



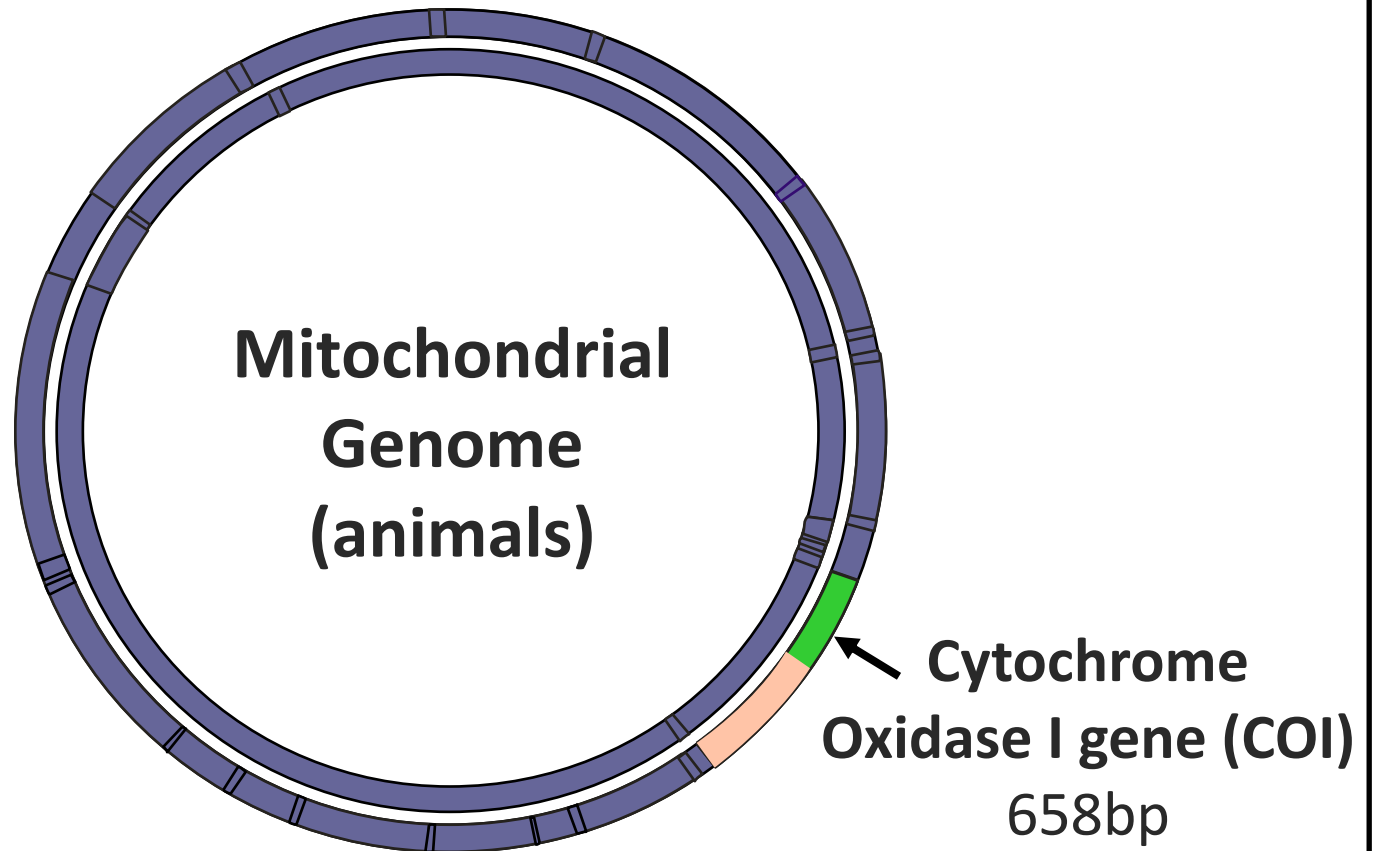
No more PCR: Mitochondrial metagenomics in action

Kat Bruce
Douglas Yu
Alfried Vogler

Min Tang
Chloe Hardman
Xin Zhou

DNA Barcoding

Established since 2003



Same within species; differs between species

DNA Barcoding



PCR amplify
barcode region



GTTATACCTATTATAATTGGAGGATTGGA

BOLDSYSTEMS | Databases | Taxonomy | Identification | Workflows

Advancing species identification and discovery by providing an integrated environment for the assembly and application of DNA barcodes.

Sequence statistics

Phylum	Barcode Sequences
Fungi & Other Life	2,328

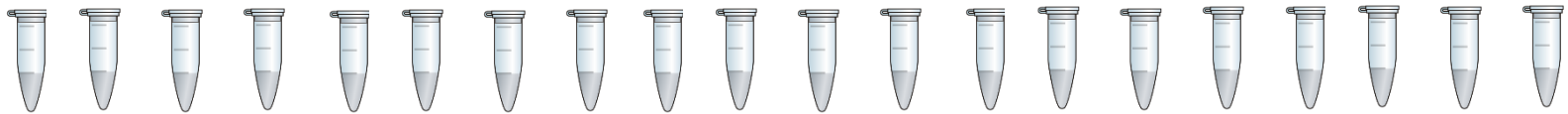
Taxonomy Search

SWALLOWTAIL BUTTERFLY

DNA Barcoding

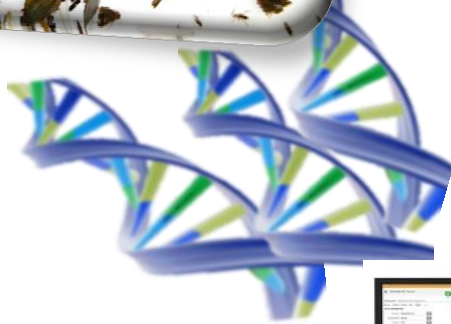
slow

expensive



Separate sequencing reaction for each specimen

Metabarcoding



PCR amplify
barcode region



ATTTGGAGTTATACCTATTATAATTGGAGG

GTTATACCTATTATAATTGGAGGATTGGA

TGGAGTTATACCTATTATAATTGGAGGATT

BOLDSYSTEMS Databases Taxonomy Identification Workb

Advancing species identification and discovery by providing a user-friendly environment for the assembly and analysis of DNA barcodes.

Formally published sequences

Sequence statistics

110,258	de clusters for animals	202,779
Sequences		1,772,072
Fungi & O	Barcode Sequences	1,576,039

Search



SWALLOWTAIL BUTTERFLY
CARABID BEETLE 1
CARABID BEETLE 2

Next generation sequencers can sequence many taxa in parallel

Metabarcoding



You can sequence many
soups in a single run

	Site 1	Site 2	Site 3	Site 4
<i>Carabus sp.</i>	0	0	1	15
Mosquito 345	426	33	0	2
<i>Lasius niger</i>	25	100	2	5

Metabarcoding



PCR amplify
barcode region



ATTGAGTTATACCTATTATAATTGGAGG

GTTATACCTATTATAATTGGAGGATTGGA

TGGAGTTATACCTATTATAATTGGAGGATT

BOLDSYSTEMS Databases | Taxonomy | Identification | Workflows

Advancing species identification and discovery by providing a global environment for the assembly and analysis of DNA barcodes.

Formally published sequences

Sequence statistics

110,258	Barcode clusters for animals	202,779
1,772,072	Sequences	1,772,072
1,576,039	Barcode Sequences	1,576,039

Search



SWALLOWTAIL BUTTERFLY
CARABID BEETLE 1
CARABID BEETLE 2

Next generation sequencers can sequence many taxa in parallel

The Problem(s) with PCR

Amplification bias

Primer design

Contamination

These represent barriers to uptake



100s to 1000s of wild bee species per country

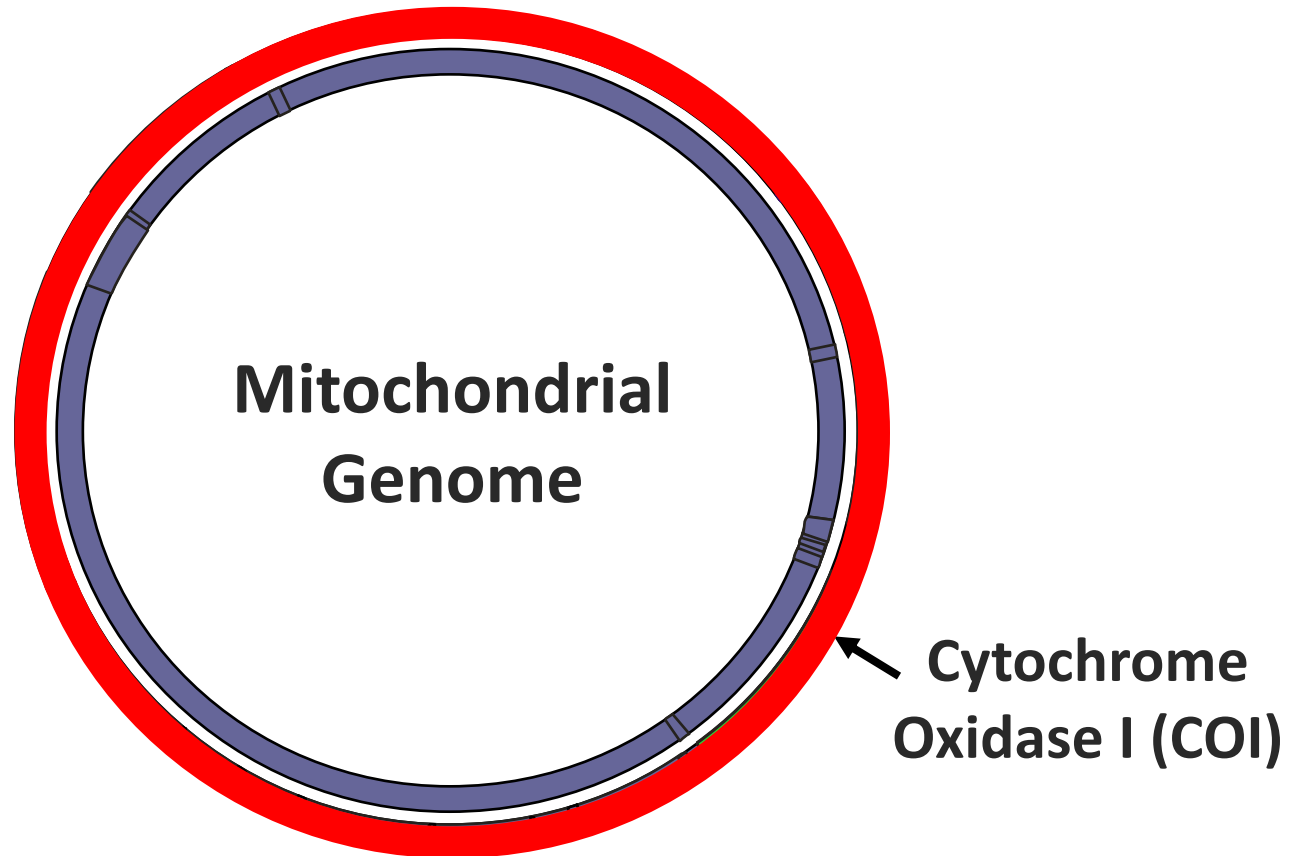


Necessary for pollinating wild and crop plants



© Jane Adams

Metagenomics



Metagenomics



**No PCR
amplification**



Metagenomics



**No PCR
amplification**



GAGTTAT AATTGG TGGAGG
AATTGGA CCTAT TTGG

Metagenomics



Species 1

ATTGAGTTATACCTATTATAATTGGAGGTATAATTGGAGG

Species 2

GTTATACCTATTATAATTTATAATTGGAGGGGAGGATTTGGA

CCTATTA

AATTGGA

ATTTGG

Species 3

TGGAGTTATACCTATAATTGGAGGTATTATAATTGGAGGATT

GAGTTAT

TTGG

TGGAGG

No PCR
amplification



GAGTTAT

ATTTGG

TGGAGG

AATTGGA

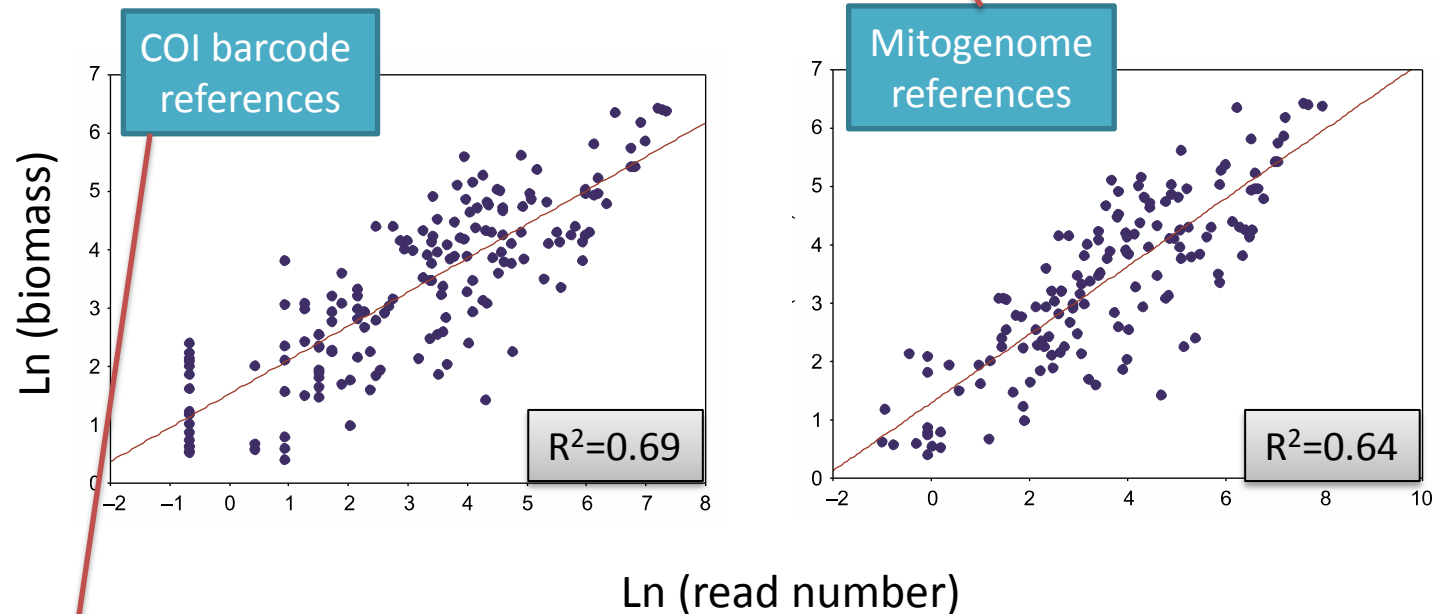
AATTGGA

CCTAT

TTGG

Validating the power of mitochondrial metagenomics for community ecology and **phylogenetics** of complex assemblages

Carola Gómez-Rodríguez^{1,2*}, Alex Crampton-Platt^{1,3}, Martijn J. T. N. Timmermans^{1,4,5},
Andrés Baselga² and Alfried P. Vogler^{1,4}



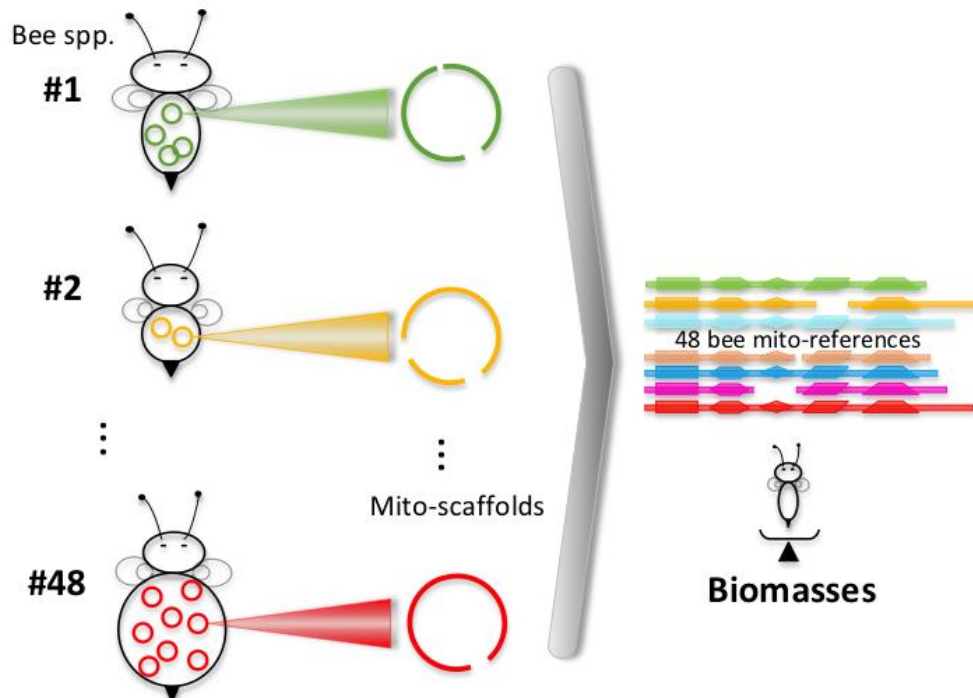
More false absences

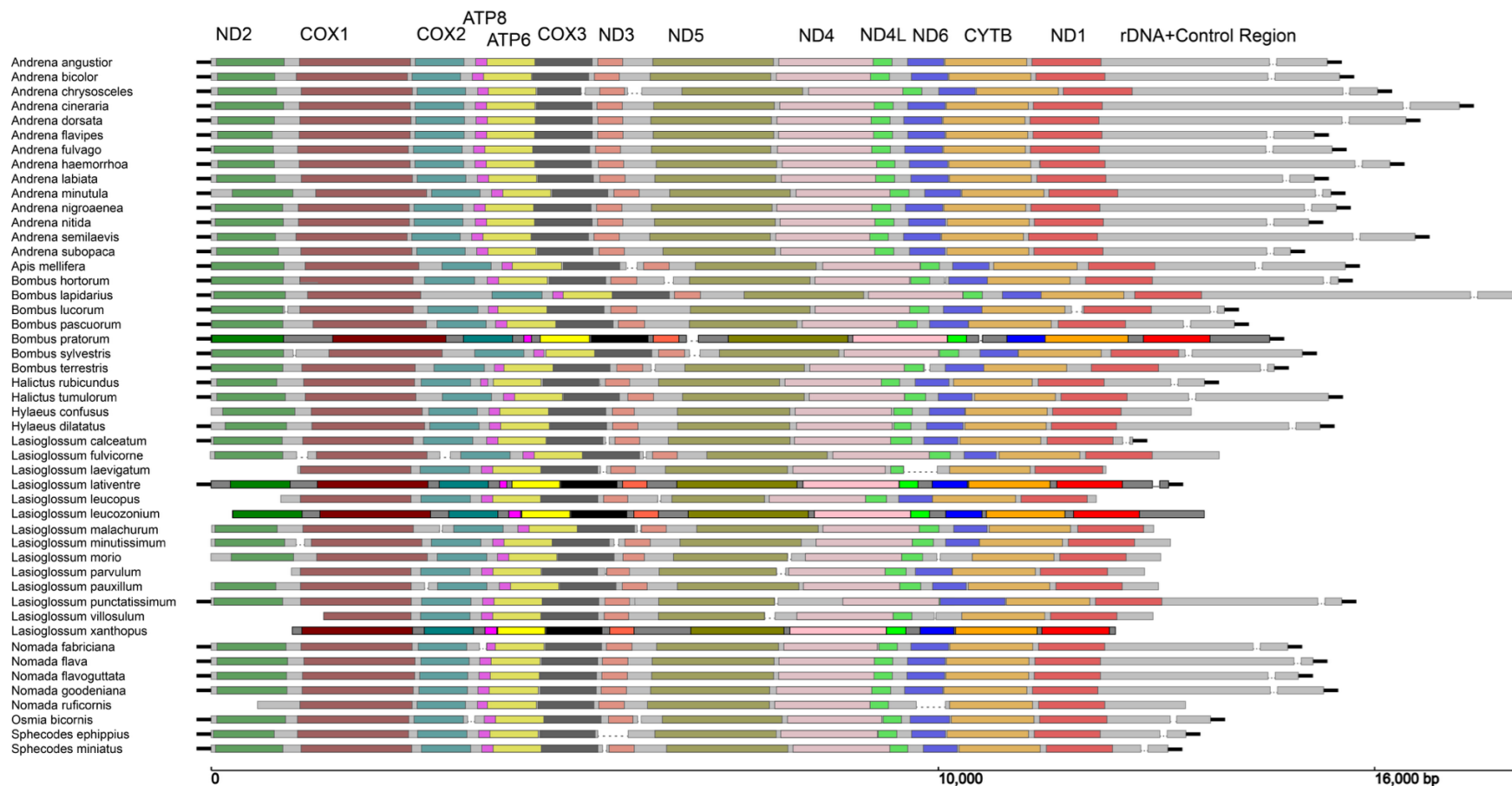
High-throughput monitoring of wild bee diversity and abundance via mitogenomics

Min Tang^{1†}, Chloe J. Hardman^{2†}, Yinqiu Ji^{3†}, Guanliang Meng¹, Shanlin Liu¹, Meihua Tan^{1,4}, Shenzhou Yang¹, Ellen D. Moss⁵, Jiaxin Wang³, Chenxue Yang³, Catharine Bruce⁶, Tim Nevard^{7,8}, Simon G. Potts², Xin Zhou^{1*} and Douglas W. Yu^{3,6*}

1. Mitogenomic skimming

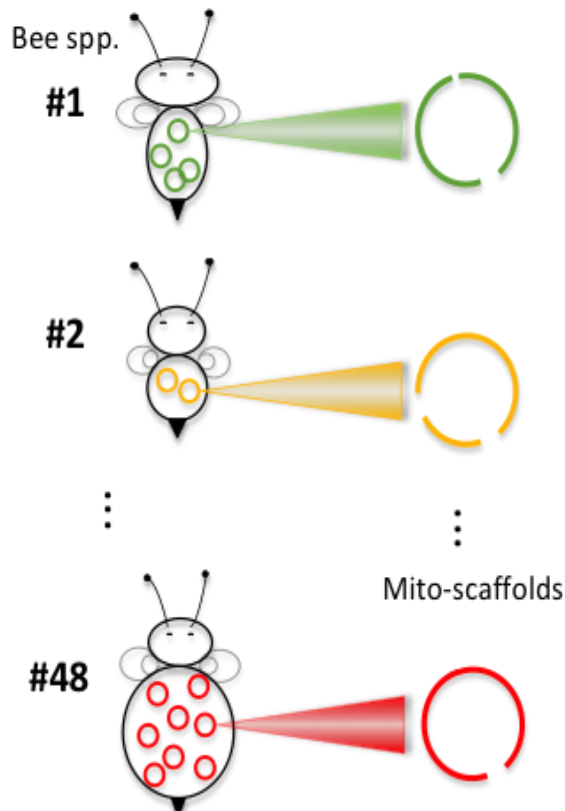
DNA extraction, sequencing (Hiseq 2000), assembly (SOAPdenovo-Trans), mito-annotation, protein-coding-gene extraction alignment (ClustalW2, MEGA6), and verification with reads (BWA)





1. Reference construction

DNA extraction, sequencing (Hiseq 2000), assembly (SOAPdenovo-Trans), mito-annotation, protein-coding-gene extraction alignment (ClustalW2, MEGA6), and verification with reads (BWA)



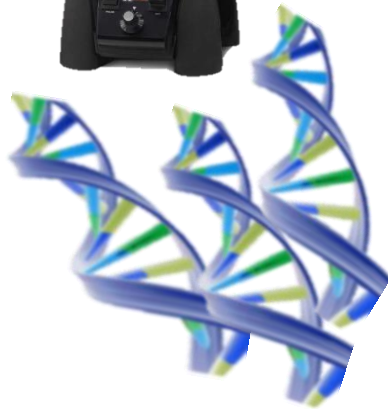
Bulk bee samples



Sample 1, Sample 2 ... Sample 10



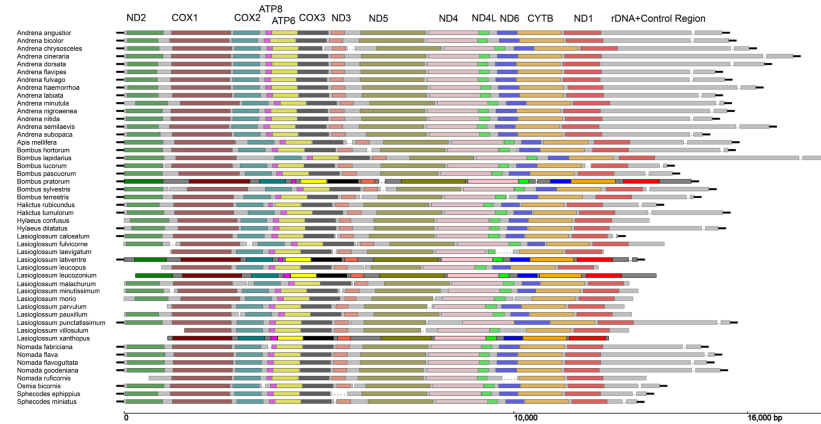
Biomasses



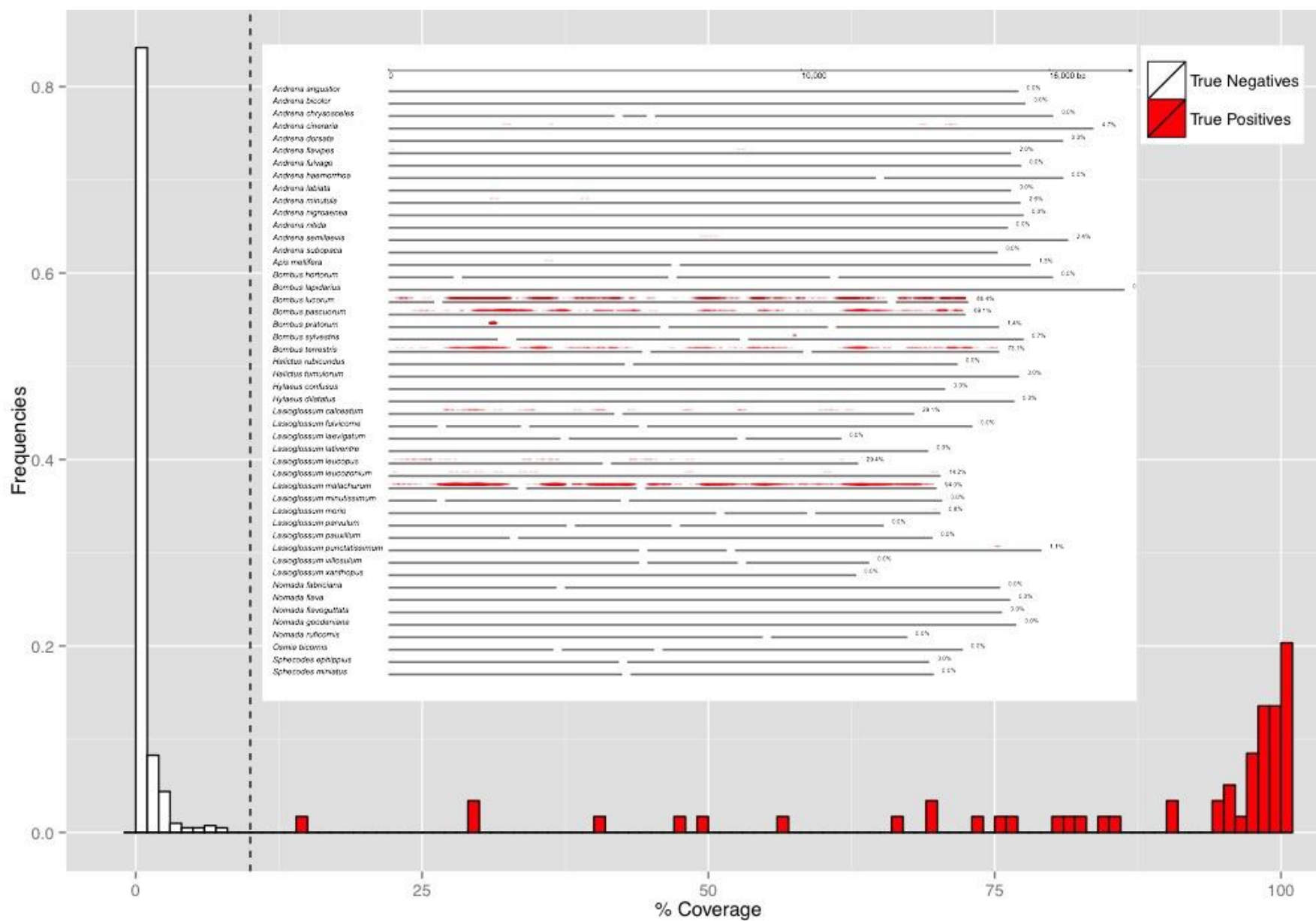
No PCR amplification



Metagenomics



A collection of yellow sticky notes with various 6-letter DNA sequences. The sequences include GAGTTAT, TTTTCC, TGGAGG, AATTGGA, CCTAT, TTGG, and others.



We can detect bee species in bulk samples (93.7% detection rate)

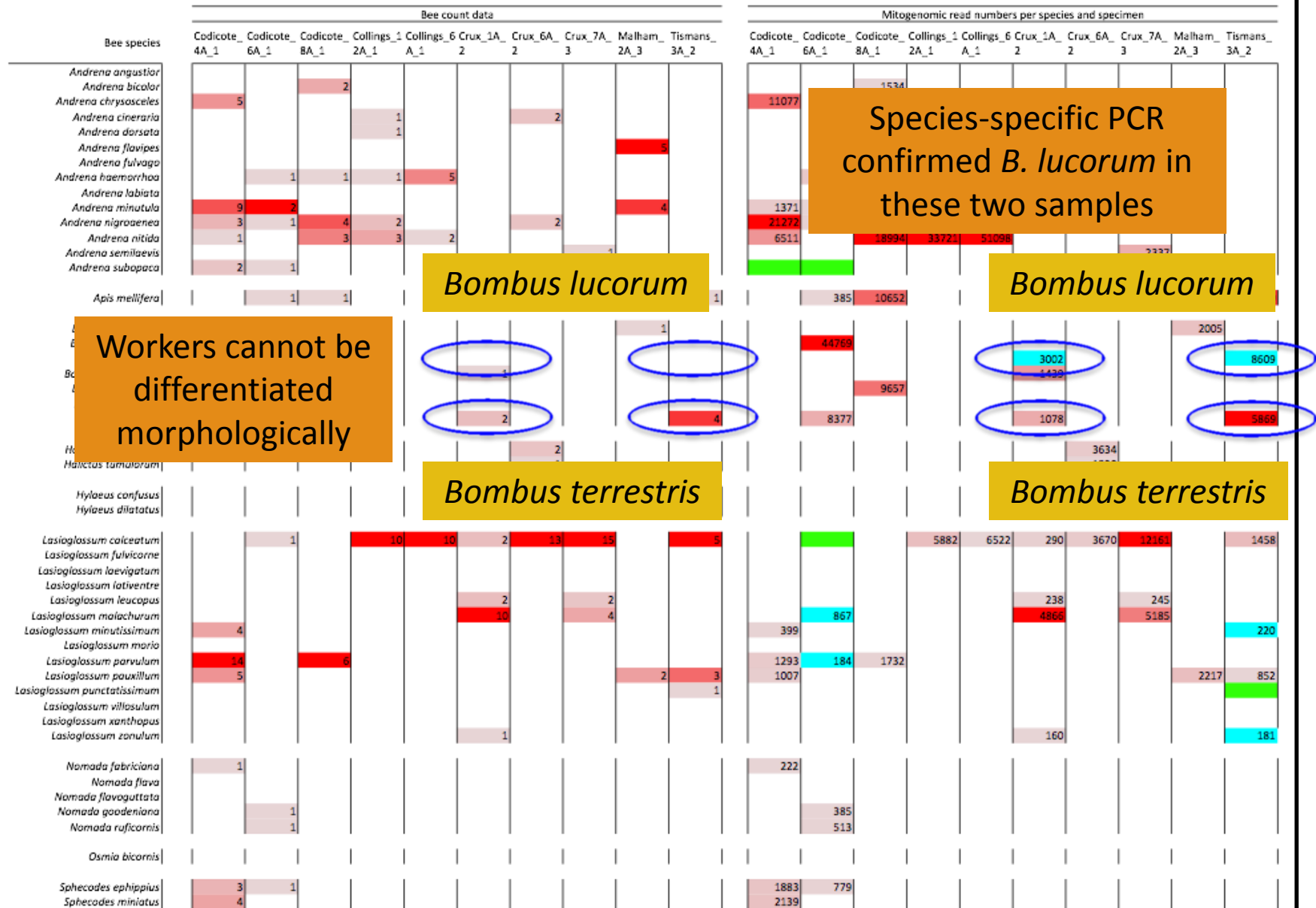
[illegible][illegible]

We can detect bee species in bulk samples (93.7% detection rate)

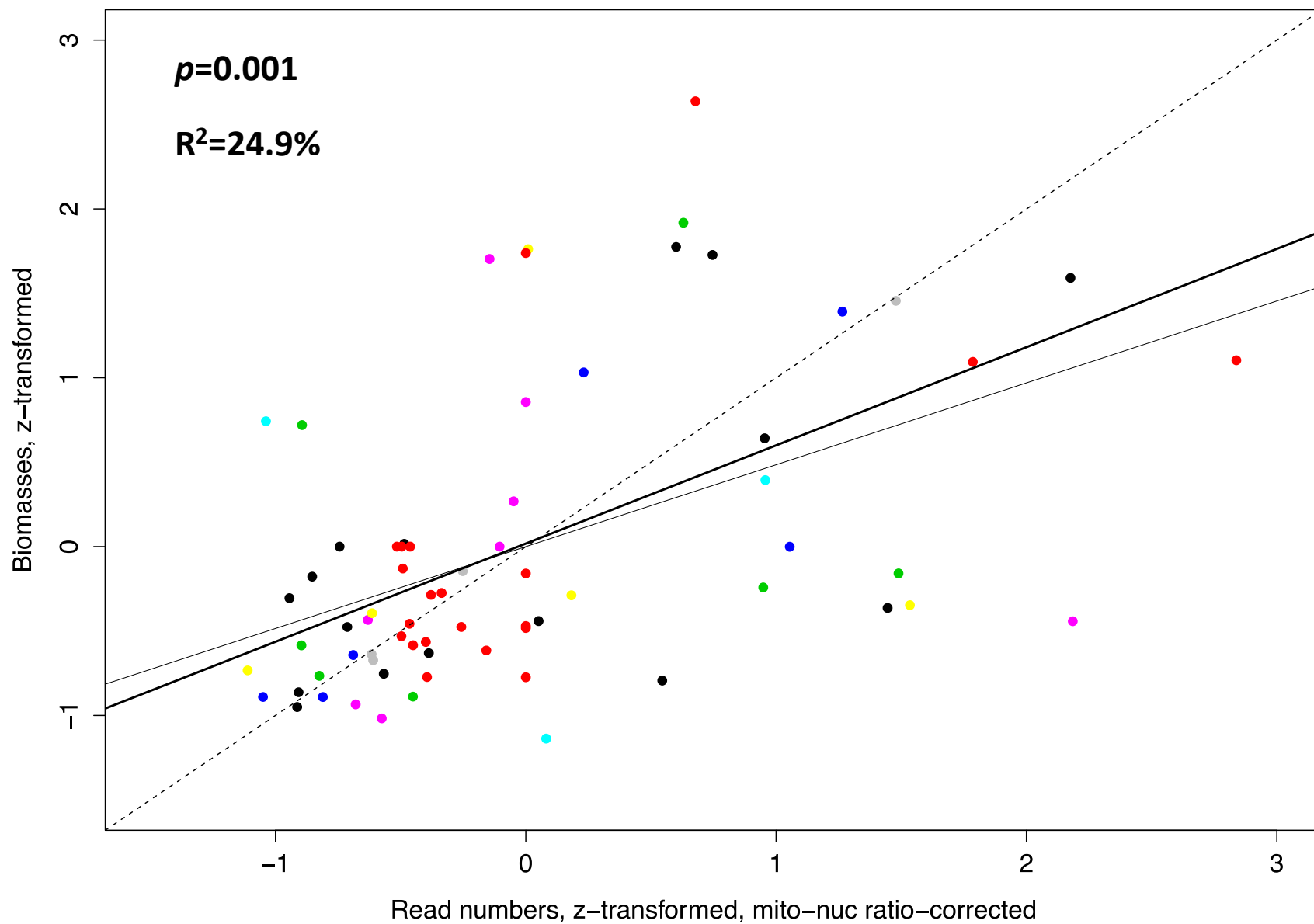
	Codicote_4A_1	Codicote_6A_1	Codicote_8A_1	Collings_2A_1	Collings_A_1	Crux_Crux_1A_2	Crux_Crux_6A_2	Crux_Crux_7A_3	Melham_Melham_2A_3	Tismans_Tismans_3A_2
Bee species										
<i>Andrena angustior</i>										
<i>Andrena bicolor</i>										
<i>Andrena chrysaeseles</i>		5								
<i>Andrena cineraria</i>										
<i>Andrena dorsata</i>										
<i>Andrena flavipes</i>										
<i>Andrena fulvago</i>										
<i>Andrena haemorrhoa</i>										
<i>Andrena labiata</i>		9								
<i>Andrena minutula</i>		3								
<i>Andrena nigroaenea</i>		1								
<i>Andrena nitida</i>		2								
<i>Andrena semilaevis</i>										
<i>Andrena subopaca</i>										
<i>Apis mellifera</i>										
<i>Bombus hortorum</i>										
<i>Bombus lapidarius</i>										
<i>Bombus lucorum</i>										
<i>Bombus pascuorum</i>										
<i>Bombus pratorum</i>										
<i>Bombus sylvestris</i>										
<i>Bombus terrestris</i>										
<i>Halictus rubicundus</i>										
<i>Halictus tumularum</i>										
<i>Hylaeus confusus</i>										
<i>Hylaeus dilatatus</i>										
<i>Lasioglossum calceatum</i>										
<i>Lasioglossum fulvicorne</i>										
<i>Lasioglossum laevigatum</i>										
<i>Lasioglossum lativentre</i>										
<i>Lasioglossum leucopus</i>										
<i>Lasioglossum malachurum</i>										
<i>Lasioglossum minutissimum</i>		4								
<i>Lasioglossum morio</i>		10								
<i>Lasioglossum parvulum</i>		5								
<i>Lasioglossum pauxillum</i>										
<i>Lasioglossum punctatissimum</i>										
<i>Lasioglossum villasolum</i>										
<i>Lasioglossum xanthopus</i>										
<i>Lasioglossum zonulum</i>										
<i>Nomada fabriciana</i>		1								
<i>Nomada flava</i>										
<i>Nomada flavoguttata</i>										
<i>Nomada goodeniana</i>										
<i>Nomada ruficornis</i>										
<i>Osmia bicornis</i>										
<i>Sphecodes ephippius</i>		3								
<i>Sphecodes miniatus</i>		4								

[illegible]

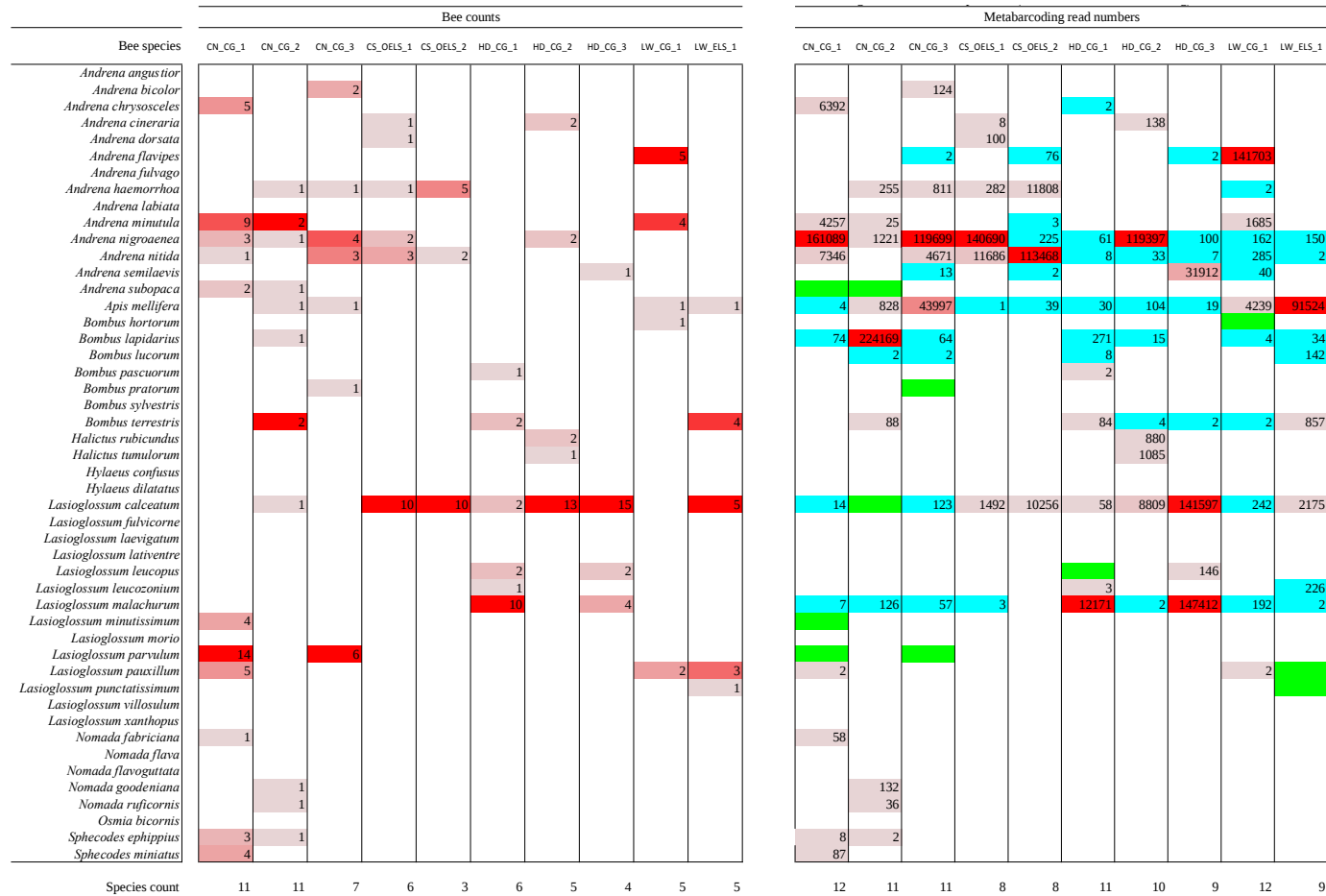
We can detect bee species in bulk samples (93.7% detection rate)



Biomass versus Read numbers



Metabarcoding gives lots of false positive (blue) and false negatives (green)



319 bp amplicon, primers modified from Leray et al. (2013)



Thank You



Imperial College
London

