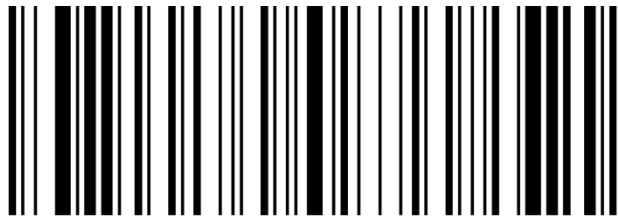




Metabarcoding

Sten Anslan <sten.anslan@ut.ee>

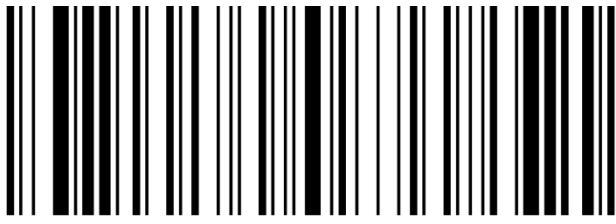


Barcoding and metabarcoding

DNA barcoding - identification of specimens based on the specific DNA sequence.
first proposed by Hebert et al (2003).

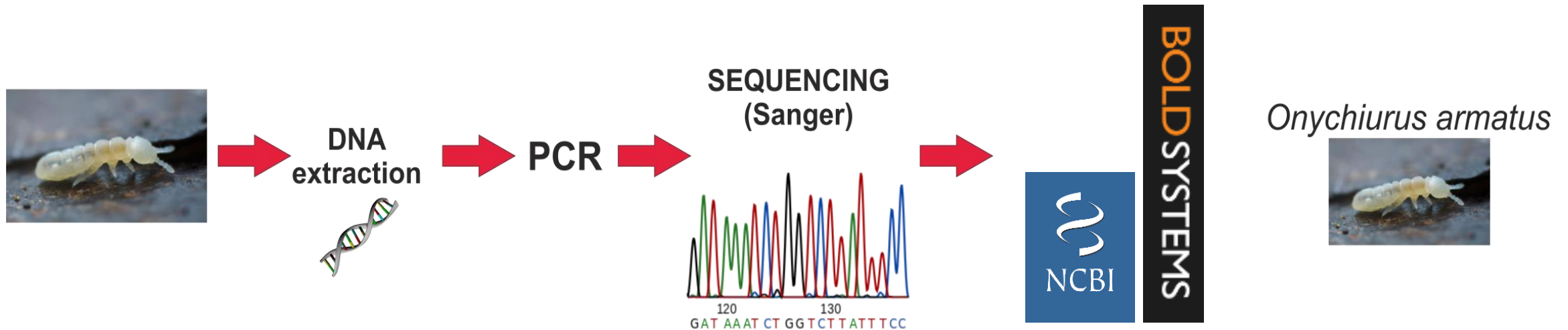


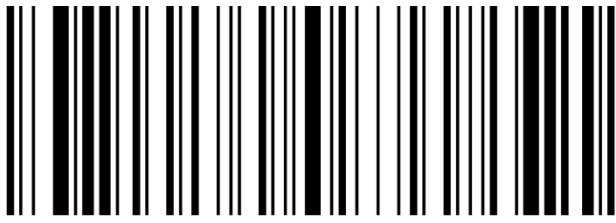
Taberlet et al. 2018



Barcoding and metabarcoding

DNA barcoding - identification of specimens based on the specific DNA sequence.
first proposed by Hebert et al (2003).





Barcoding and metabarcoding

beyond
DNA metabarcoding – identification of multiple species from a single bulk sample



DNA
extraction



PCR

HIGH-THROUGHPUT
SEQUENCING

ATGCTAGCTAGCTACAG
ATCACGTACGTACGTAC
CTAGCTAGCTACGAATT
CGTAGCTAGCTAGCTAT
CGTAGCTAGCTAGCTAT
CGTAGCTAGCTAGCTAT



illumina.com




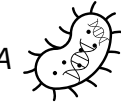
BOLD
SYSTEMS

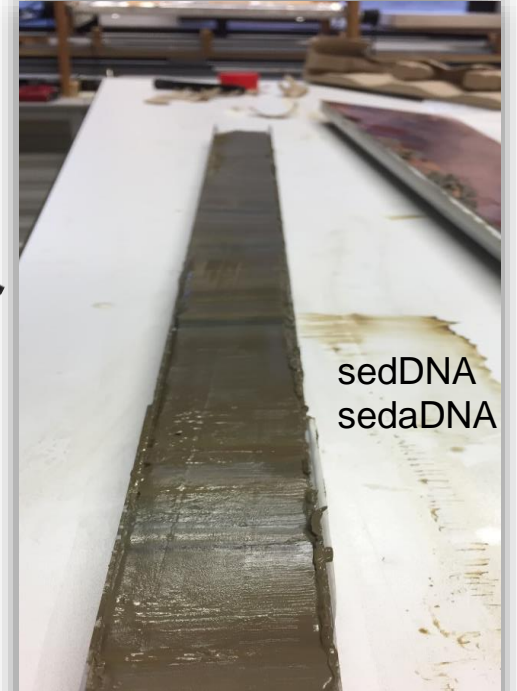
Onychiurus armatus
Orchesella cincta
Entomobrya nivalis
Protaphorura fimata
Megalothorax minimus
Isotomiella sp.



Environmental DNA (eDNA)

eDNA is a complex **mixture of genomic DNA** from many different organisms found in an environmental sample (Taberlet et al 2012).

 *extracellular DNA + intracellular DNA* 



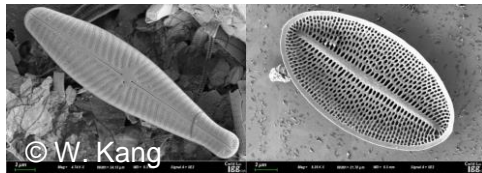
bulk samples
wocDNA

Biomonitoring

1.0



Morph. id



2.0



DNA metabarcoding



identifying species and
biological communities;

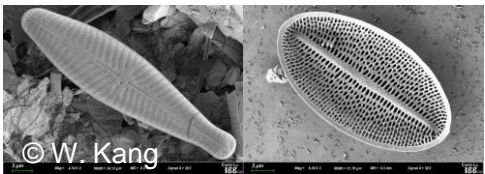
monitoring diseases,
ecosystem health,
water quality,
species invasions etc.

Biomonitoring



Morph. id

identifying species ->
sex ratios,
body condition (e.g. age, size),
absolute abundance (no. of individuals)



rare species,
cryptic species,
juveniles,
DNA traces,

relative abundance(?)

DNA metabarcoding

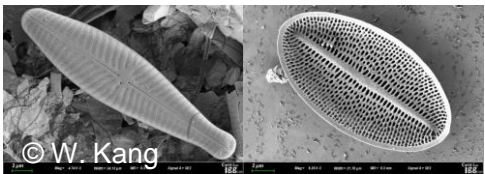


Biomonitoring



Morph. id

identifying species ->
sex ratios,
body condition (e.g. age, size),
absolute abundance (no. of individuals)



rare species,
cryptic species,
juveniles,
DNA traces,

relative abundance(?)

DNA metabarcoding



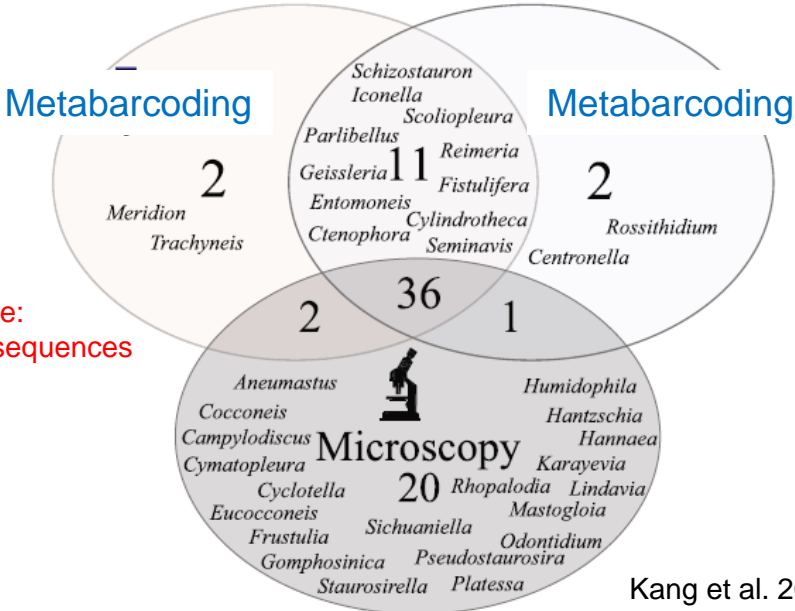
Biomonitoring



vs.



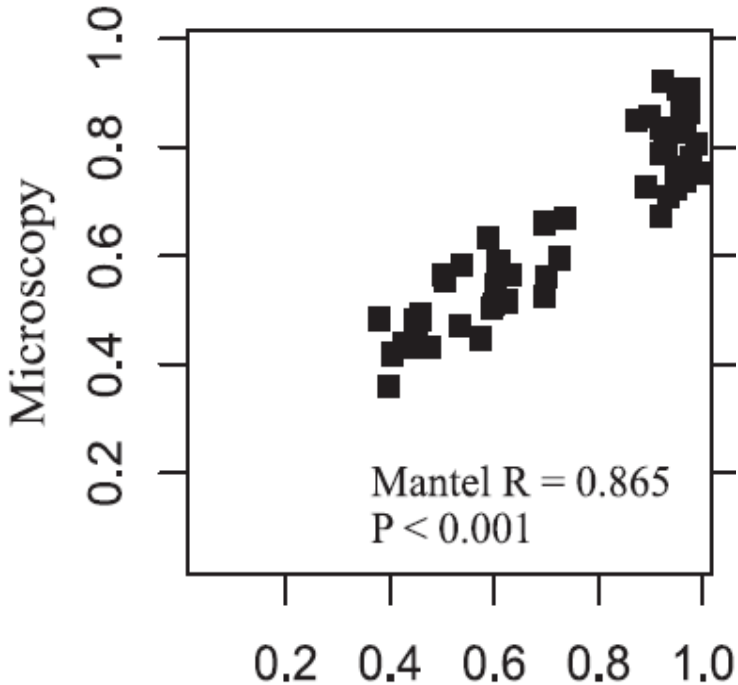
Not completely overlapping patterns is species identifications,
but **highly overlapping community structure** patterns!



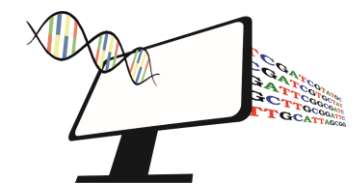
Major issue:
lack of reference sequences

Kang et al. 2021

Metabarcoding

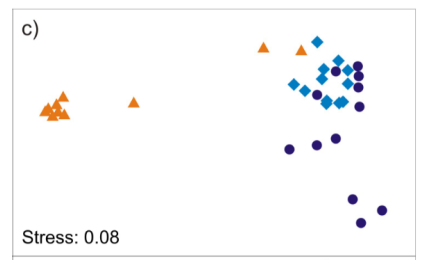


An outline of the metabarcoding approach



Genus	Species
Staurosira	0.96 Staurosira_brevistriata
Amphora	0.23 Amphora_aff_atomoides
Amphora	0.62 Amphora_pediculus
Navicula	0.94 Navicula_cryptocephala
Neidium	0.32 Neidium_productum
Anomoeo	0.47 Anomoeoneis_fogedii
Gomphon	0.21 Gomphonema_capitatum

Biological interpretation



An outline of the metabarcoding approach

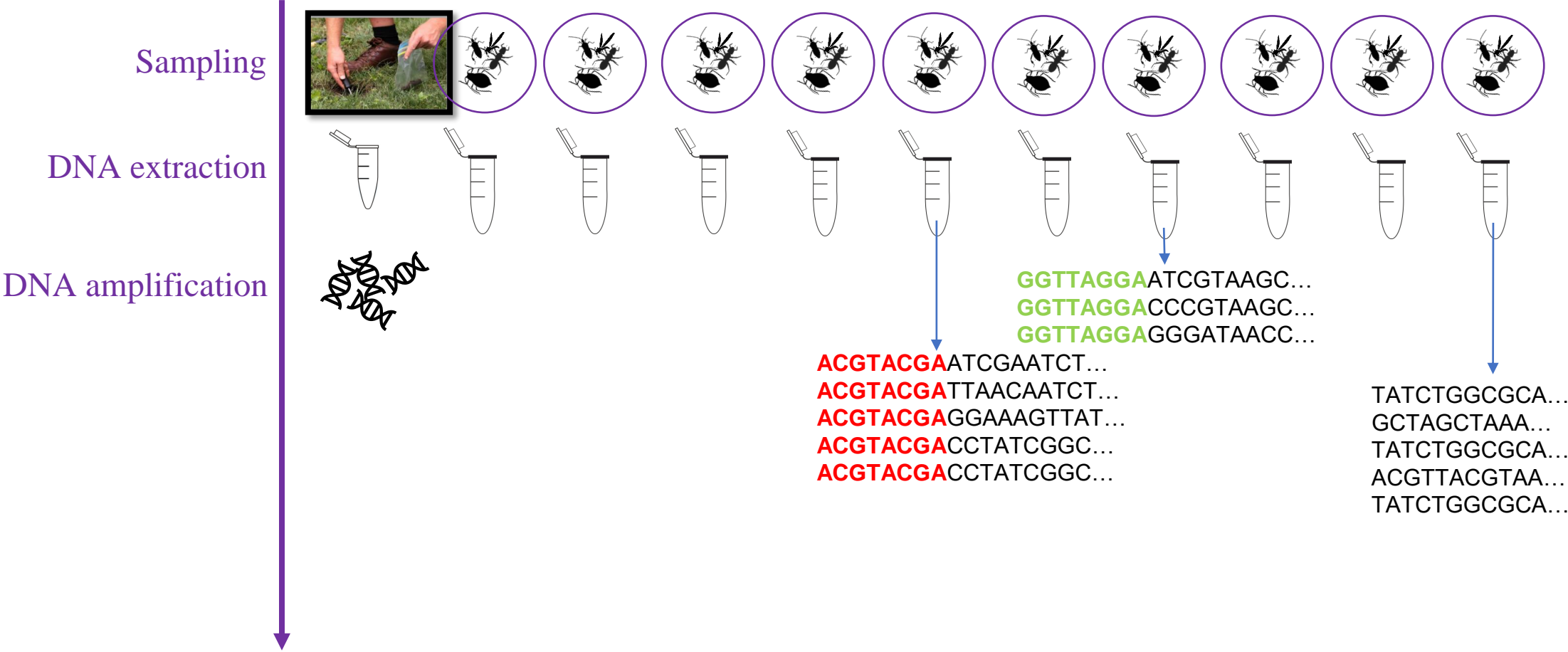
Sampling



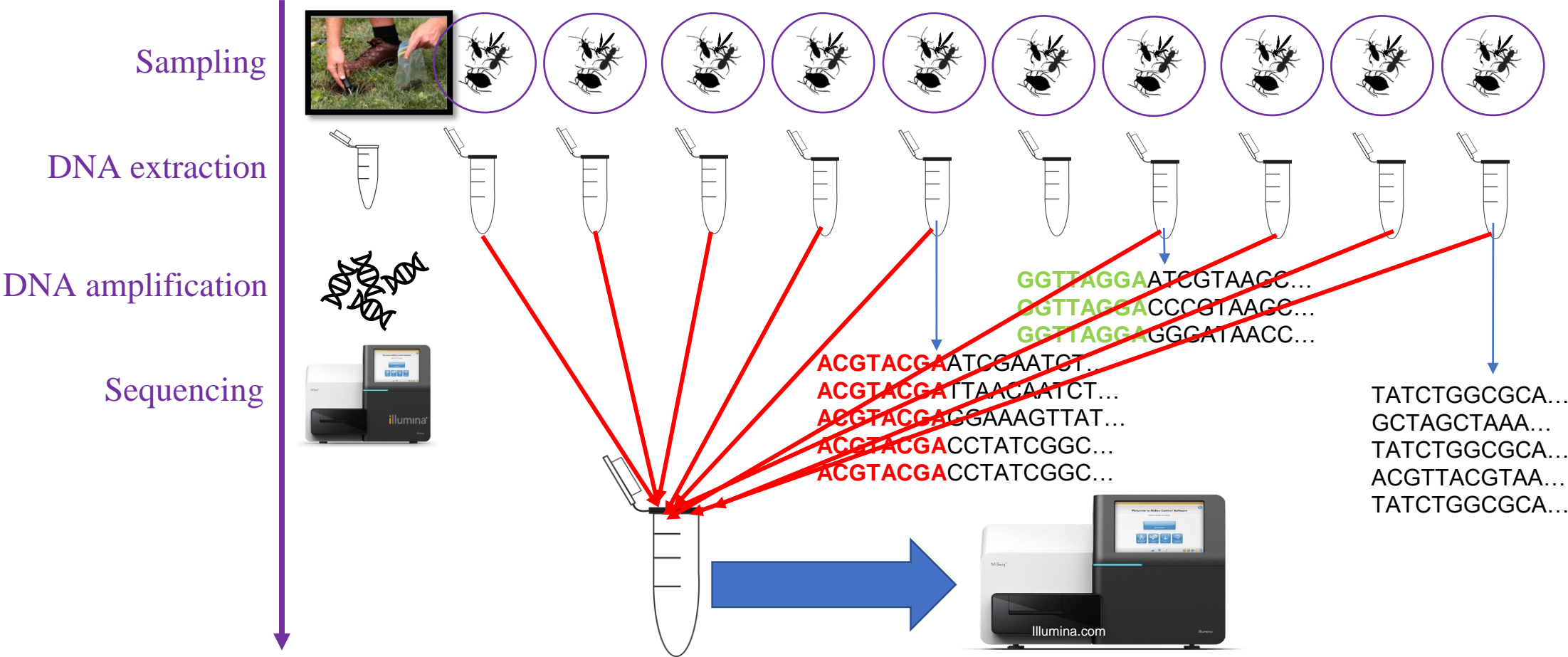
An outline of the metabarcoding approach



An outline of the metabarcoding approach



An outline of the metabarcoding approach



An outline of the metabarcoding approach

Sampling



DNA extraction



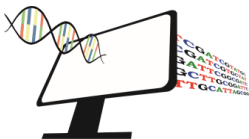
DNA amplification



Sequencing



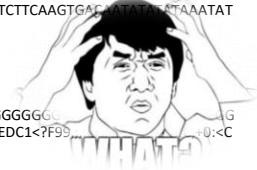
Sequence analyses



@M01338:44:000000000-AFFTE:1:1101:9082:1061 1:N:0:5
CTCGCCGTGCTCCTGCGCTTATTGATATGCTTAAGTTCANCGGGTAATCTCACTTGATTCGAGCCCGAAGAAAAAGAGAAAAGGGAAGTATATTTTAAAAAACAGCCCTTTTGAGAGA
GAGAAGAATGGATGGAATGAAAAAGAGAGGTTCTTACACAGATTGAATGTGCCCTTACTTCACTCTATCTTTCTTTTACCCTTTCTCTGTTCTTTCTTCAAGTGACATATATATAAATAT
TGATTGACATCCAACCTTTCACAAAAAACAGTGTGATTTCTTGTTT

+

8BCC@EGGGEGEFFGGGGGGGGFFGAGGGGG<FGGCH#>CFGFGGGGEGG9FGGGCAFEFGFEFCFGGGGF<CF7EFFGGDFFFGCDGFGGGGGGGGG<4?,,E<+BEFFGGGGGGGGF
GE<FFDF9<@FCF7B=ABFCF8F8FGGFCF@F?FFGAADBFFCBGCFEFD<DFCFFGCFCC<<CFGG,E;FGGDCF9@DC9BCD;=E9>7;DB,2;;?C,<?,?D;EDC1<?F9>,,;CC,>CC<,,11=CC,+0:<C
75D?+2++302*2)1957?CF*:*02:D::6::2*.)



@M01338:44:000000000-AFFTE:1:1101:10307:1063 1:N:0:5
TCGCAGACACGGAAGGATCATTACACACAAAAAATTNCACGTGAATGTACTCCGTATGAGGTTTTGTGTTGCTCATTTATGGAGTGAAGTCCGGAAGGTTTCGTATGAAACCGATGTA
TCTTTTAACTCCCTTTTAAATTAATTACTGAACATAATTGCAGACAGAAATGTGTGCATAAAATTGAAATACAACCTTCAACAGTGGATGTCTAGGCTCGCACATCGATGAAGAACTCTCGGAA
CTGCGATACGTAATGCGAATTGCAGAAATCAGTGAGTCAACAAAAATTTTGACC

+

8BCCGGEGFGCGGGGGCEC<FDGFFGGGEGGGFFGGGGG#>CFGFFFGGGG9FFEFCE<FGFG,,CDFGFG8FDGGGGC,EFFDGGGFEEAFGGC@7C+@CF,EFF<EFFGGGGFFEGFCFGE
GG?F@,B,?FDGFEFEFGFC9??C<FFFFG,<F,<E9DDD8DF8,7FFBEDFFC,7CFBFGCGGG=C@CFAFFAB98E,DFFF9>;>EC@,::CGCCCEFGCEGFG:8,4>79=5/*+=C=F5*79+=E7:ED
C77EG4CGGFGGCG+7+02207*/;)20*01

@M01338:44:000000000-AFFTE:1:1101:10533:1065 1:N:0:5
TCGCAGACACGGAAGGATCATTACACACATATGAAACNAACCTAAACGTAGTCTTATTTTCTAAGCGGGCGTGCACCGGCTCCAGTGCAGTGTGTTGGTGTAGGCCGACCGA
ACTCTCTCTAGAAATATGTGCTAAACCTTCAGTTGTAAACACCAACTTTTATCAAGGCAACCAACCAATATTTTCTAATCTGAAAAATTTTCGACGATGATACCTTGGTTCCCATATCATGAA
GAACGACGAAATGCGATACGCAATGCGAATTGCATAATCCGAGAGTCATC

+

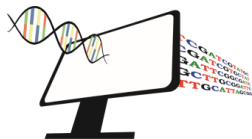
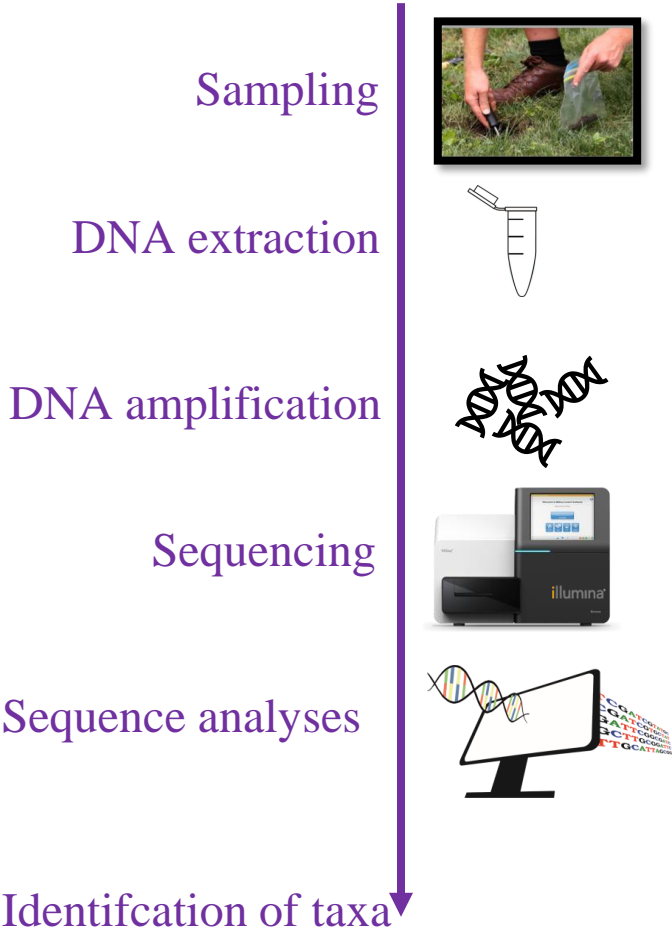
8ACCGGGGGGGEGGGGGGGGGGGGGGG@>FCFFGGGGG#>CFGGGGGGGGGGGGGGGGGGG>FGGGGGGGGGGG>FEGFGCFGCCGGGFFGG@FF=@FFD9FGGGDFGFBEG
FGDEGGGGGGGGCFGGFFGGGGFFGGGGGGGGGGFF7FGGGFGG;FFGGGGGDFAGGCCFGGGGGGGGGF>*CCFFGCFDGG:FFC@9B9B@8<F:18,*<C>*8@FFGCFGF78<CFGG6C
+;CFCCFGDGCCEEGEE5++*95>5EDG*69)1)73*2C+*2*7*F*)1)*0/@M01338:44:000000000-AFFTE:1:1101:9082:1061 1:N:0:5
CTCGCCGTGCTCCTGCGCTTATTGATATGCTTAAGTTCANCGGGTAATCTCACTTGATTGAGCCCGAAGAAAAAGAGAAAAAGGGAAGTATATTTTAAAAAACAGCCCTTTTGAGAGA
GAGAAGAATGGATGGAATGAAAAAGAGAGGTTCTTACACAGATTGAATGTGCCCTTACTTCACTCTATCTTTCTTTTACCCTTTCTCTGTTCTTTCTTCAAGTGACATATATAAATAT
TGATTGACATCCAACCTTTCACAAAAAACAGTGTGATTTCTTGTTT

GEFFFGGGGGGGGGFFGAGGGGG<FGGCH#>CFGFGGGGEGG9FGGGCAFEFGFEFCFGGGGF<CF7EFFGGDFFFGCDGFGGGGGGGGG<4?,,E<+BEFFGGGGGGGGF
GF7B=ABFCF8F8FGGFCF@F?FFGAADBFFCBGCFEFD<DFCFFGCFCC<<CFGG,E;FGGDCF9@DC9BCD;=E9>7;DB,2;;?C,<?,?D;EDC1<?F9>,,;CC,>CC<,,11=CC,+0:<C
2)1957?CF*:*02:D::6::2*.)

@M01338:44:000000000-AFFTE:1:1101:10307:1063 1:N:0:5
TCGCAGACACGGAAGGATCATTACACACAAAAAATTNCACGTGAATGTACTCCGTATGAGGTTTTGTGTTGCTCATTTATGGAGTGAAGTCCGGAAGGTTTCGTATGAAACCGATGTA
TCTTTTAACTCCCTTTTAAATTAATTACTGAACATAATTGCAGACAGAAATGTGTGCATAAAATTGAAATACAACCTTCAACAGTGGATGTCTAGGCTCGCACATCGATGAAGAACTCTCGGAA
CTGCGATACGTAATGCGAATTGCAGAAATCAGTGAGTCAACAAAAATTTTGACC

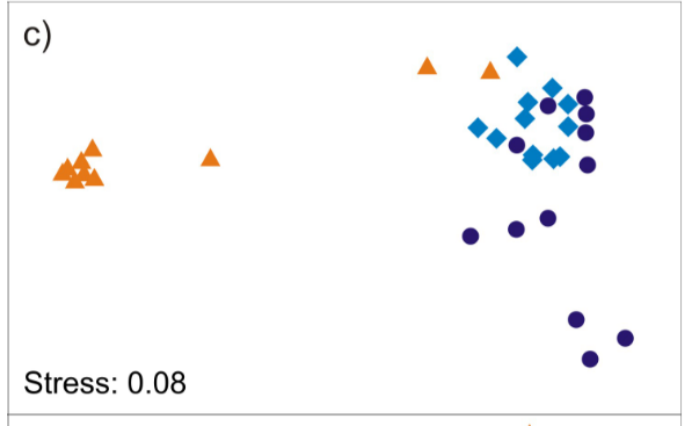
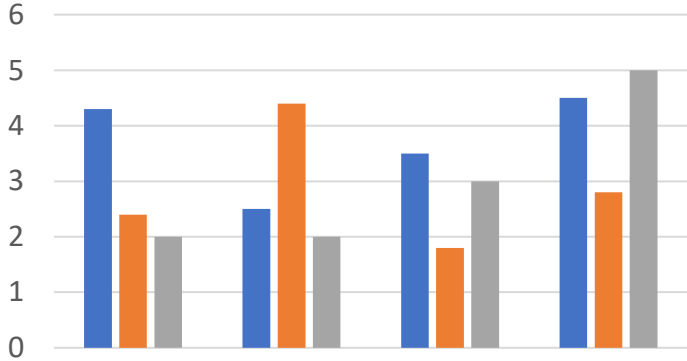
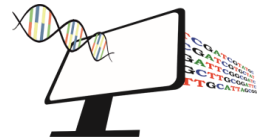
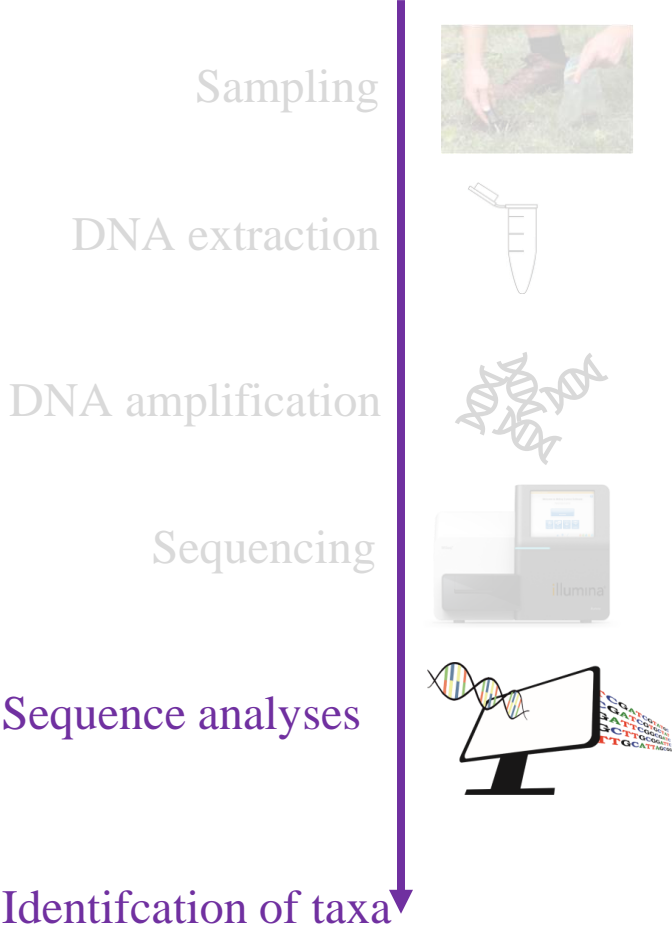


An outline of the metabarcoding approach



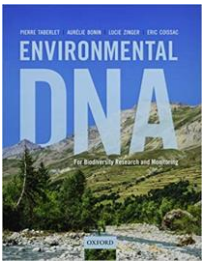
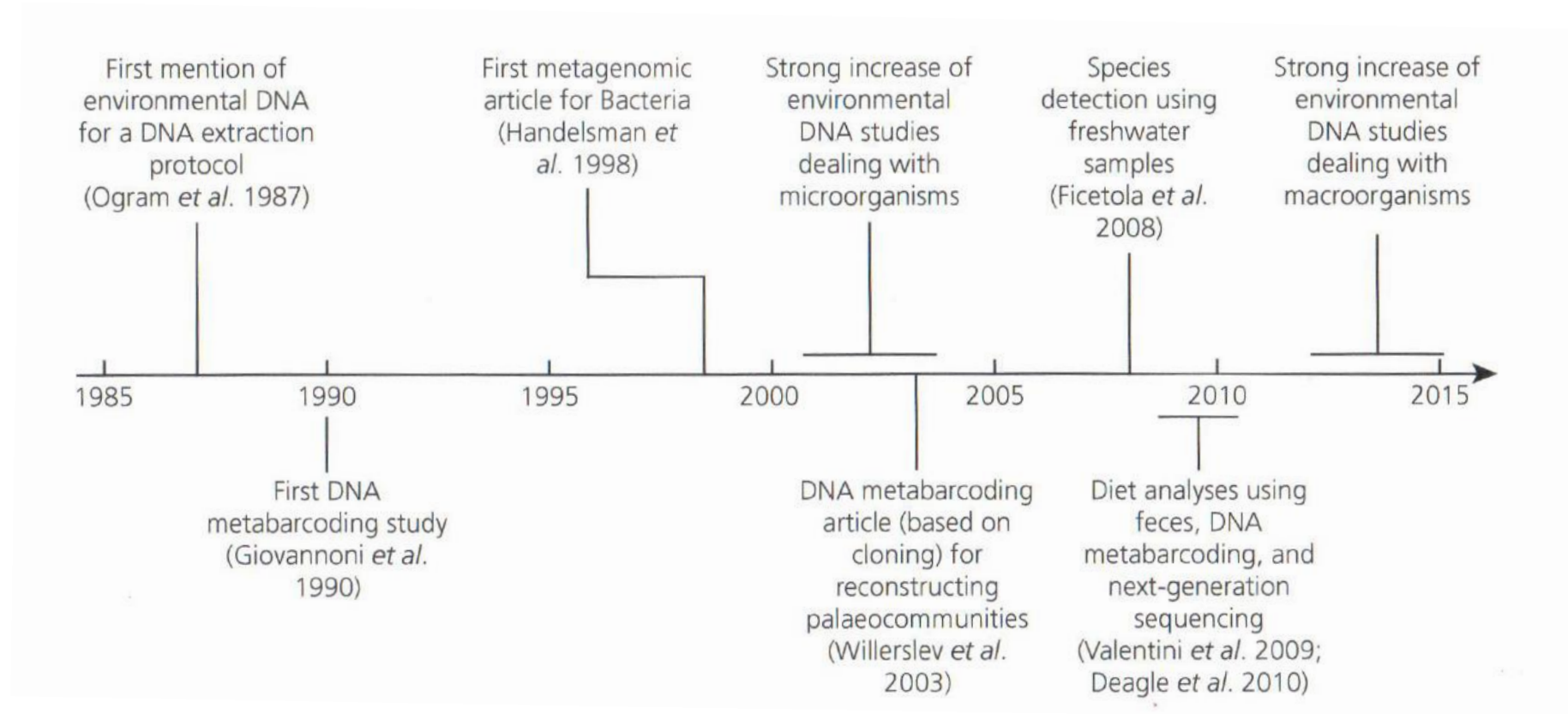
	A	B	C	D	E	F	G	H
1	Kingdom	Phylum	Order	Class	Family	Genus	Species	OTU
2	Fungi	Zygomycota	Mucoromycotina	Mucorales	Cunninghamellaceae	Absidia	Absidia_glauca	otu4
3	Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Aspergillus	Aspergillus_amstelodami	otu25
4	Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Aspergillus	Aspergillus_niger	otu26
5	Fungi	Zygomycota	Zygomycota_cls_Ir	Basidiobolales	Basidiobolaceae	Basidiobolus	Basidiobolus_magnus	otu5
6	Fungi	Zygomycota	Zygomycota_cls_Ir	Basidiobolales	Basidiobolaceae	Basidiobolus	Basidiobolus_ranarum	otu6
7	Fungi	Basidiomycota	Agaricomycetes	Cantharellales	Cantharellaceae	Cantharellus	Cantharellus_decolorans	otu13
8	Fungi	Basidiomycota	Agaricomycetes	Cantharellales	Cantharellaceae	Cantharellus	Cantharellus_decolorans	otu15
9	Fungi	Ascomycota	Dothideomycetes	Hysteriales	Gloniaceae	Cenococcum	Cenococcum_geophilum	otu33
10	Fungi	Ascomycota	Dothideomycetes	Capnodiales	Davidiellaceae	Cladosporium	Cladosporium_cladosporioides	otu44
11	Fungi	Ascomycota	Dothideomycetes	Capnodiales	Davidiellaceae	Cladosporium	Cladosporium_oxysporum	otu31

An outline of the metabarcoding approach



Biological interpretation

Overview of the emergence of eDNA studies



Taberlet et al. 2018

- Sediments
- Water
- Biofilms
- Bulk specimens
- Air
- Snow
- Soil
- Litter
- Roots and other plant parts
- Dust
- Skin
- Gut
- Feces



Sampling



DNA extraction



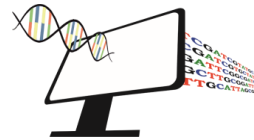
DNA amplification



Sequencing



Sequence analyses



Identification of taxa

Genus	Species
Staurosira	0.96 Staurosira_brevistriata
Amphora	0.23 Amphora_aff_atomoides
Amphora	0.62 Amphora_pediculus
Navicula	0.94 Navicula_cryptocephala

Shotgun sequencing, **metagenomics**

Species-specific DNA amplification,
(quantitative, digital droplet) **PCR**

Sampling



DNA extraction



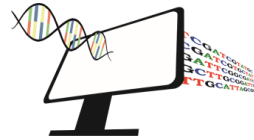
DNA amplification



Sequencing



Sequence analyses



Identification of taxa

Genus	Species
Staurosira	0.96 Staurosira_brevistriata
Amphora	0.23 Amphora_aff_atomoides
Amphora	0.62 Amphora_pediculus
Navicula	0.94 Navicula_cryptocephala
Neidium	0.32 Neidium_productum
Anomoeo	0.47 Anomoeoneis_fogedii
Gomphon	0.21 Gomphonema_capitatum

Lab work sessions

Bioinformatics sessions



Sten Anslan <sten.anslan@ut.ee>