	***
C : H	molar	0.77 ± 0.02	1.00 ± 0.06	***
PLFA bacteria	nmol g^{-1}	486.6 ± 106.1	205.3 ± 48.8	**
PLFA fungi	nmol g^{-1}	189.0 ± 32.3	26.7 ± 7.1	***
fungi / bacteria (PLFA)		0.39 ± 0.03	0.13 ± 0.03	***
ergosterol	µg g^{-1}	0.509 ± 0.257	0.020 ± 0.012	***
rDNA bacteria	10^3 x copy / ng DNA	18.4 ± 3.3	11.3 ± 1.0	***
rDNA fungi	10^3 x copy / ng DNA	19.5 ± 4.4	3.4 ± 0.7	***
fungi / bacteria (rDNA)		1.11 ± 0.27	0.30 ± 0.06	***
rRNA bacteria	10^5 x copy / ng cDNA	58.4 ± 23.6	3.8 ± 1.8	***
ITS fungi	10^3 x copy / ng cDNA	9.61 ± 0.80	0.11 ± 0.06	***
β-glucosidase	µmol min^{-1} g^{-1} DM	26.5 ± 10.4	11.0 ± 6.6	***
α-glucosidase	µmol min^{-1} g^{-1} DM	3.2 ± 1.1	1.0 ± 0.7	***
cellobiohydrolase	µmol min^{-1} g^{-1} DM	5.3 ± 2.2	2.4 ± 2.2	**
β-xylosidase	µmol min^{-1} g^{-1} DM	6.7 ± 1.8	8.1 ± 1.9	
N-acetylglucosaminidase	µmol min^{-1} g^{-1} DM	11.4 ± 5.4	4.9 ± 5.6	*
arylsulfatase	µmol min^{-1} g^{-1} DM	0.6 ± 0.1	1.3 ± 0.6	**
phosphomonoesterase	µmol min^{-1} g^{-1} DM	114.4 ± 24.7	90.3 ± 22.4	*

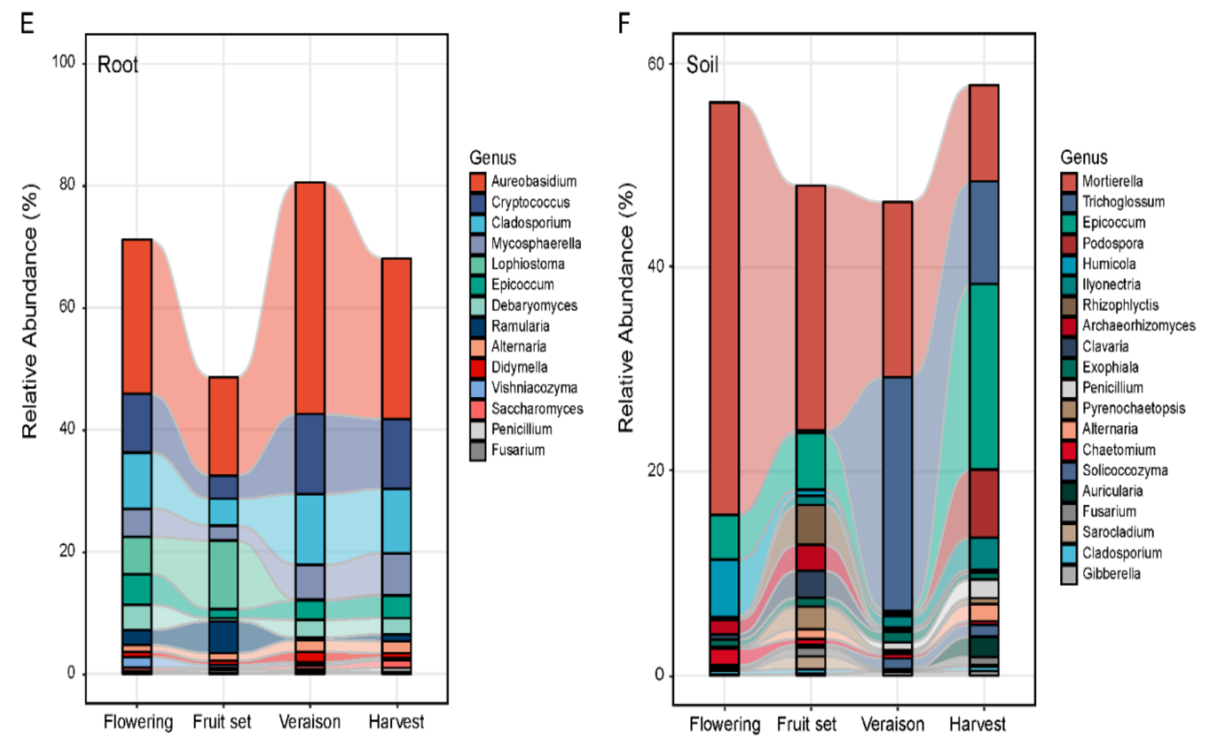
* Significant differences among soil horizons (one-way ANOVA followed by Tukey post-hoc test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).



príprava vzoriek pôdnych húb

sezónnosť a rôzne substráty





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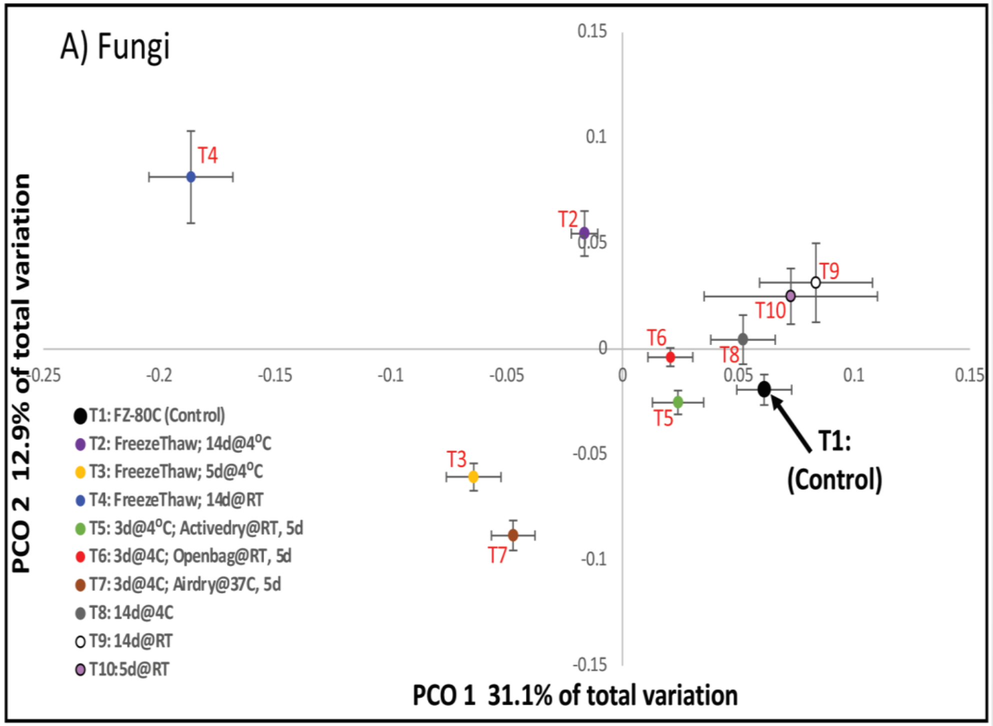
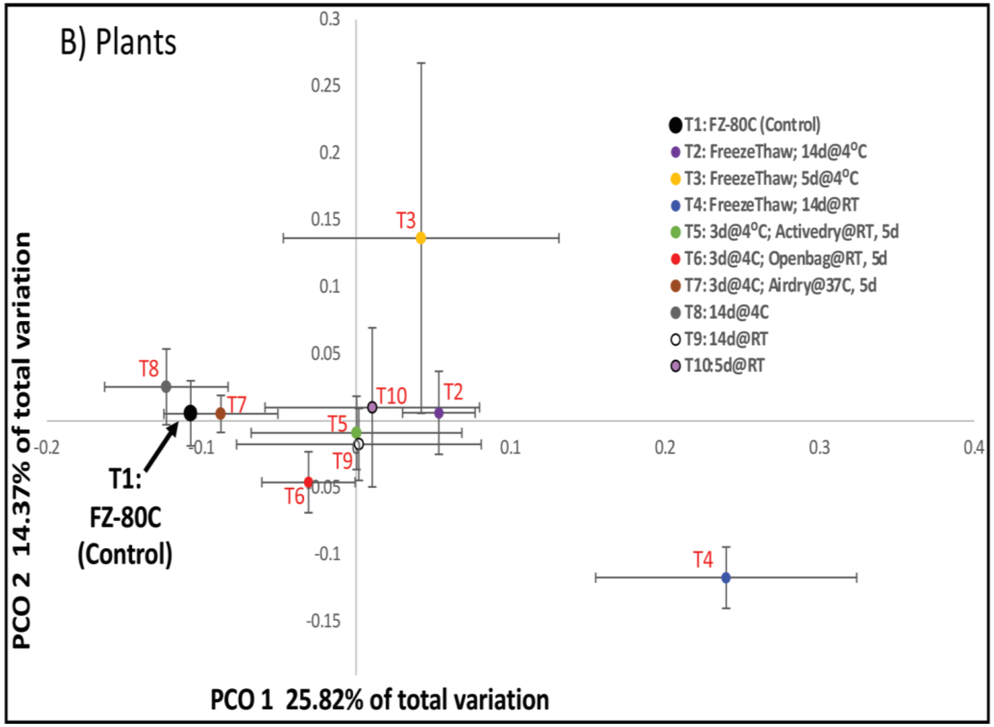
príprava vzoriek pôdných húb

skladovanie vzoriek





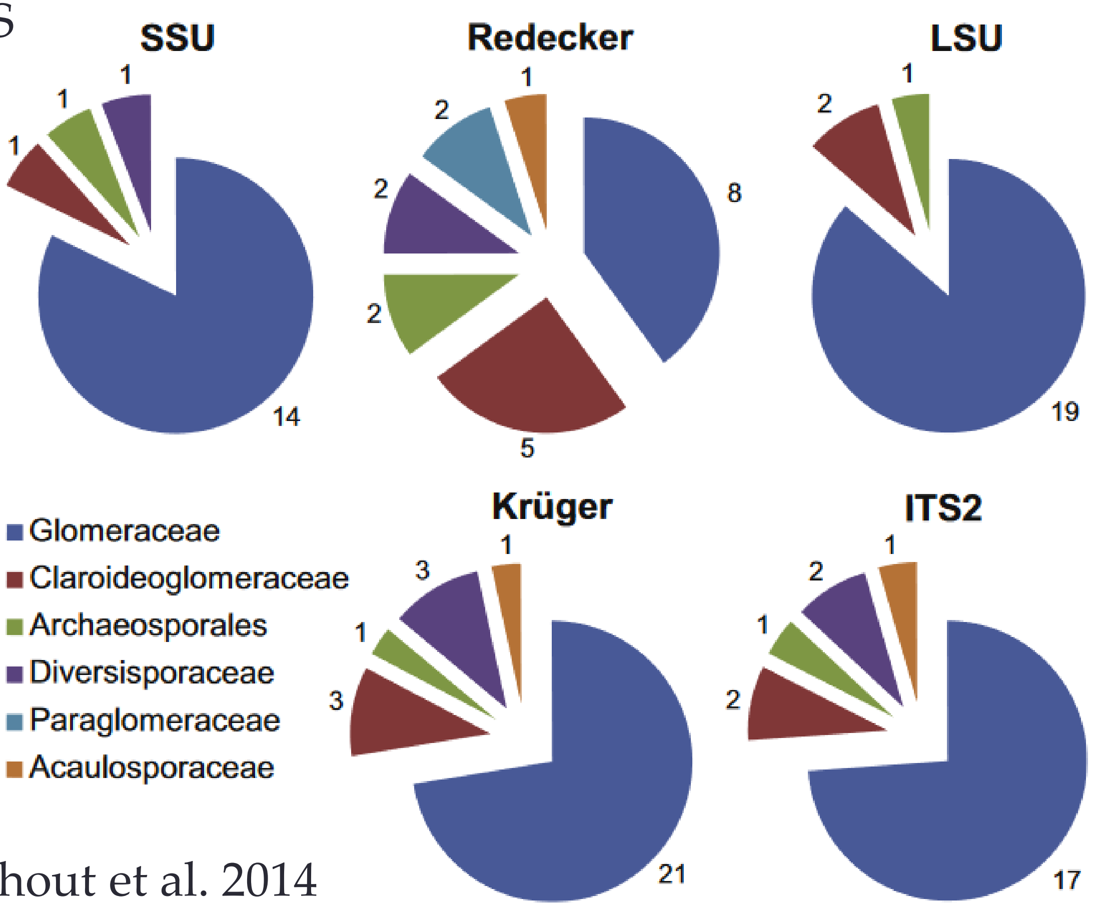
Clasen et al. 2020

príprava vzoriek pôdných húb

výber primerov a barcode sekvencií

pre viaceré skupiny ekologicky dôležitých húb ako sú patogény rastlín a endofyty, ITS region neposkytuje dostatok informácií na odlíšenie druhov (Vu et al. 2018)

štúdie vodných húb a arbuskulárnych mykoríz preto používajú často small subunit (SSU) (18S) a large subunit (LSU) (28S) nuclear rRNA gény

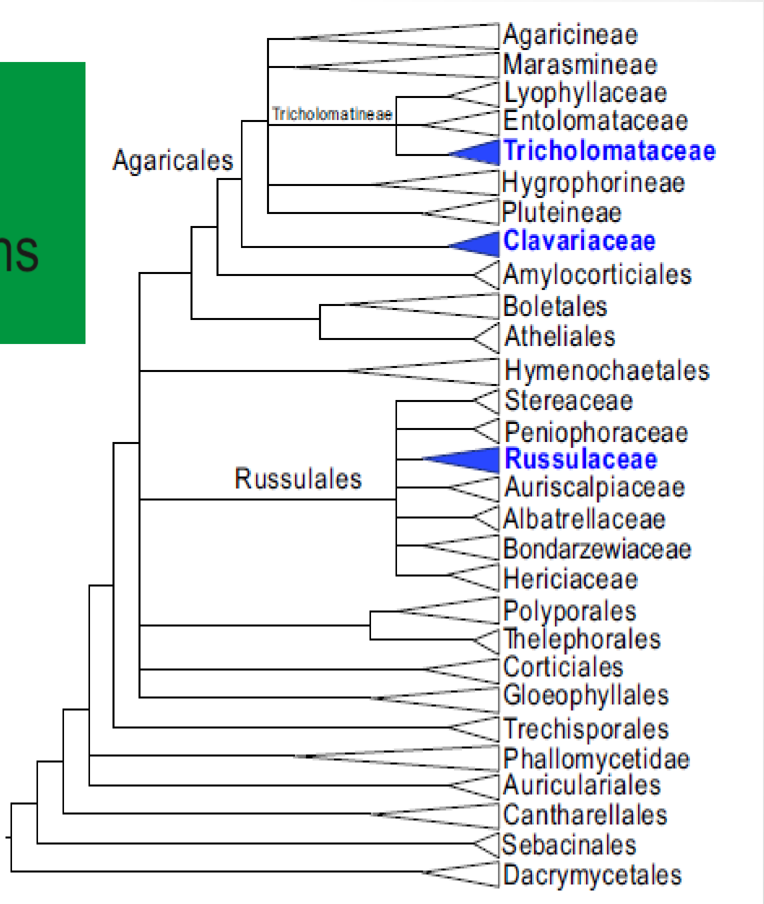


Kohout et al. 2014

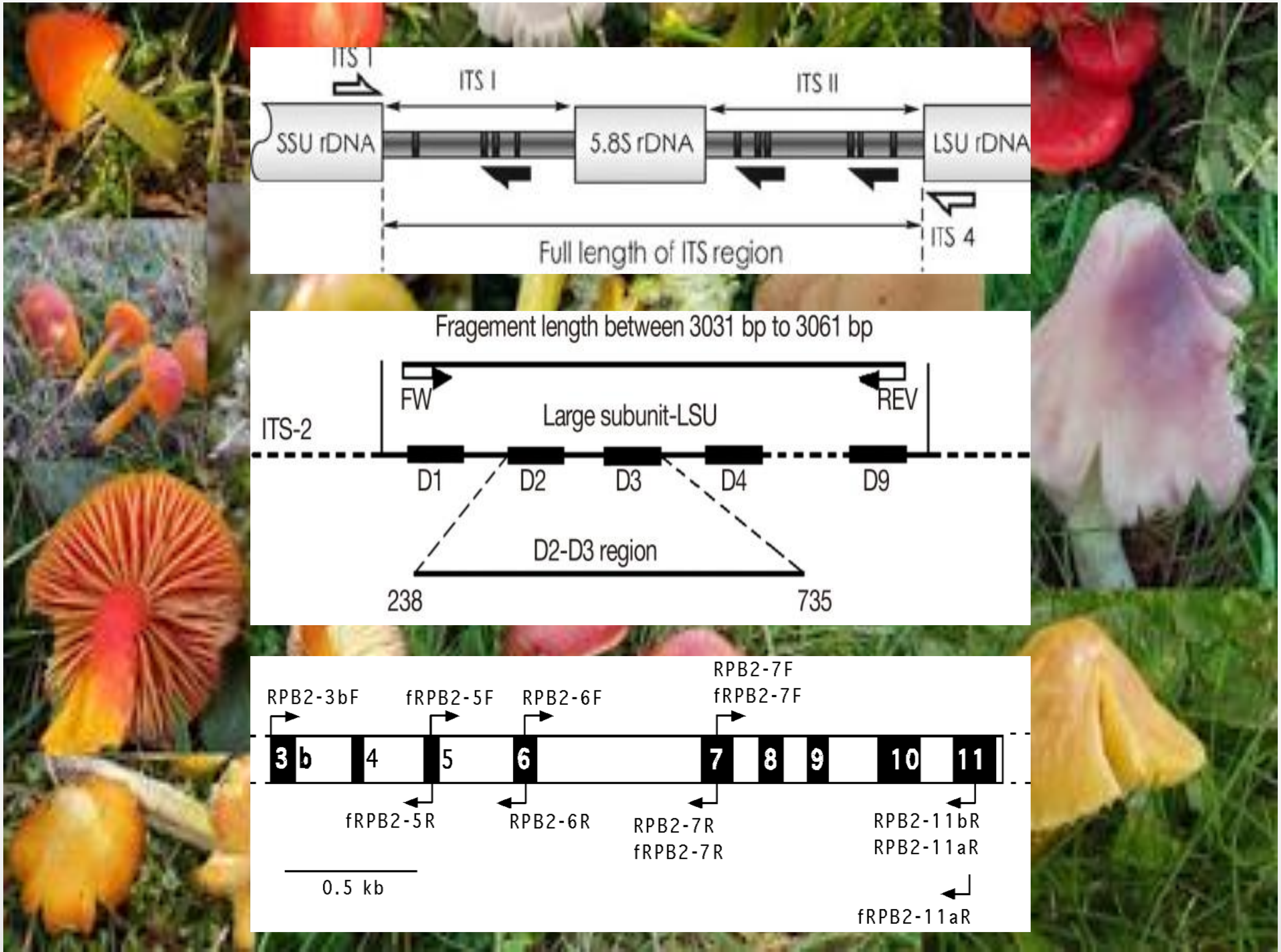
alternatívne barcode regióny

Recovery of species-rank OTUs of agarics (Agaricomycotina, fungi) in metagenomic datasets based on various nrDNA amplicon lengths and positions

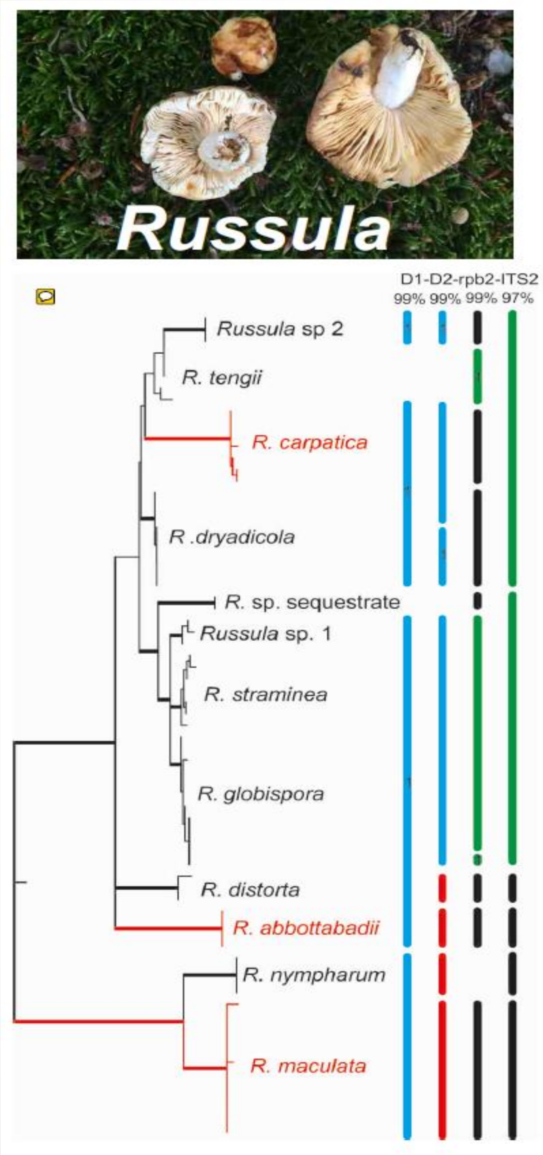
Slavomir Adamčík, Brian P. Looney, Miroslav Kolařík, Marisol Sánchez-García, Katarína Adamčíková, Miroslav Caboň, Gareth W. Griffith

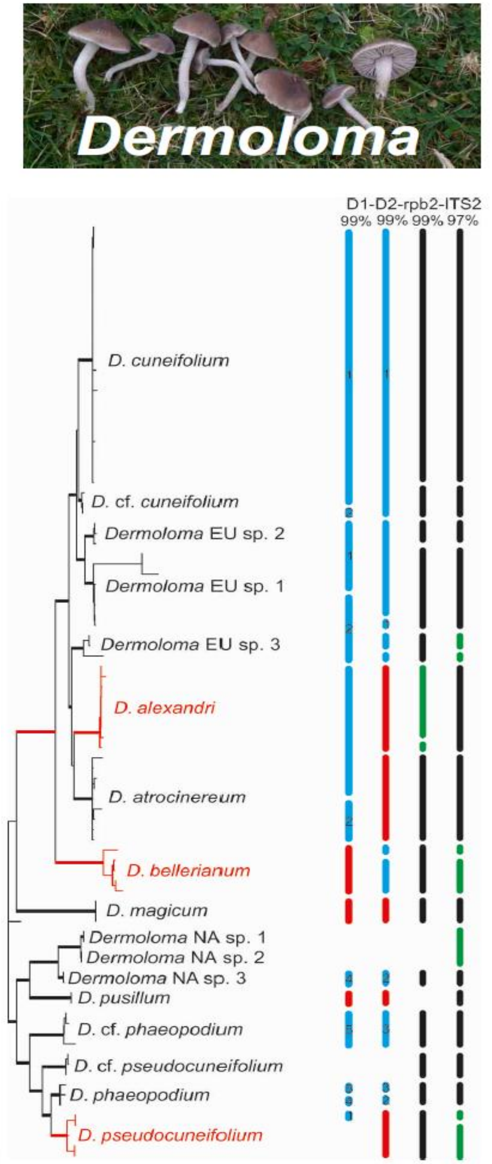


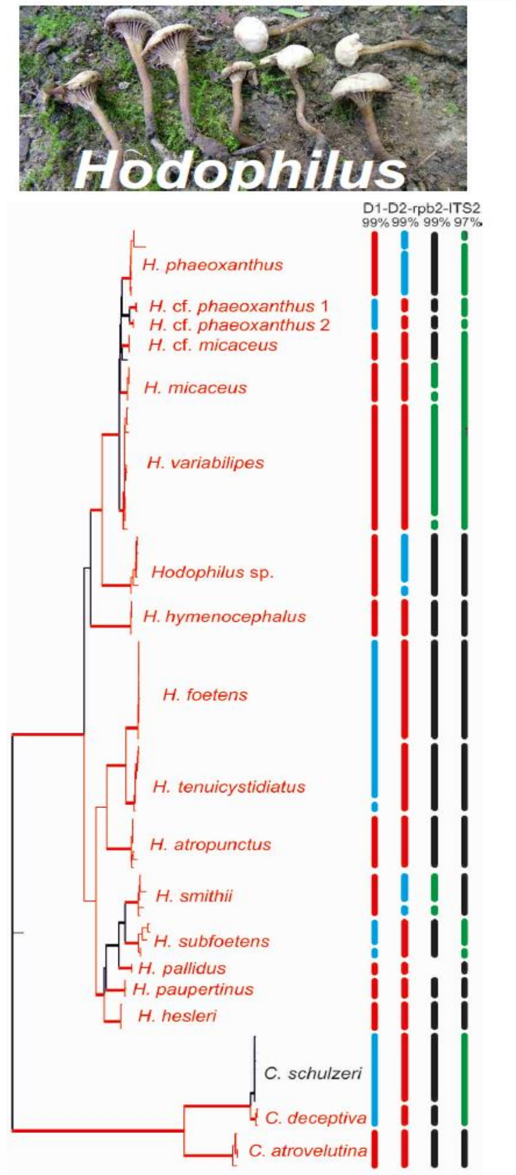
alternatívne barcode regióny



testovanie alternatívnych barcoding regiónov







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Mulching has negative impact on fungal and plant diversity in Slovak oligotrophic grasslands

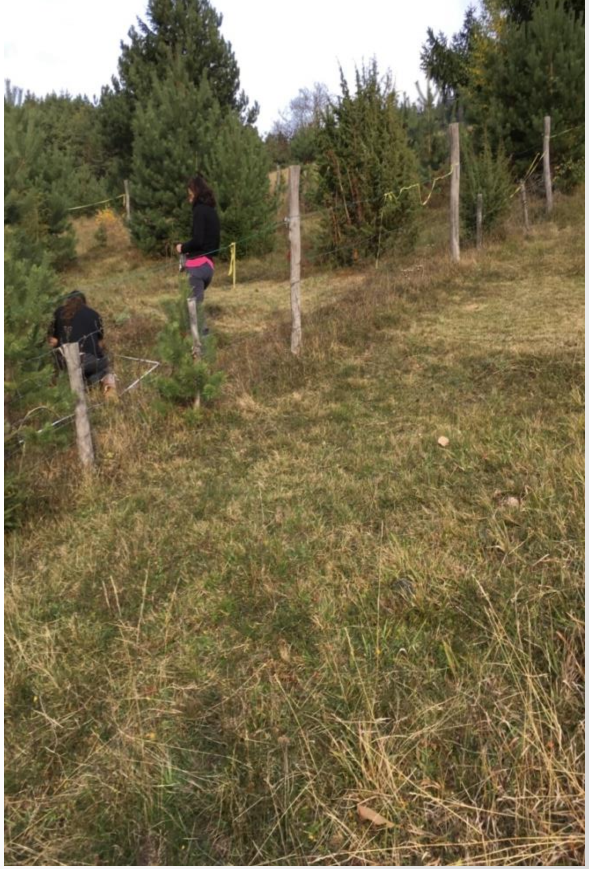
Check for updates

Miroslav Caboň[a,*], Dobromil Galvánek[a], Andrew P. Detheridge[b],
Gareth W. Griffith[b], Silvia Maráková[a], Slavomír Adamčík[a]

mulčovanie: môže spôsobiť sezónne obohatenie živinami spôsobené rozkladom rastlinnej biomasy saprofytickými organizmami

hypotéza: kompetitívne a antagonistické interakcie pôdnych mikroorganizmov a zmeny v dostupných živinách pravdepodobne mení štruktúru spoločenstiev pôdných húb v travinných biotopoch

ciele: zistiť zmeny v mikrobiálnych spoločenstvách húb v Slovenských oligotrofných travnných biotopoch spôsobené dlhodobou (osem rokov) aplikáciou šiestich
- rôznych manažmentových postupov



dizajn štúdie

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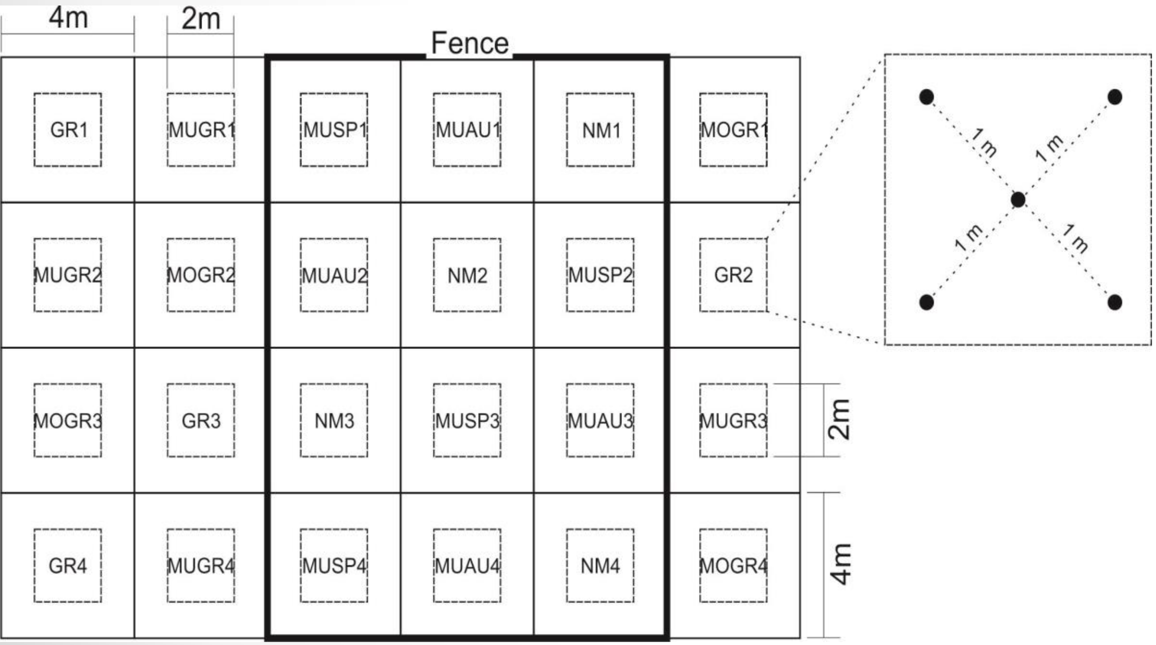
Miroslav Caboň[a,]*, Dobromil Galvánek[a], Andrew P. Detheridge[b], Gareth W. Griffith[b], Silvia Maráková[a], Slavomír Adamčík[a]

experiment založený v roku 2009 aplikáciou šiestich manažmentových postupov v štyroch opakovaniach



- GR: pasenie (pozitívna kontrola
- MOGR: tradičné kosenie kosou kombinované s pasením (seno odstránené)
- MUAU: mulčovanie v jeseni (prvá polovica septembra)
- MUSP: mulčovanie v jari (druhá polovica júna až prvá polovica júla)
- MUGR: pasenie kombinované s jesenným mulčovaním
- NM: bez zásahu (negatívna kontrola)

pozorované parametre a dáta

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- pôda: organický uhlík(Cox), humus, obsah N a P, pôda odobratá 7. novembra 2016
- rastlinné spoločenstvá: geobotanické zápisy zaznamenané v máji až júni 2018
- huby: odobraných 5 pôdnych vzoriek pre metabarcoding 12 októbra 2017 a 3 mája 2018, sekvenované Illumina MiSeq, ITS2 rDNA za použitia primerov ITS3F, ITS4R
- CHEGD huby: identifikované pomocou referenčných databáz Clavariaceae (Birkebak et al. 2016), Hygrophoraceae (Ainsworth, Cannon & Dentinger 2013, Wang et al. 2018), Entolomataceae (Morozova, Noordeloos & Vila 2014), Geoglossaceae (Fedosova et al. 2017) a Tricholomataceae (Sánchez-García et al. 2021)

abiotické pôdne parametre


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		Cox [g/kg]	Humus [g/kg]	N [g/kg]	P [mg/kg]
GR	GR1	27,44	47,3	2,97	2,31
GR	GR2	32,18	55,47	3,10	2,53
GR	GR3	25,2	43,45	2,90	2,80
GR	GR4	28,55	49,22	2,82	2,40
GR (average)		28,3425	48,86	2,9475	2,51 ab
MOGR	MOGR1	21,58	37,2	2,67	2,54
MOGR	MOGR2	27,71	47,78	2,64	2,45
MOGR	MOGR3	29,67	51,15	3,08	3,79
MOGR	MOGR4	36,92	63,65	3,42	3,18
MOGR (average)		28,97	49,945	2,9525	2,99 b
MUAU	MUAU1	31,06	53,55	2,92	3,27
MUAU	MUAU2	33,29	57,4	3,24	2,62
MUAU	MUAU3	32,46	55,96	3,22	2,78
MUAU	MUAU4	36,64	63,17	3,21	3,76

iba obsah fosforu ukázal signifikantné rozdiely medzi manažmentami

príklad OTU húb

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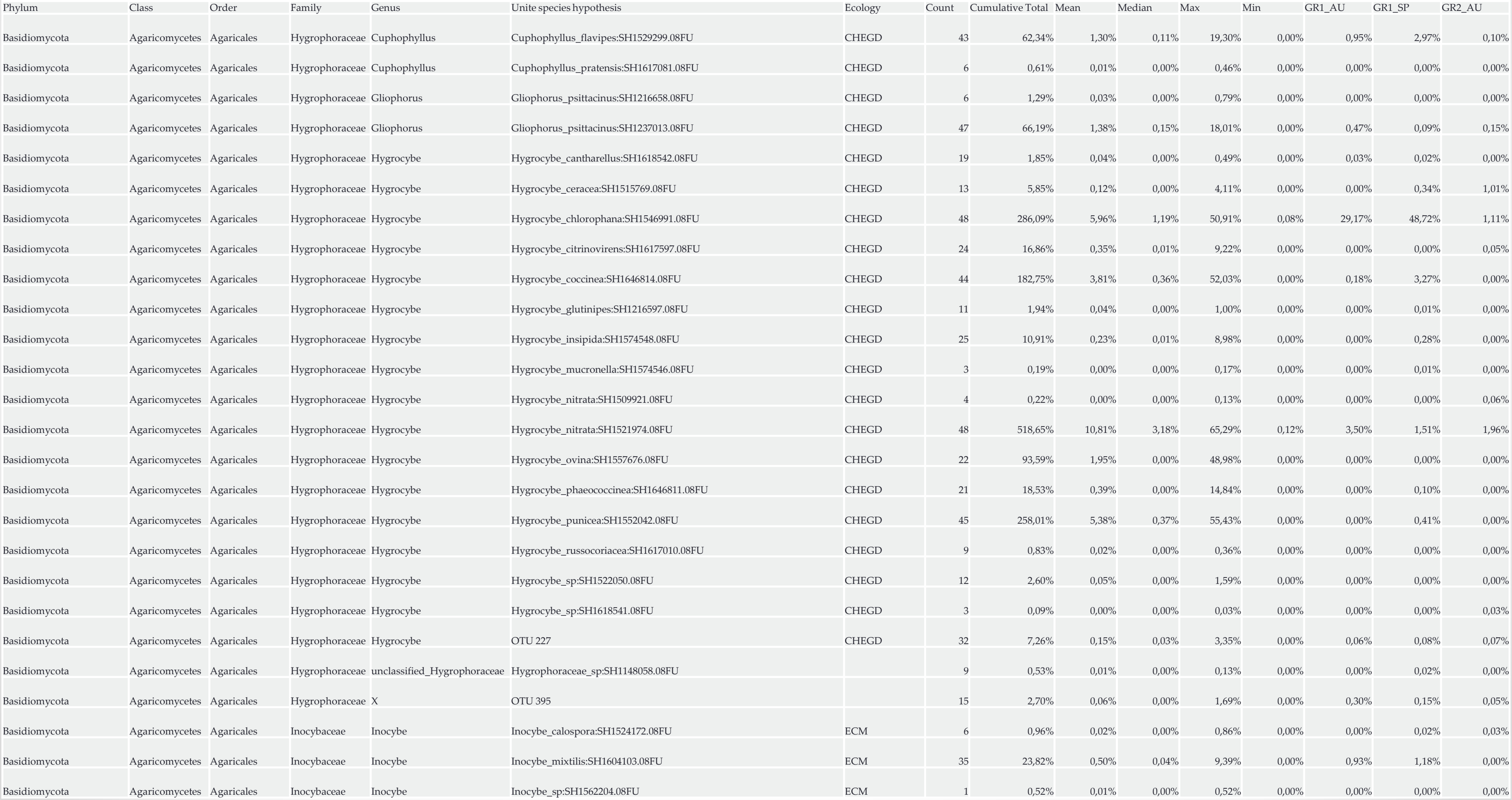

Phylum	Class	Order	Family	Genus	Unite species hypothesis	Ecology	Count	Cumulative Total	Mean	Median	Max	Min	GR1_AU	GR1_SP	GR2_AU
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Cuphophyllus	Cuphophyllus_flavipes:SH1529299.08FU	CHEGD	43	62,34%	1,30%	0,11%	19,30%	0,00%	0,95%	2,97%	0,10%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Cuphophyllus	Cuphophyllus_pratensis:SH1617081.08FU	CHEGD	6	0,61%	0,01%	0,00%	0,46%	0,00%	0,00%	0,00%	0,00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Gliophorus	Gliophorus_psittacinus:SH1216658.08FU	CHEGD	6	1,29%	0,03%	0,00%	0,79%	0,00%	0,00%	0,00%	0,00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Gliophorus	Gliophorus_psittacinus:SH1237013.08FU	CHEGD	47	66,19%	1,38%	0,15%	18,01%	0,00%	0,47%	0,09%	0,15%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe	Hygrocybe_cantharellus:SH1618542.08FU	CHEGD	19	1,85%	0,04%	0,00%	0,49%	0,00%	0,03%	0,02%	0,00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe	Hygrocybe_ceracea:SH1515769.08FU	CHEGD	13	5,85%	0,12%	0,00%	4,11%	0,00%	0,00%	0,34%	1,01%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe	Hygrocybe_chlorophana:SH1546991.08FU	CHEGD	48	286,09%	5,96%	1,19%	50,91%	0,08%	29,17%	48,72%	1,11%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe	Hygrocybe_citrinovirens:SH1617597.08FU	CHEGD	24	16,86%	0,35%	0,01%	9,22%	0,00%	0,00%	0,00%	0,05%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe	Hygrocybe_coccinea:SH1646814.08FU	CHEGD	44	182,75%	3,81%	0,36%	52,03%	0,00%	0,18%	3,27%	0,00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe	Hygrocybe_glutinipes:SH1216597.08FU	CHEGD	11	1,94%	0,04%	0,00%	1,00%	0,00%	0,00%	0,01%	0,00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe	Hygrocybe_insipida:SH1574548.08FU	CHEGD	25	10,91%	0,23%	0,01%	8,98%	0,00%	0,00%	0,28%	0,00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe	Hygrocybe_mucronella:SH1574546.08FU	CHEGD	3	0,19%	0,00%	0,00%	0,17%	0,00%	0,00%	0,01%	0,00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe	Hygrocybe_nitrata:SH1509921.08FU	CHEGD	4	0,22%	0,00%	0,00%	0,13%	0,00%	0,00%	0,00%	0,06%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe	Hygrocybe_nitrata:SH1521974.08FU	CHEGD	48	518,65%	10,81%	3,18%	65,29%	0,12%	3,50%	1,51%	1,96%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe	Hygrocybe_ovina:SH1557676.08FU	CHEGD	22	93,59%	1,95%	0,00%	48,98%	0,00%	0,00%	0,00%	0,00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe	Hygrocybe_phaeococcinea:SH1646811.08FU	CHEGD	21	18,53%	0,39%	0,00%	14,84%	0,00%	0,00%	0,10%	0,00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe	Hygrocybe_punicea:SH1552042.08FU	CHEGD	45	258,01%	5,38%	0,37%	55,43%	0,00%	0,00%	0,41%	0,00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe	Hygrocybe_russocoriacea:SH1617010.08FU	CHEGD	9	0,83%	0,02%	0,00%	0,36%	0,00%	0,00%	0,00%	0,00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe	Hygrocybe_sp:SH1522050.08FU	CHEGD	12	2,60%	0,05%	0,00%	1,59%	0,00%	0,00%	0,00%	0,00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe	Hygrocybe_sp:SH1618541.08FU	CHEGD	3	0,09%	0,00%	0,00%	0,03%	0,00%	0,00%	0,00%	0,03%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe	OTU 227	CHEGD	32	7,26%	0,15%	0,03%	3,35%	0,00%	0,06%	0,08%	0,07%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	unclassified_Hygrophoraceae	Hygrophoraceae_sp:SH1148058.08FU		9	0,53%	0,01%	0,00%	0,13%	0,00%	0,00%	0,02%	0,00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	X	OTU 395		15	2,70%	0,06%	0,00%	1,69%	0,00%	0,30%	0,15%	0,05%
Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	Inocybe	Inocybe_calospora:SH1524172.08FU	ECM	6	0,96%	0,02%	0,00%	0,86%	0,00%	0,00%	0,02%	0,03%
Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	Inocybe	Inocybe_mixtilis:SH1604103.08FU	ECM	35	23,82%	0,50%	0,04%	9,39%	0,00%	0,93%	1,18%	0,00%
Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	Inocybe	Inocybe_sp:SH1562204.08FU	ECM	1	0,52%	0,01%	0,00%	0,52%	0,00%	0,00%	0,00%	0,00%

cievnaté rastliny

geobotanické zápisy

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Tax_ID	GR1	GR2	GR3	GR4	MOGR1	MOGR2	MOGR3	MOGR4	MUAU1	MUAU2	MUAU3	MUAU4	MUGR1
Acetosa pratensis	0,5		0,5	0,5	0,5	0,5	0,5		0,5	2	0,5	1	
Acetosella vulgaris				0,5				0,5				0,5	0,5
Agrimonia eupatoria				1			0,5						
Agrostis capillaris	0,5	2	1	0,5	1	1	1	10	2	1	20	3	1
Achillea millefolium	7	1	4	8	3	1	10	3	4	1	5	8	3
Ajuga genevensis													
Ajuga reptans													
Alchemilla sp.	0,5	1	0,5	1	1	2	7	2	0,5	2	2	2	1
Antennaria dioica													
Anthoxanthum odoratum	0,5	1	0,5	1	15	1	3	6	0,5	1	1	1	1
Avenula praeusta		5			5								
Avenula pubescens					0,5								3
Betula pendula			1			0,5		0,5					
Betula_pendula_E2													
Brachypodium pinnatum		1			0,5		1	20		1		2	
Briza media	1	2	2	0,5	15	2	2	2	1	5	3	5	5
Campanula patula		0,5			0,5				0,5			1	1
Campanula persicifolia													
Carex caryophyllea	2	1	1	2	0,5	2	0,5		0,5	1		0,5	1
Carex montana													
Carex pallescens	5	0,5	1	0,5	1	0,5	0,5	0,5	0,5	1	3	7	2
Carex panicea	5	1	8	0,5	1	1	2	0,5	2	1	1	2	1
Carex pilulifera	10	7	20	15	2	3	10	6	5	2	10	1	5
Carlina acaulis									4				
Carlina vulgaris	0,5						0,5					0,5	
Carpinus betulus													0,5

- priemerne 66898 nepárovaných amplikónov na vzorku
- priemerne 21148 „pairended“ amplikónov húbamplicons
- celkovo1191 hubových MOTUs
- 94.8% všetkých hubových amplikónov identifikovaných do čelade alebo presnejšie
- priemerný počet hubových MOTUs na manažment podobný pre všetky manažmenty (353–402)


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huby -výsledok

	GR	MOGR	MUAU	MUGR	MUSP	NM	Average all plots
Fungi identified to family [%]	94.7 (2.4)	94.0 (1.8)	94.8 (3.1)	95.1 (1.2)	95.7 (2.9)	95.1 (1.9)	94.8 (2.3)
Fungi identified to genus [%]	94.3 (2.7)	93.4 (1.7)	94.6 (3.2)	94.7 (1.2)	94.9 (3)	94.8 (2)	94.4 (2.5)
Fungi identified to species [%]	86.7 (5.2)	84.2 (4.3)	89.5 (4.8)	88.6 (2.5)	90.6 (4.8)	89.3 (2.8)	88.2 (4.7)
Shannon Index	3.88 (0.49)	4.21 (0.55)	3.49 (0.73)	3.71 (0.62)	3.52 (0.66)	4.05 (0.6)	3.81 (0.67)
Inverse Simpson Index	17.84 (12.43)	23.59 (13.05)	12.03 (9.09)	13.02 (11.48)	11.25 (13.58)	21.88 (13.17)	16.6 (13.15)
Average fungal MOTU richness	353.1 (47.2)	372.3 (83.9)	358.1 (56.5)	385.3 (54.6)	402 (46.8)	401.5 (74.5)	378.7 (65.1)

klasifikácia húb

- viac ako 80% amplikónov Basidiomycota a Ascomycota
- tieto dve skupiny takmer rovnako zastúpené v GR and MOGR

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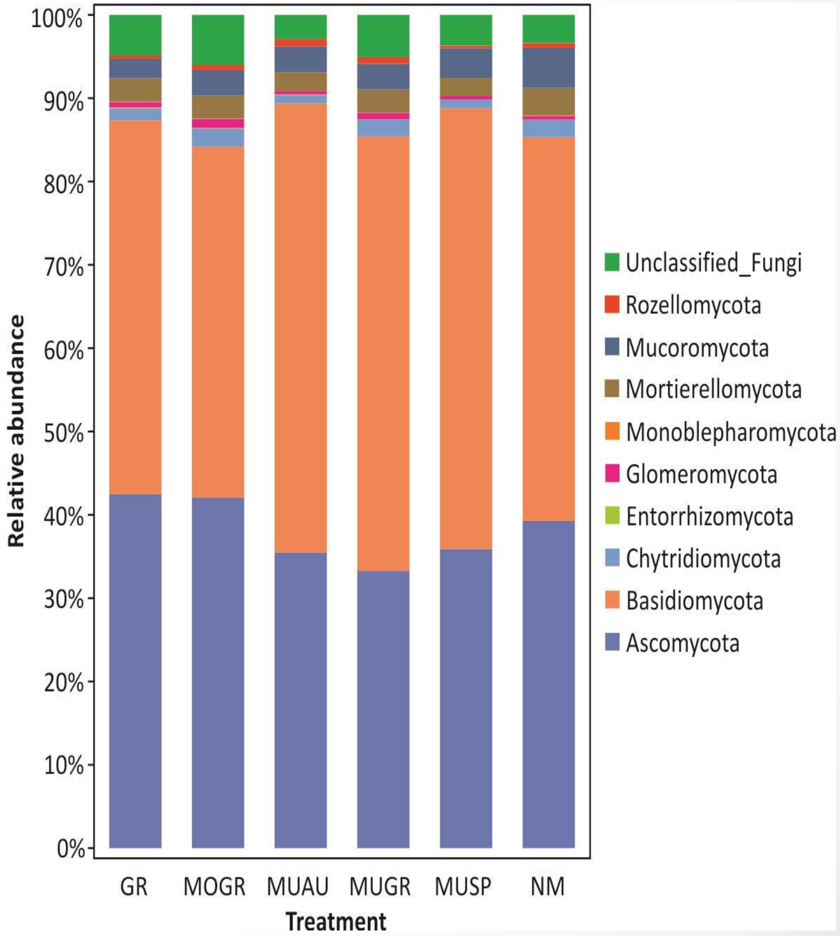

ekologické funkčné skupiny (guilds)

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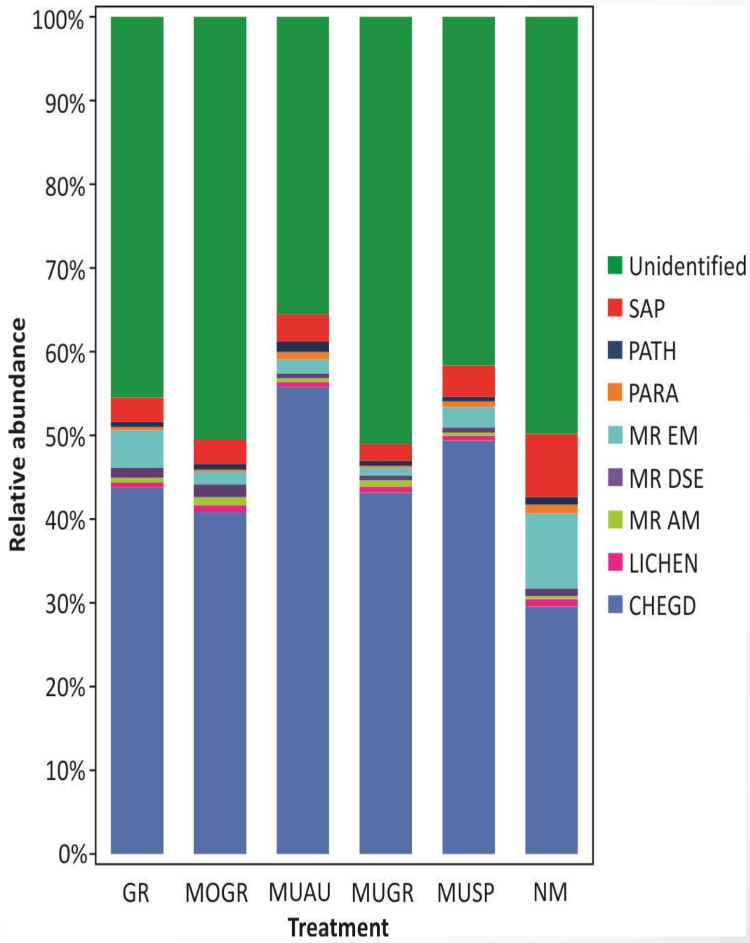
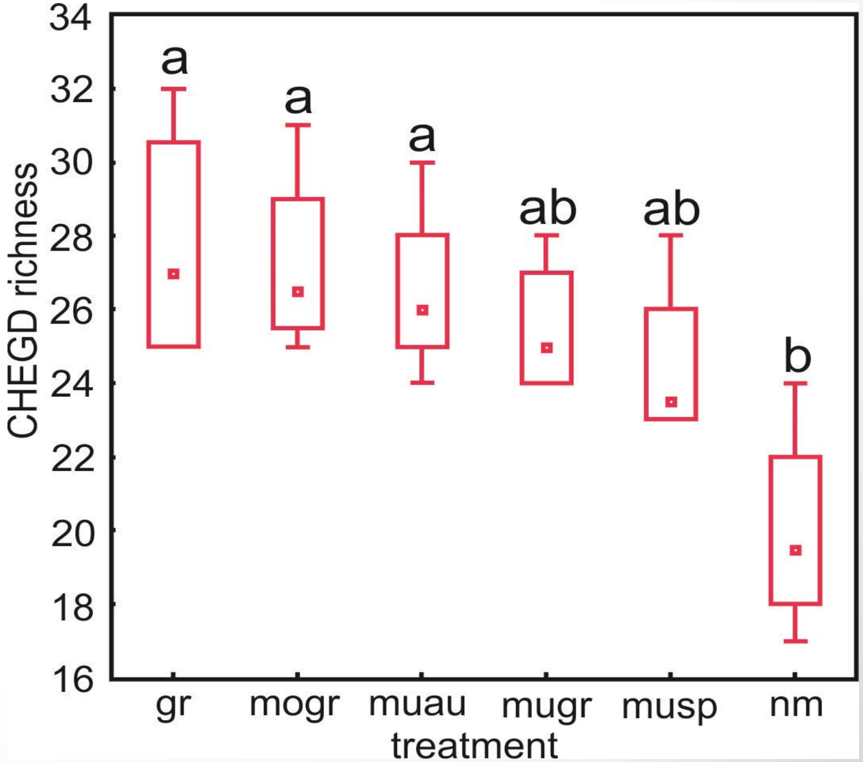
- najviac početnou (abundant) funkčnou skupinou sú CHEGD huby, zastúpené od 28.8% (NM) do 54.1% (MUAU)
- NM mal najvyššiu relatívnu abundanciu ektomykoríznych (MR EM 8.7%), parazitických (PARA 1.1%) and saprofytických (SAP 7.4%) húb



CHEGD fungi

- nekultivovateľné
- dominujú v prirodzených rastlinných spoločenstvách
- citlivé na manažment

List of recognized OTUs	Frequency of occurence					
	GR	MOGR	MUAU	MUGR	MUSP	NM
Geoglossum atropurpureum	0	1	1	1	1	0
Geoglossum sp1	0	0	1	2	1	0
Geoglossum sp2	4	4	4	4	4	4
Geoglossaceae	3	0	1	0	2	1
Hemileucoglossum sp1	1	0	2	0	1	0
Trichoglossum sp1	2	4	3	4	2	0
Trichoglossum sp2	0	1	0	0	1	0
Microglossum olivaceum	2	1	3	3	1	0
Microglossum sp1	3	4	3	3	3	2
Camarophyllopsis schulzeri	4	4	4	4	3	4
Clavaria falcata	4	4	4	4	4	4
Clavaria flavipes	0	1	2	1	1	0
Clavaria fragilis	2	0	1	2	0	0
Clavaria fumosa	0	2	4	2	1	0
Clavaria sp1	0	2	0	0	0	1
Clavariaceae sp2	0	0	0	1	2	2
Clavariaceae sp3	0	1	3	3	1	3
Clavariaceae sp4	1	0	0	0	0	0
Clavariaceae sp5	4	3	3	3	3	1
Clavariaceae sp6	0	2	1	0	1	1
Clavariaceae sp7	4	4	4	4	4	4
Clavulinopsis helvola	1	2	2	3	3	2
Clavulinopsis sp1	1	0	0	1	0	0
Clavulinopsis luteoalba	0	0	0	1	0	0
Clavulinopsis sp2	0	0	0	0	1	0
Clavulinopsis sp3	3	1	2	1	2	0
Clavulinopsis sp4	1	0	0	0	0	0
Ramariopsis sp1	4	4	4	4	4	4
Ramariopsis sp2	4	3	4	4	4	1
Entoloma ameides	0	0	0	0	1	1
Entolomataceae sp1	4	4	2	4	1	1
Entoloma sp1	3	2	2	0	1	1
Entoloma exile	1	0	1	0	1	0
Entoloma formosum	0	0	0	1	0	0
Entoloma glaucobasis	1	3	0	2	1	0
Entoloma griseocyaneum	4	4	0	2	0	0
Entoloma chalybeum	4	4	3	4	3	0
Entoloma infula	0	0	0	0	0	1
Entoloma madidum	1	1	3	1	0	0
Entoloma mougeotii	0	0	0	0	2	1
Entoloma ochreoprunuloides	0	0	0	0	0	1
Entoloma prunuloides	1	0	0	0	1	1
Hygrophoraceae sp1	0	0	0	1	0	1
Gliophorus psittacinus	1	3	4	3	2	4
Gliophorus sp	3	4	3	3	2	4
Hygrocybe cantharellus	1	2	0	2	0	0
Hygrocybe ceracea	2	0	0	0	0	0
Hygrocybe citrinovirens	1	1	1	0	3	2
Hygrocybe coccinea	4	4	4	4	4	4
Hygrocybe conica	3	2	4	3	1	1
Hygrocybe chlorophana	4	4	4	4	4	4
Hygrocybe insipida	3	1	2	0	2	1
Hygrocybe nitrata	4	4	4	4	4	4
Hygrocybe ovina	1	1	2	0	4	4
Hygrocybe nigrescens	0	1	0	0	0	0
Hygrocybe phaeococcinea	4	4	1	1	2	2
Hygrocybe punicea	4	4	4	4	4	4
Hygrocybe sp1	0	0	1	0	0	0
Hygrocybe sp2	0	0	0	0	2	0
Hygrocybe sp3	4	2	0	0	0	0
Hygrocybe sp4	1	1	0	0	0	0
Hygrophoraceae sp2	2	2	2	0	1	2
Hygrophoraceae sp3	0	2	1	1	0	0
Dermoloma sp1	1	1	1	2	1	1
Dermoloma sp2	1	0	0	0	0	0
Pseudobaeospora pyrifera	0	0	1	0	2	1

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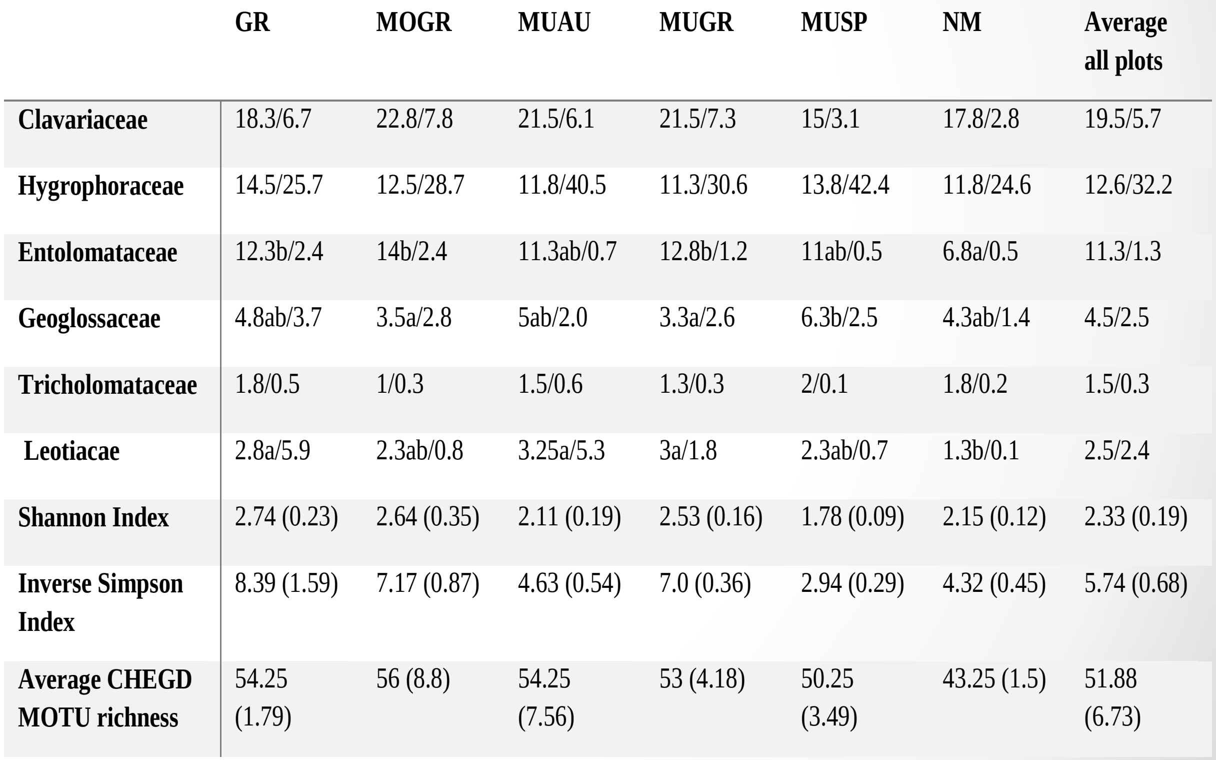
CHEGD huby štatistika

- 121 MOTUs CHEGD huby
- 51 Clavariaceae
- 23 Hygrophoraceae
- 30 Entolomataceae
- 10 Geoglossaceae,
- 4 Leotiaceae
- 3 Tricholomataceae
- Clavariaceae vždy s najväčšou species richness, nasledované Hygrophoraceae alebo Entolomataceae
- najväčšiu relative (sequence) abundance mali Hygrophoraceae, od 24.6% (NM) do 42.4% (MUSP)



	GR	MOGR	MUAU	MUGR	MUSP	NM	Average all plots
Clavariaceae	18.3/6.7	22.8/7.8	21.5/6.1	21.5/7.3	15/3.1	17.8/2.8	19.5/5.7
Hygrophoraceae	14.5/25.7	12.5/28.7	11.8/40.5	11.3/30.6	13.8/42.4	11.8/24.6	12.6/32.2
Entolomataceae	12.3b/2.4	14b/2.4	11.3ab/0.7	12.8b/1.2	11ab/0.5	6.8a/0.5	11.3/1.3
Geoglossaceae	4.8ab/3.7	3.5a/2.8	5ab/2.0	3.3a/2.6	6.3b/2.5	4.3ab/1.4	4.5/2.5
Tricholomataceae	1.8/0.5	1/0.3	1.5/0.6	1.3/0.3	2/0.1	1.8/0.2	1.5/0.3
Leotiacae	2.8a/5.9	2.3ab/0.8	3.25a/5.3	3a/1.8	2.3ab/0.7	1.3b/0.1	2.5/2.4
Shannon Index	2.74 (0.23)	2.64 (0.35)	2.11 (0.19)	2.53 (0.16)	1.78 (0.09)	2.15 (0.12)	2.33 (0.19)
Inverse Simpson Index	8.39 (1.59)	7.17 (0.87)	4.63 (0.54)	7.0 (0.36)	2.94 (0.29)	4.32 (0.45)	5.74 (0.68)
Average CHEGD MOTU richness	54.25 (1.79)	56 (8.8)	54.25 (7.56)	53 (4.18)	50.25 (3.49)	43.25 (1.5)	51.88 (6.73)

CHEGD huby sú najviac abundantné OTU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Neohygrocybe nitrata
priemerná relatívna
abundancia na manažment
10.81%

Hygrocybe punicea 5.38%


Daniel Sugny

Hygrocybe chlorophana
5.96%

most abundant MOTUs – ostatné CHEGD huby


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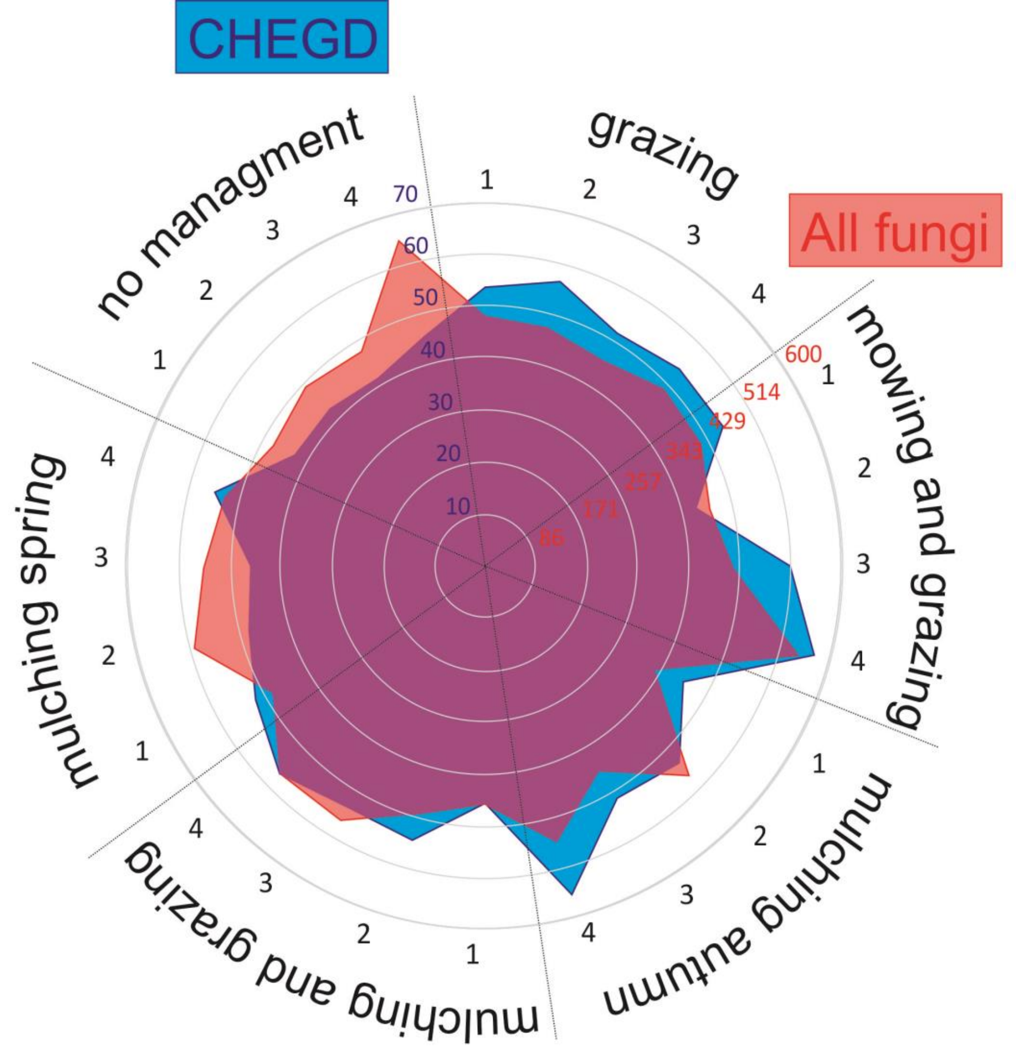
Clavariaceae = *Camarophyllopsis schulzeri* (1.53%)

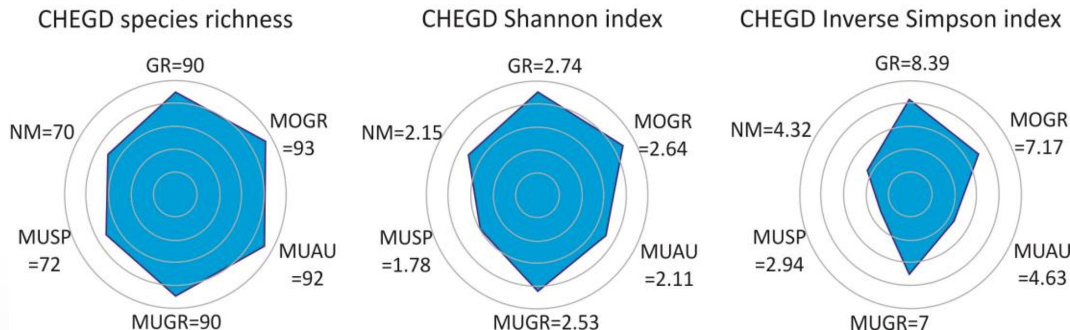
Geoglossaceae = *Geoglossum barlae* (1.97%)

Entolomataceae MOTU = *E.* cf. *bloxamii* (0.3%)

CHEGD porovnanie so všetkými hubami

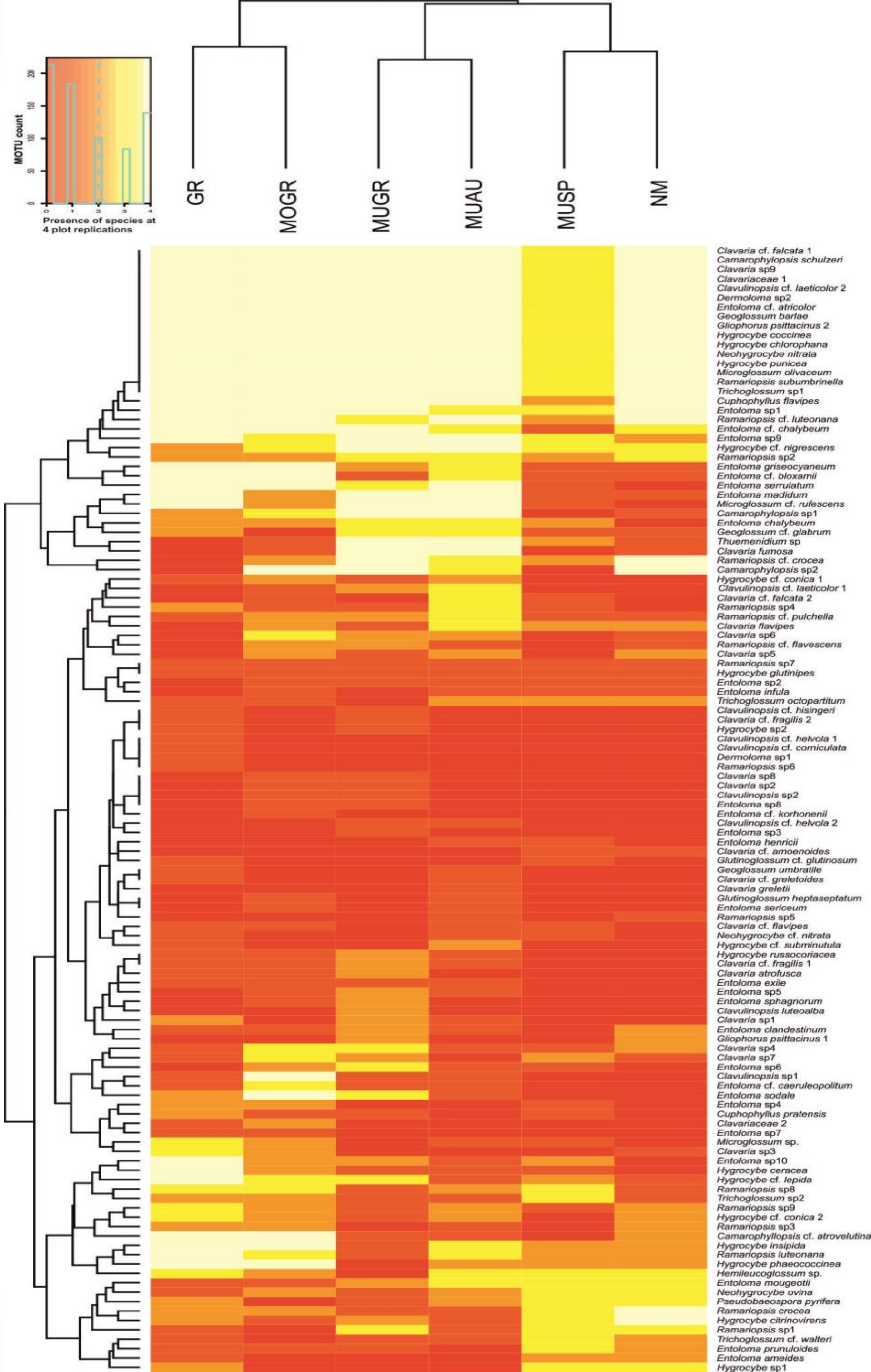
- NM a MUSP s najnižšou CHEGD richness
- relatívny pomer všetkých húb a CHEGD richness bol najvyšší v NM a MUSP
- GR majú najvyšší pomer CHEGD húb v pomere ku kompletnej hubovej diverzite





CHEGD species richness

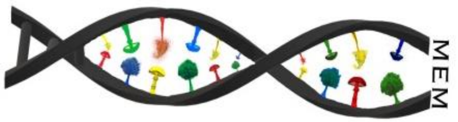
- žiadna CHEGD MOTU sa nevyskytovala na všetkých 24 výskumných plochách
- 40 MOTU zaznamenaných na aspoň jednej ploche každého manažmentu
- desať CHEGD MOTU zaznamenané iba z jedného manažmentu a deväť z nich iba z jedinej lokality



CHEGD relatívna abundancia

Hierarchické zhlukovanie založené na abundancii amplikónov húb

- manažmenty sú zoskupené do troch skupín na základe manažmentových štýlov, (mulčovanie, pasenie a no management)



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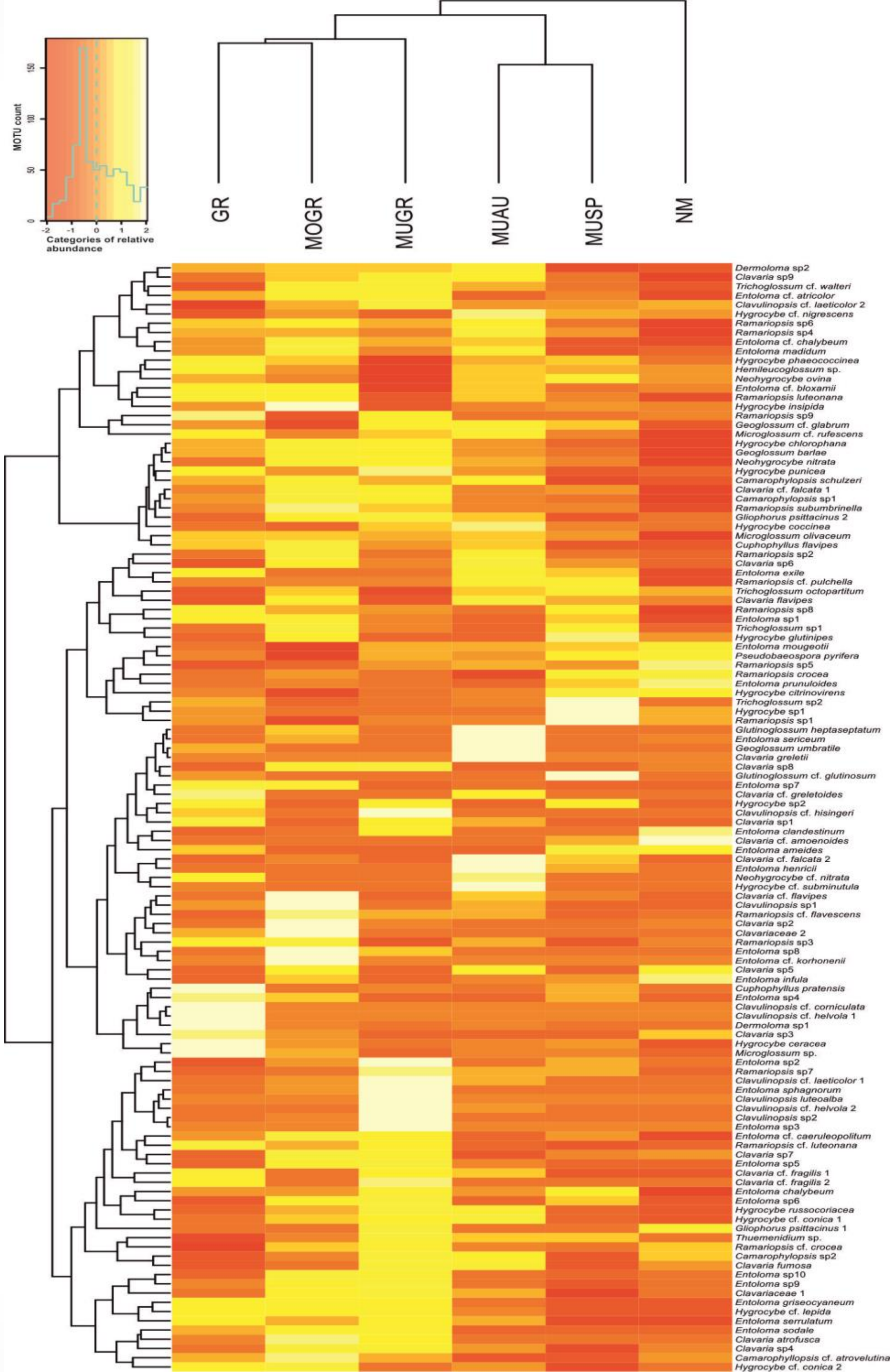

cievnaté rastliny

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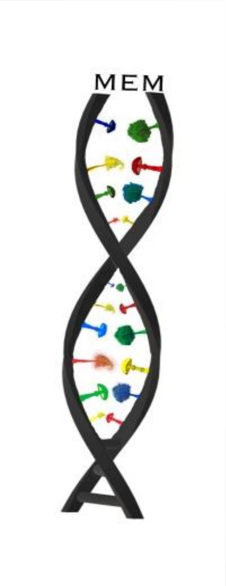
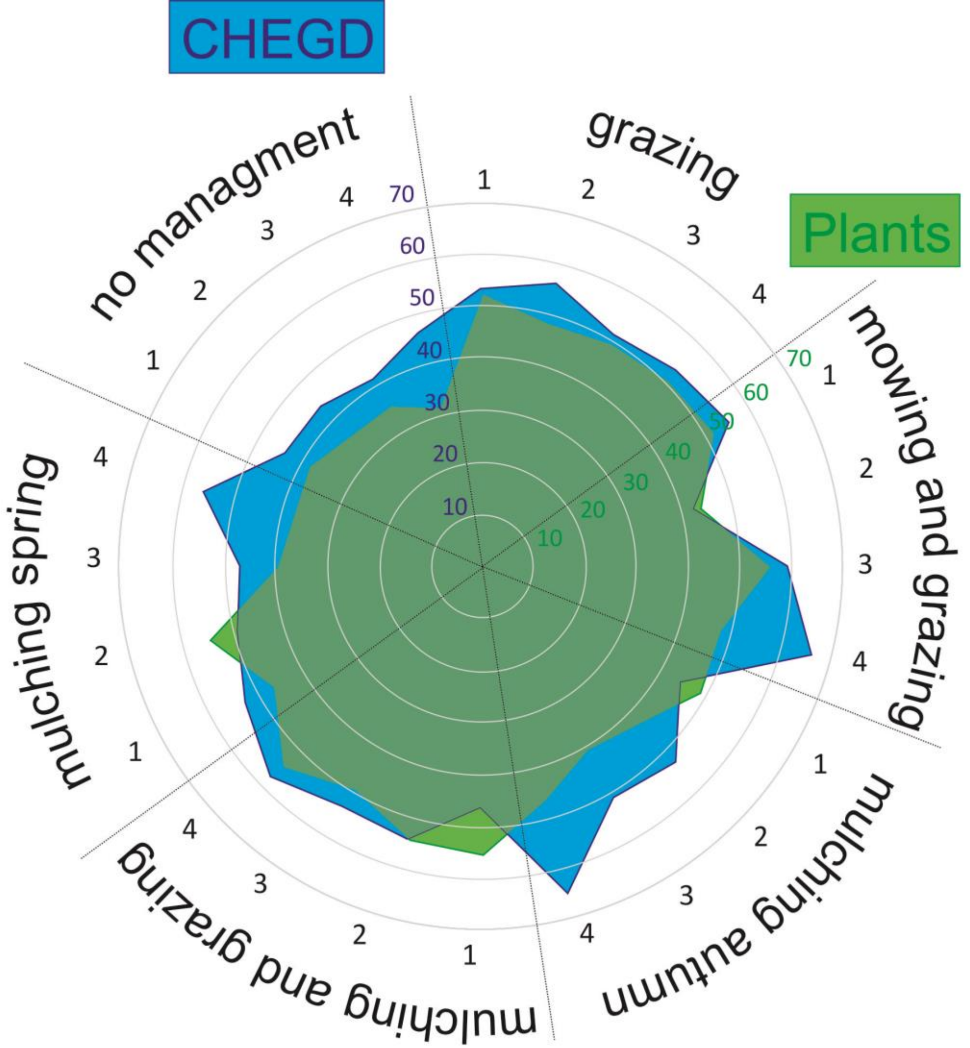
- celkovo 97 druhov
- druhová bohatosť na manažment od 61 do 76 druhov
- (total 1191 hubových MOTUs)

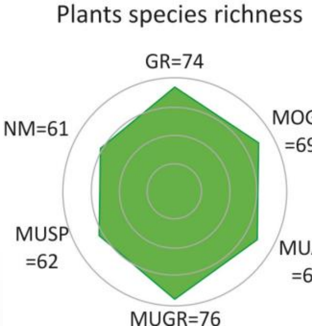
	GR	MOGR	MUAU	MUGR	MUSP	NM	Average all plots
Shannon Index	3.13b (0.17)	3.25b (0.09)	3.15b (0.22)	3.32b (0.15)	3.16b (0.16)	2.37a (0.23)	3.06 (0.36)
Inverse Simpson Index	14.21ab (3.81)	15.62ab (2.39)	12.50ab (5.48)	15.40ab (4.11)	14.74b (4.05)	5.31a (2.11)	11.21 (5.25)
Average plant species richness	50.8b (1.5)	50b (4.5)	45ab (3.2)	54ab (2.4)	45b (6.7)	35.8a (2.5)	46.8 (7)

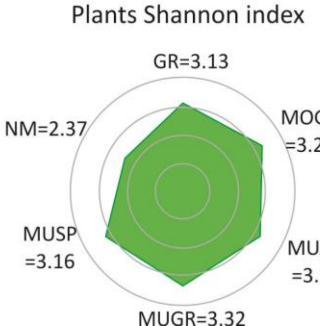


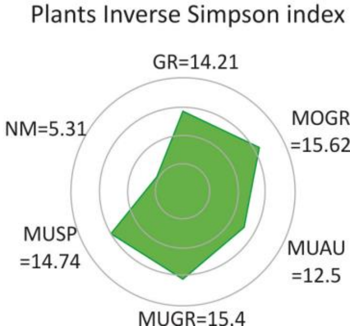
CHEGD vs. cievnaté rastliny

- cievnaté rastlina aj CHEGD huby ukazujú vyššiu druhovú diverzitu pre pasenie (pozitívna kontrola)
- obe skupiny nižšiu pre NM manažment (negatívna kontrola)




Plants species richness


Plants Shannon index


Plants Inverse Simpson index

korelačné analýzy

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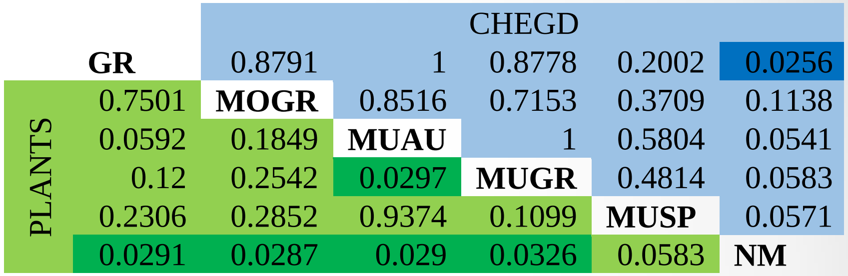
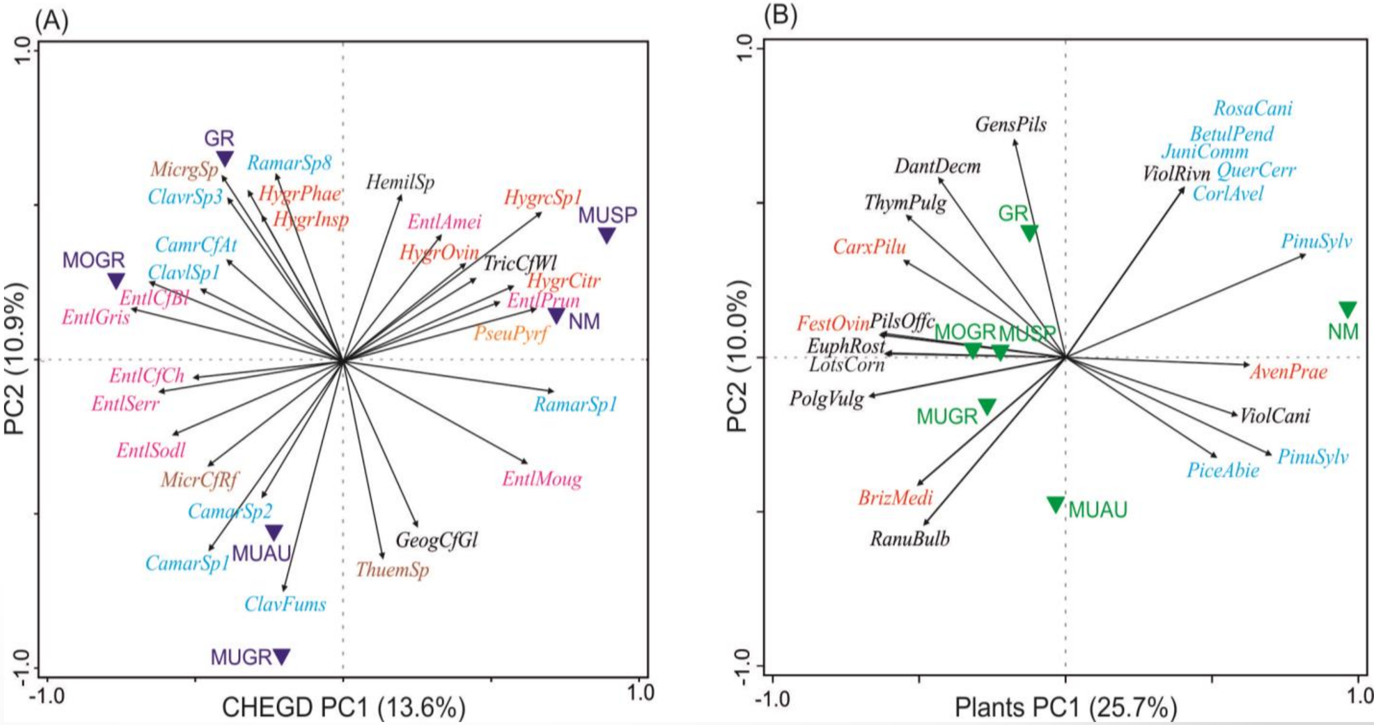
- PERMANOVA párové porovnanie manažmentov založené na prezencii MOTU CHEDG húb na jednotlivých plochách
- PCA analýza odhalila podobnú štruktúru spoločenstiev CHEGD húb NM a MUSP
- PCA cievnatých rastlín ukázala izolovanú pozíciu NM

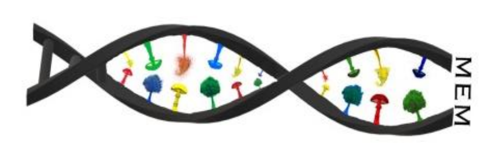
PERMANOVA



		CHEGD				
	GR	0.8791	1	0.8778	0.2002	0.0256
PLANTS	0.7501	**MOGR**	0.8516	0.7153	0.3709	0.1138
	0.0592	0.1849	**MUAU**	1	0.5804	0.0541
	0.12	0.2542	0.0297	**MUGR**	0.4814	0.0583
	0.2306	0.2852	0.9374	0.1099	**MUSP**	0.0571
	0.0291	0.0287	0.029	0.0326	0.0583	**NM**

PCA





Závery

- mulčovanie nie je vhodnou náhradou za tradičné manažmentové postupy
- všetky manažmentové postupy vrátane mulčovania a NM prispeli nejakými unikátnymi CHEGD MOTU jednotkami
- kombinácia všetkých týchto manažmentových postupov v jednej oblasti by prispela k zvýšeniu celkovej diverzity CHEGD húb
- CHEGD huby sa ukázali byť vhodnou skupinou na pochopenie manažmentových vplyvov v ochrane prírody

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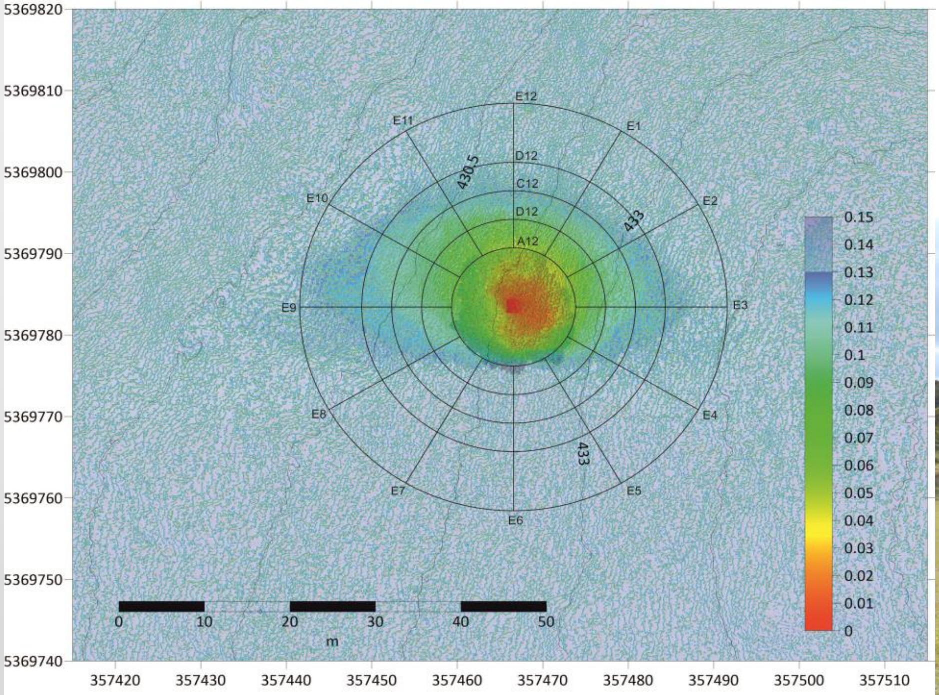
Strom a krajina – STRAKA (APVV 20-257)



radial circle diameter: 7.25-10.75-14.25-17.75-25

laboratórium
Molekulárnej
Ekológie a
Metagenomiky

... práca s e DNA dáva ekológii nový rozmer

Centrum biológie rastlín a biodiverzity SAV