

Spider webs as a source of eDNA

LifeWatch ERIC worskop

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Matjaž Gregorič (matjaz.gregoric@zrc-sazu.si)

Institute of Biology ZRC SAZU, Ljubljana, Slovenia



Introduction: Institute of Biology ZRC SAZU

Vegetation

- Vegetation classification and community diversity
- Synsystematics
- Dynamics
- Macroecology
- Nature conservation (habitat types)
- Databases

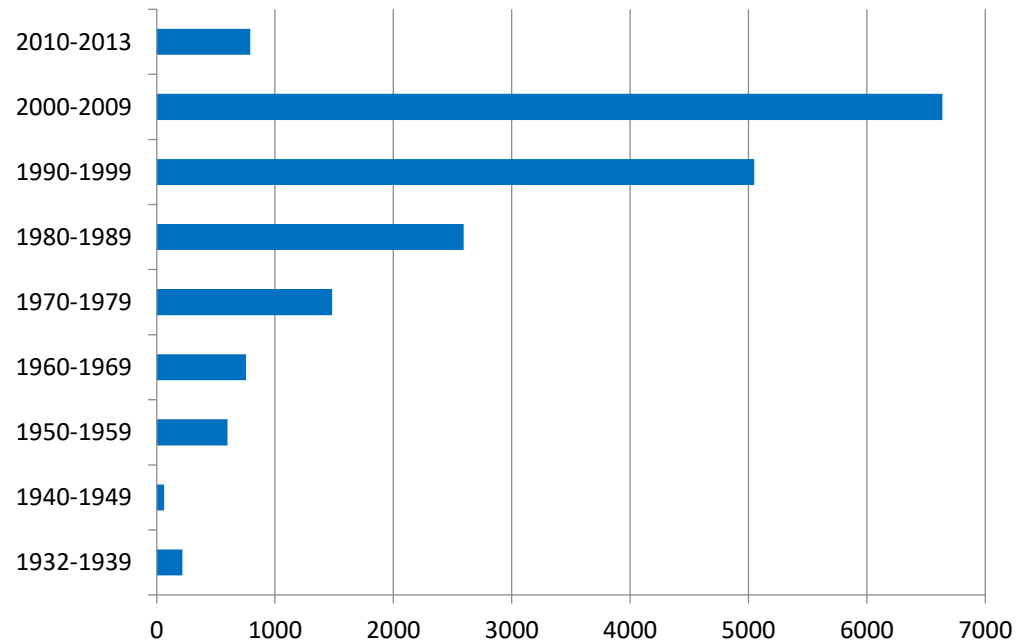
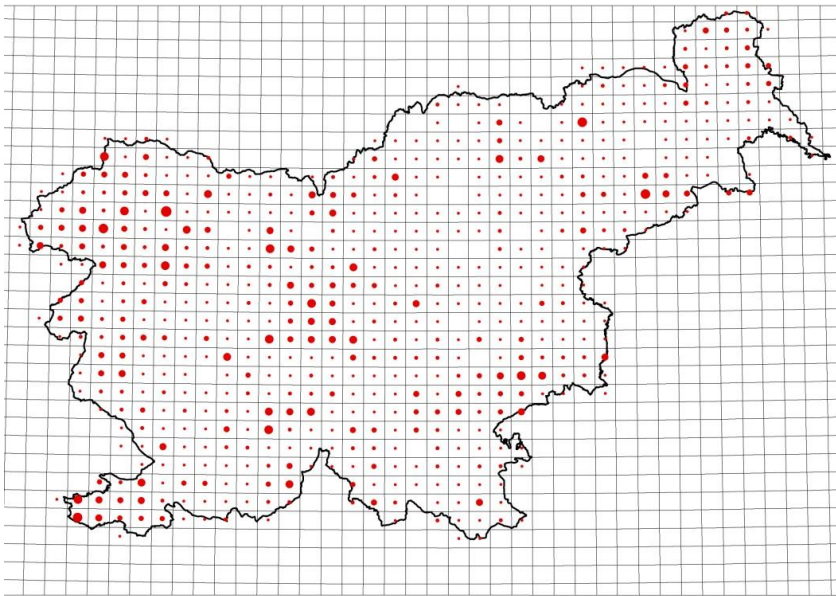


Arachnology

- Systematics & taxonomy
- Evolution of traits: sexual size dimorphism, behavior, webs, silk material properties
- Phylogeography & speciation
- DNA barcoding
- eDNA from webs

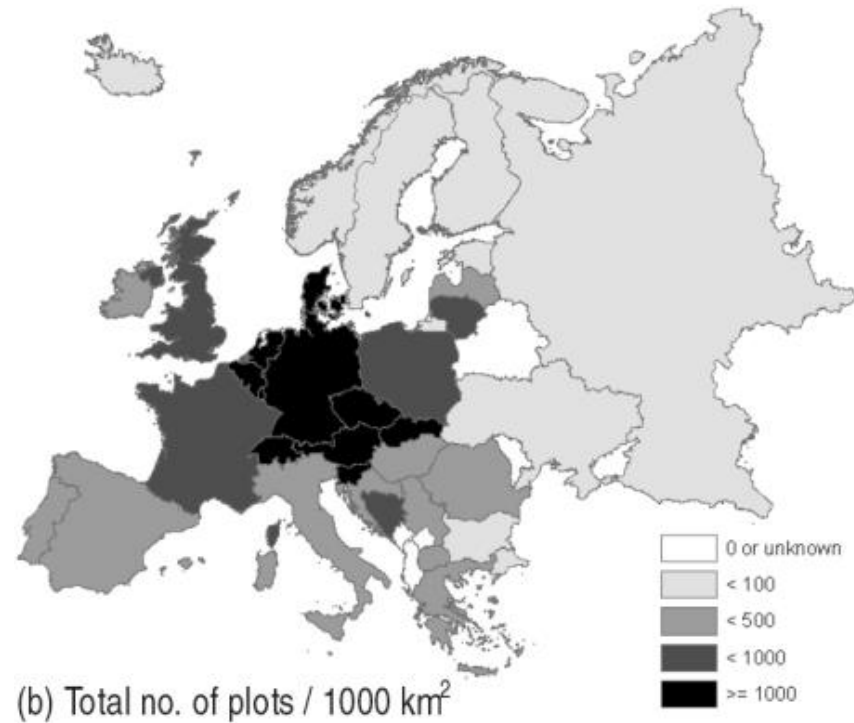
Vegetation: Slovenia

- Database – Institute of Biology ZRC SAZU
- Mostly Braun-Blanquet method
- Oldest relevé 1932
- 21.000 relevés



Vegetation: European vegetation archive – EVA

All relevés > 4.3 million



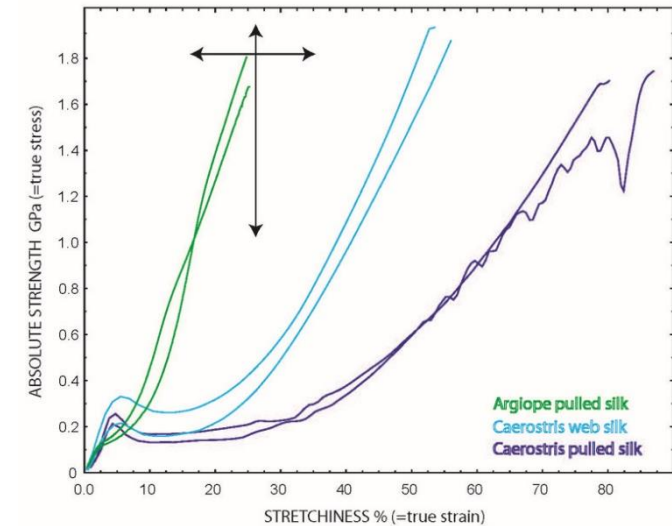
Nr relevés / 1000 km²
(Schaminée et al. 2009)

International synergy



Arachnology: Web & silk evolution

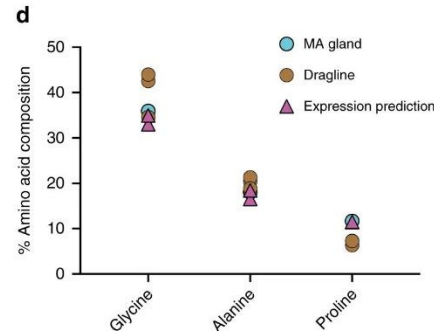
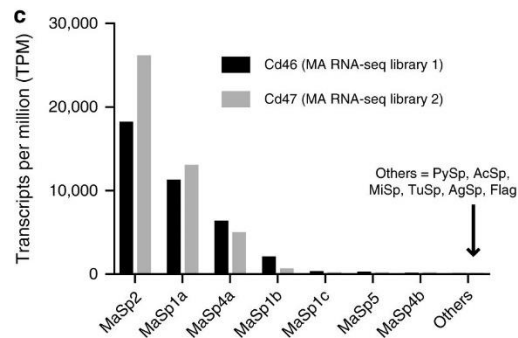
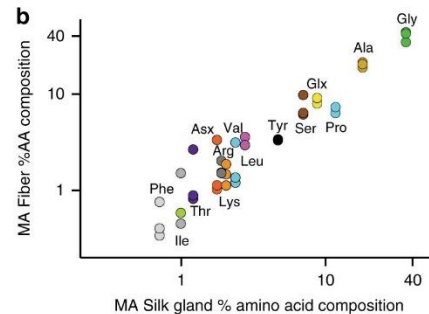
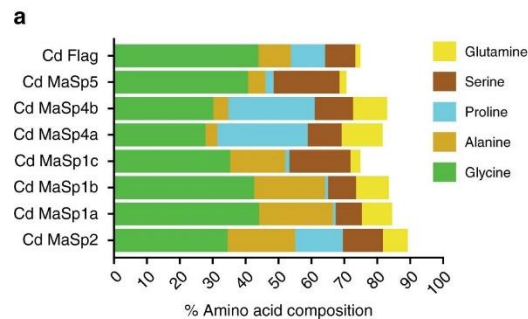
- Web biology & evolution
- Silk – toughest biomaterial



Agnarsson et al. 2010 PLoS ONE
Kuntner & Agnarsson 2010 J Arachnol
Gregorič et al. 2011 J Arachnol
Gregorič et al. 2011 PLoS ONE

Arachnology: Web & silk evolution

- Web biology & evolution
- Silk – toughest biomaterial
- Comparative genomics
- Gland specific gene expression



N. clavipes: 28+

Major ampullate :	8	
Minor ampullate :	4	
Piriform :	1	
Aciniform :	1	
Tubuliform :	1	
Venom-gland expressed :	1	
Flagelliform :	1	
Aggregate :	4	
Other / unknown :	7	

Babb et al. 2017 Nature Genetics



C. darwini: 33+

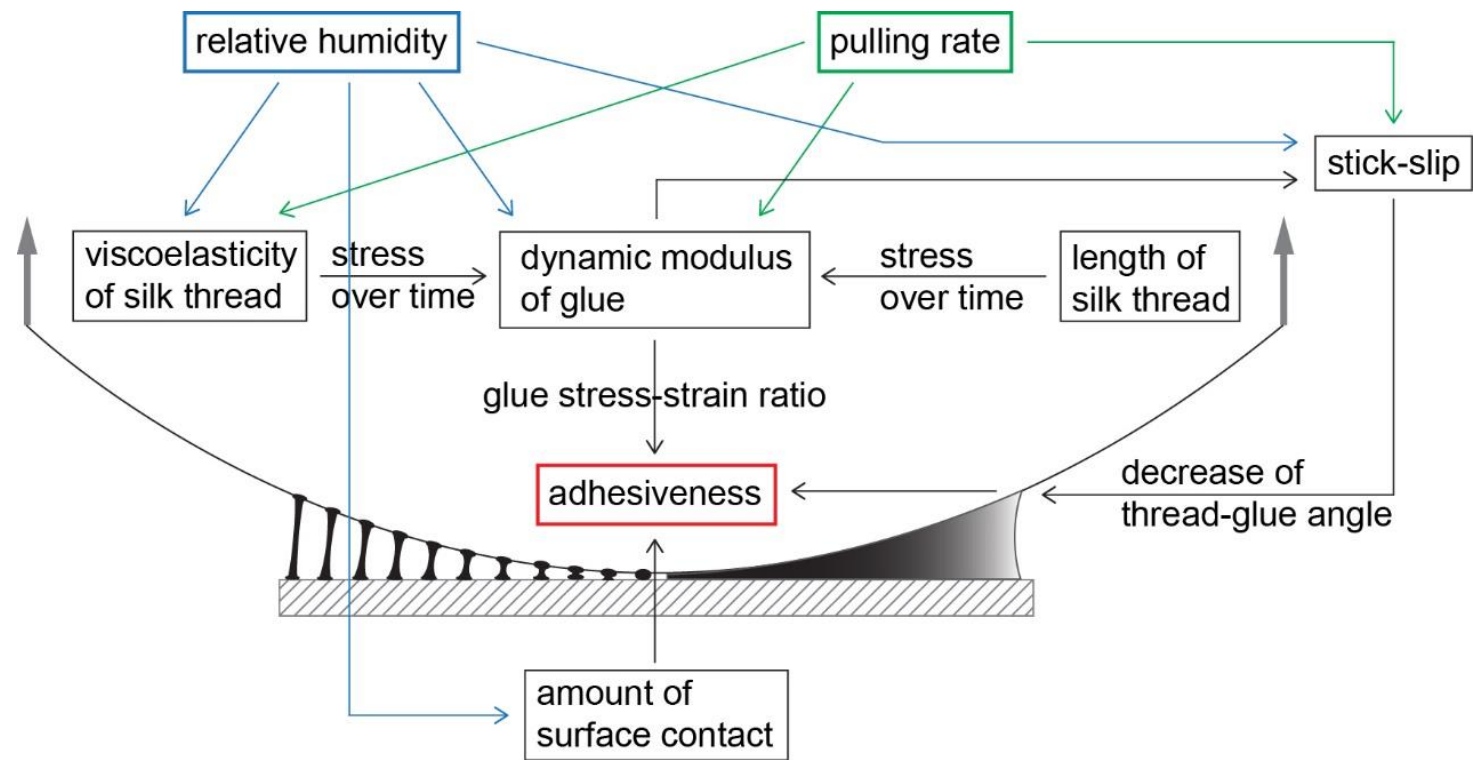
Major ampullate :	8	
Minor ampullate :	5	
Piriform :	1	
Aciniform :	1	
Tubuliform :	1	
Venom-gland expressed :	0 (not present)	
Flagelliform :	6	
Aggregate :	4	
Other / unknown :	7	

Babb et al. In prep.

Garb et al. 2019 Communications Biology

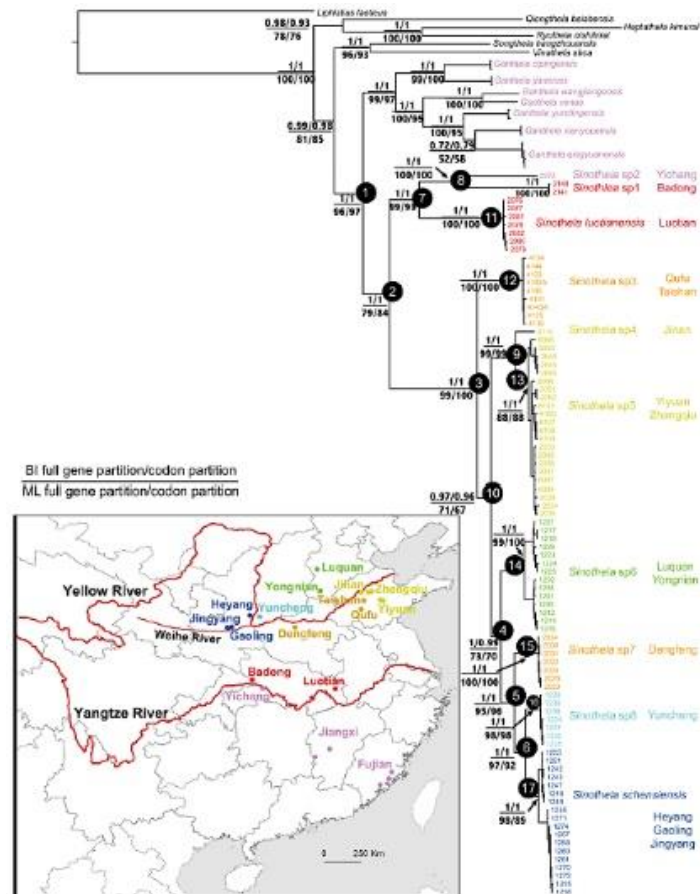
Arachnology: Web & silk evolution

- Web biology & evolution
- Silk – toughest biomaterial
- Comparative genomics
- Gland specific gene expression
- Glue – responsive adhesive system

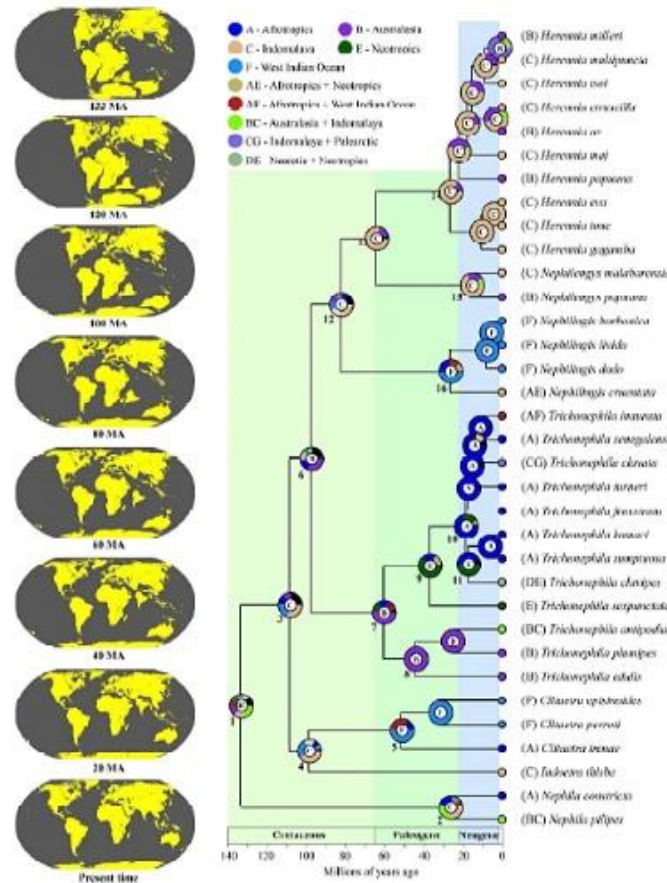


Gregorič et al. In prep.

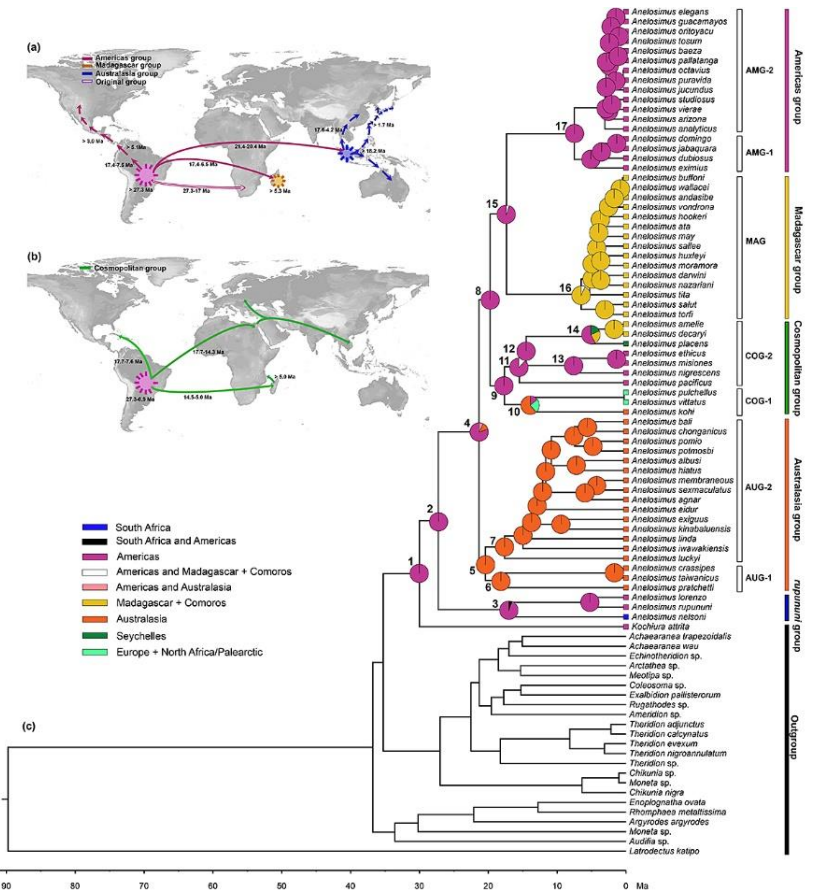
Arachnology: Phylogeography



Xu et al. 2017 J Biogeography



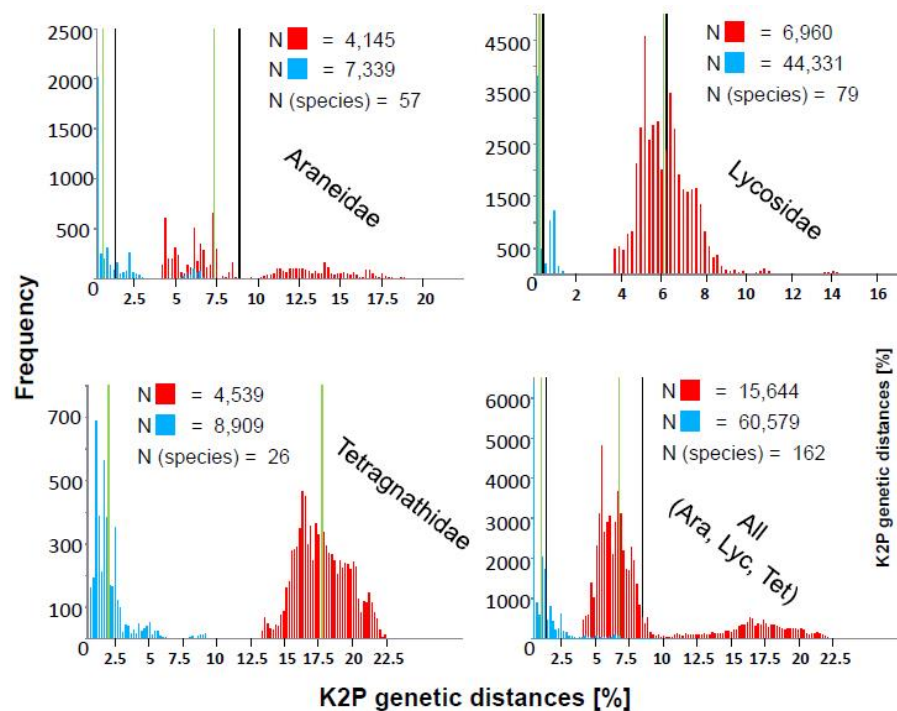
Turk et al. J Biogeography in press



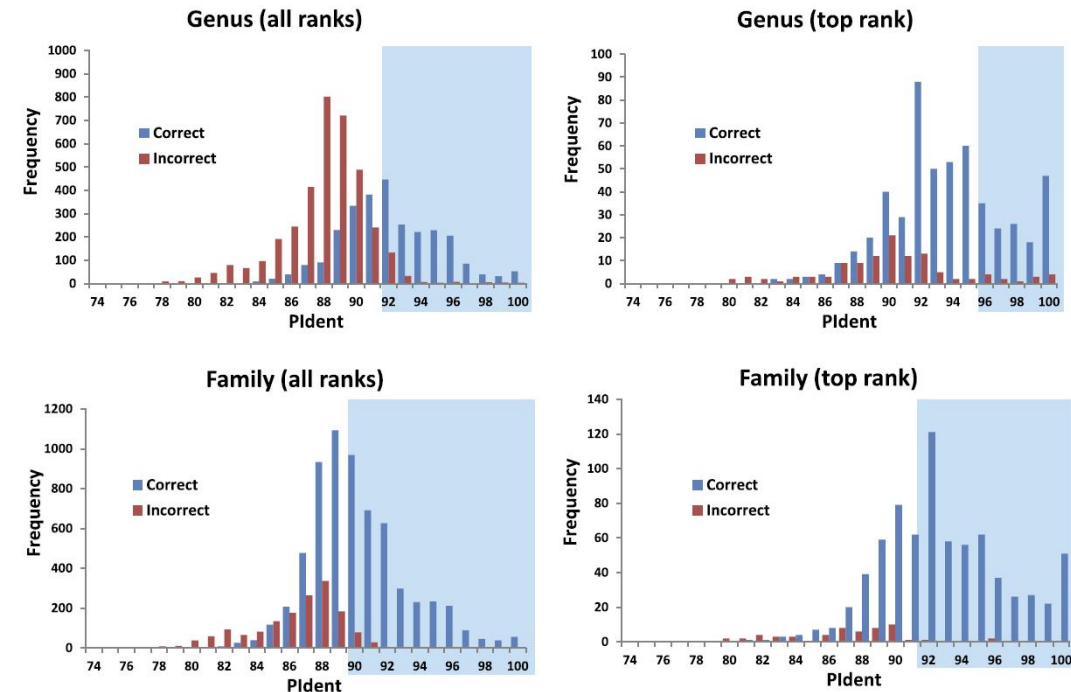
Luo et al. Systematic Biology in press

Arachnology: DNA barcoding & preservation

- DNA barcoding & utility
- Cryopreservation (Global Genome Initiative)



Čandek et al. 2015 Molec Ecol Res



Coddington et al. 2016 PeerJ

eDNA: Spider webs

Xu et al. 2015 Plos One

- *Latrodectus* silk contains host/prey DNA



RESEARCH ARTICLE

Spider Web DNA: A New Spin on Noninvasive Genetics of Predator and Prey

Charles C. Y. Xu¹*, Ivy J. Yen¹, Dean Bowman², Cameron R. Turner¹†

1 Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana, United States of America, **2** Potawatomi Zoo, South Bend, Indiana, United States of America

*a Current address: Animal Ecology Research Group, Department of Ecology and Genetics, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden

†b Current address: ecoSystem Genetics LLC, South Bend, Indiana, United States of America

* charles.cong.xu@gmail.com

Abstract

Noninvasive genetic sampling enables biomonitoring without the need to directly observe or disturb target organisms. This paper describes a novel and promising source of noninvasive spider and insect DNA from spider webs. Using black widow spiders (*Latrodectus* spp.) fed with house crickets (*Acheta domesticus*), we successfully extracted, amplified, and sequenced mitochondrial DNA from spider web samples that identified both spider and prey to species. Detectability of spider DNA did not differ between assays with amplicon sizes from 135 to 497 base pairs. Spider and prey DNA remained detectable at least 88 days after living organisms were no longer present on the web. Spider web DNA as a proof-of-concept may open doors to other practical applications in conservation research, pest management, biogeography studies, and biodiversity assessments.



OPEN ACCESS

Citation: Xu CCY, Yen IJ, Bowman D, Turner CR (2015) Spider Web DNA: A New Spin on Noninvasive Genetics of Predator and Prey. PLoS ONE 10(11): e0142503. doi:10.1371/journal.pone.0142503

Editor: Matjaž Kunther, Scientific Research Centre, Slovenian Academy of Sciences and Arts, SLOVENIA

Blake et al. 2016 Conservation Genet Resour

- *Pholcus* & *Psalmopoeus* silk contains host DNA

Conservation Genet Resour (2016) 8:219–221
DOI 10.1007/s12686-016-0537-8



TECHNICAL NOTE

DNA extraction from spider webs

Max Blake¹ · Niall J. McKeown¹ · Mark L. T. Bushell² · Paul W. Shaw¹

Received: 5 November 2015 / Accepted: 20 April 2016 / Published online: 26 April 2016
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Abstract Many spider species produce webs that represent a potential non-invasive source of DNA for conservation genetic analysis. Reported here is the successful isolation of target DNA from members of two families (Theraphosidae and Pholcidae) using a standard CTAB phenol–chloroform–isoamyl protocol. The isolated DNA was of sufficient quality to permit routine PCR amplification and sequencing of mtDNA COI fragments of various sizes (maximum 710 bp attempted). This adds to other studies in demonstrating that webbing offers an excellent resource for genetic studies of spiders across families. Applications of the technique include species identification and monitoring, faunistic surveys, population connectivity, subpopulation structuring, and ex situ breeding programs.

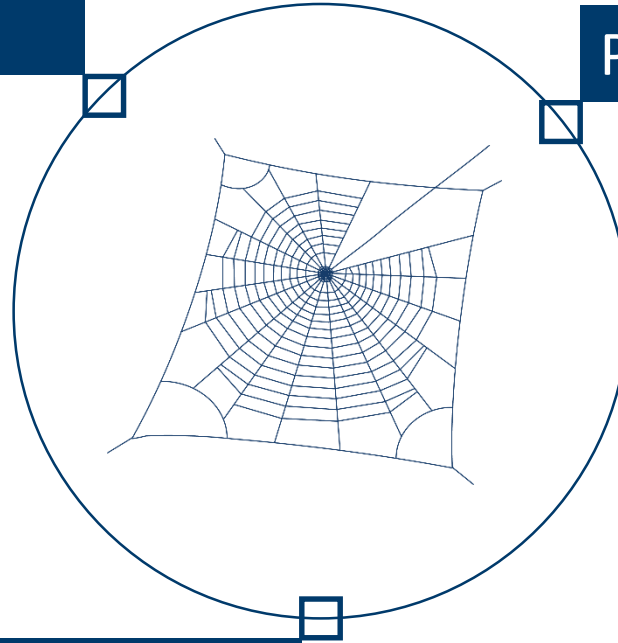
bulk environmental samples, not necessarily targeted toward a taxonomic group (Barnes and Turner 2016)). Spider webbing represents a potential source of DNA for such applications (Xu et al. 2015).

DNA isolation was tested on samples of webbing from two species (*Psalmopoeus cambridgei* Pocock 1895, Theraphosidae, and *Pholcus phalangioides* Fuesslin 1775, Pholcidae) that produce different web forms. *Psalmopoeus* construct vertical sheet webs in enclosed spaces in trees, which are then covered in loose material surrounding the web structure; primarily detritus and leaves (Bushell pers. obs.). *Pholcus* build 'space webs' which are used as prey-detection structures from which the spider hunts prey (Jackson and Brassington 1987). Both species produce

eDNA: Spider webs

Diverse & abundant

- Architecture
- Size
- Microhabitat



Potential application

- Biodiversity monitoring: arthropods, plants, fungi, bacteria
- Invasive species
- Fungal pathogens

Sources of eDNA?

- Easily collected
- Filters of air column
- Spatial & temporal scales

Research goals

Goal 1

- Precise laboratory protocols
- Webs in laboratory

Goal 2

- Confirm laboratory protocols for webs in nature

Goal 3

- DNA metabarcoding



Photo: Visualhunt.com

Goal 1: Methods – prey

Acheta domestica

- Used in *Xu et al. 2015*

2 COI markers

- Adom1
- Adom2



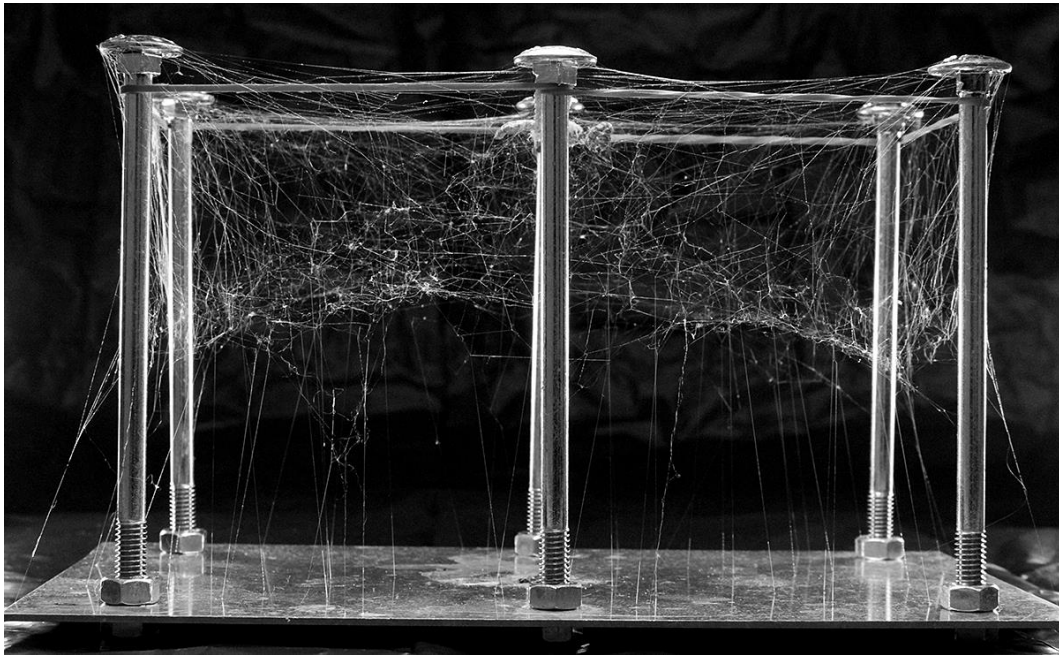
DNA Sequences	Translated Protein Sequences
Species/Abbrv	
1. Acheta_domestic	T A A C A G A A G A A T A G T G A A A A T G G T G C A G G G A C A G G A T G A A C A G T T T A T C C A C C T T T A T C A A C A G G A A T C G C C A C G C G G A G C A T C T G T T G A T T A G C C A T T T T C A T T A C A C T
2. Gryllus_sp.1	T A A C A G A A G A A T A G T T G A A A A T G G T G C A G G G A C A G G A T G A A C A G T A T A T C C C A C C T T A T C A A C A G G A A T C G C C A C G C A G G A G C A T C C G T C G A T T T A G C T A T C T T C T C A T T A C A T T
3. Teleogryllus_inferi	T A A C A G A A G A A T A G T T G A A A A T G G T G C A G G T A C C G G A T G A A C A G T T T A T C C A C C T T T T C A A C A G G A A T C G C C A C G C T G G A G C A T C A G T C G A T T T A G C T A T C T T T C A T T A C A T C
4. Loxoblemmus_do	T A A C A G A A G A A T G G T T G A A A A T G G T G C A G G T A C A G G A T G A A C A G T C T A C C C A C C T T T T C A G C A G A A A T C G C T A T G C A G G A G C T T C A G T T G A T T T A G C T A T T T T C T C T T T A C A T C
5. Loxoblemmus_ari	T A A C A G A A G A A T R G T Y G A A A A Y G G T G C A G G T A C A G G A T G A A C A G T C T A C C C A C C T T T R T C A G C A G A A A T Y G C T A T G C A G G A G C W T C A S T T G A T T T A G C W A T T T T C T C T T T A C A T Y
6. Gryllus_veletis	T A A C A G A A G A A T A G T T G A A A A T G G T G C A G G A A C A G G A T G A A C A G T T T A T C C A C C T T T A T C A A C A G G A A T T G C T A C C G C T G G A G C A T C T G T T G A T T T A G C C A T T T T C T C A T T A C A T T
7. Teleogryllus_emmi	T A A C A G A A G A A T A G T T G A A A A T G G T G C A G G A A C G G A T G A A C A G T T A T A T C C A C C T Y A T C A A C A G G A A T T G C T A C C G C T G G A G C A T C A G T T G A T T A G C C A T T T T C T C A T T A C A T T
8. Teleogryllus_ocea	T G G C T A G A A G A A T A G T T G A A A A T G G A G C A G G T A C T G G A T G A A C A G T T T A T C C A C C C T T A T C A A C A G G A A T T G C T A C C G C T G G A G C A T C C G T A G A C C T G C T A T T T T C T C T T C A T T
9. Gryllus_bimaculat	T A A C A G A A G A A T A G T T G A A A A T G G T G C A G G A C A G G A T G A A C A G T T T A T C C A C C T T T A T C A A C A G G A A T T G C T A T G C A G G A G C A T C C G T T G A C T T A G C A A T T T T T C A T T A C A T T
10. Gryllus_pennsylv	T A A C A G A A G A A T A G T Y G A A A A T G G T G C A G G R A C A G G A T G A A C A G T T T A T C C A C C T T T A T C A A C A G G A A T T G C T A T G C A G G A G C A T C C G T T G A C T T A G C A A T T T T T C T C R C T A C A T T
11. Gryllus_pennsylv	T A A C A G A A G A A T A G T T G A A A A T G G G G C A G G A C A G G A T G A A C A G T A T A C C A C C C T T A T C A A C A G G C A T T T G C T A T G C A G G T G C A T C C G T T G A T T T A G C T A T T T T T C A T T A C A T T
12. Gryllus_sp.2	T A A C A G A A G A A T A A T T G A A A A T G G T G C A G G A A C A G G A T G A A C A G T A T A T C C A C C C T T A T C A A C A G G A A T T G C T A T G C T G G A G C A T C C G T T G A T T T A G C T A T C T T T T C A T T A C A T T
13. Teleogryllus_infe	A A C A G A A G A A T A G T T G A A A A T G G T G C A G G A C A C A G G A T G A A C A G T G T A T C C A C C T T T A T C A A C A G G A A T T G C T A T G C A G G A G C A T C A T T G A T C T A G C A T T T T T C A T T A C A C T

DNA Sequences	Translated Protein Sequences
Species/Abbrv	
1. Acheta_domestic	A C C C G G A G C A T C T G T T G A T T T A G C A T T T T T T T C A T T A C A C T T A G C T G G A A T T T C A T C A A T T C T G G G A G C C G T T A A T T T C A T T A C A C T A T A T C A A T A T A C G A R C C C T G G G A T G T
2. Gryllus_sp.1	A C C C A G G A G C A T C C G T T G A T T T A G C T A T T T T C T C T A C A T A C T A G C A G G A T T T C T C A A T T T T A G G G G C G T A A A T T T T A T C A C A C C A T A A T A A T A T A C G A G C C C A G G A A T A T
3. Loxoblemmus_do	T G C A G G A G C T T C A G T T G A T T T A G C T A T T T T C T C T T A C A C T A G C A G G A T T T C A T C A A T T T T A G G G G C A G T A A A T T T T A T T A C A C C A T A A T A A T A T A C G A G C C C A G G A A T A T
4. Teleogryllus_inferi	C C C G G A G C A T C A G T T G A T T T A G C A T C T T T C A T T A C A C T A G C A G G A A T C T C A T C A A T T T A G G A G C G T A A A T T T T A T T A C A C C A T A A T A A T A T A C G A G C C C T G G A A T A T
5. Gryllus_veletis	A C C C G G A G C A T C G T T G A T T T A G C A T T T T C T C A C T A C A C T A G C A G G A A T T C A T C A A T T T T A G G A G C G T A A A T T T T A T T A C T A C A T A A T A A T A T G C A G G C C G A A T A T
6. Teleogryllus_ocea	C C C G G A G C A T C G T T G A T T T A G C A T T T T C T C A C T A C A C T A G C A G G A A T T T C T C A A T T T T G C T A T T T C G T C A A T T T A G G A G C G T A A A T T T T A T T A C A C C A T A A T A A T A T A C G A G C C C T G G A A T A T
7. Teleogryllus_emmi	C C C G G A G C A T C A S T T G A T T T A G C W A T T T T T C T C T T A C A T T A C C G G A T T T C M T C A A T T T A G G R C C G T A A A T T T T A T T A C A A C C A T A A T A A T A T A C G A G C C C A G G H A T A Y
8. Loxoblemmus_ari	T G C A G G A G C W T C A S T T G A T T T A G C W A T T T T T C T C T T A C A T T A C C G G A T T T C M T C A A T T T A G G R C C G T A A A T T T T A T T A C A A C C A T A A T A A T A T A C G A G C C C A G G H A T A Y
9. Gryllus_bimaculat	T G C A G G A G C A T C C G T T G A C T T A G C A T T T T T C T C T T A C A T T T A C C G G A T T T C T C A A T T C T A G G G A T T A A A T T T T A T T A C A C C A T A A T A A T A T A C G A G C C C A G G A T A T
10. Gryllus_pennsylv	T G C A G G A G C A T C T G T T G A T T T A G C A T T T T T C T C R C T A C A T T T A C C A G G A A T T T C C T C A A T T T T A G G A G C G T A A A T T T T A T T A C T A C C A T A A T A A T A T A C G A G C C C T G G A A T A T
11. Gryllus_pennsylv	T G C A G G T C A T C C G T T G A T T T A G C A T T T T T C A T T A C A C T A G C A G G A A T C T C T C A A T T T A G G A G C G T A A A T T T T A T T A C A C C A T A A T A A T A T A C G A G C C C T G G A A T A T
12. Gryllus_sp.2	A T C C G G G A G C A T C C G T T G A T T T A G C A T T T T T T C A C A T T A G C A G G A A T C C T C T C A A T T C T A G G A G C G T A A A T T T T A T T A C T A C A T A A T A A T A T A C G A G C C C A G G A A T A T
13. Teleogryllus_infe	T G C A G G A G C A T C A G T T G A T T C T A G C A T T T T T C A T T A C A C T T A G C A G G A A T T C T C A A T T C T A G A G C G T T A A T T T T A T T A C A C C A T A A T A A T A T A C G A G C C C T G G A A T G T

Goal 1: Methods – webs

Latrodectus tredecimguttatus

- Cob web



Nephilingis cruentata

- Orb web



Goal 1: Methods

Isolation & amplification

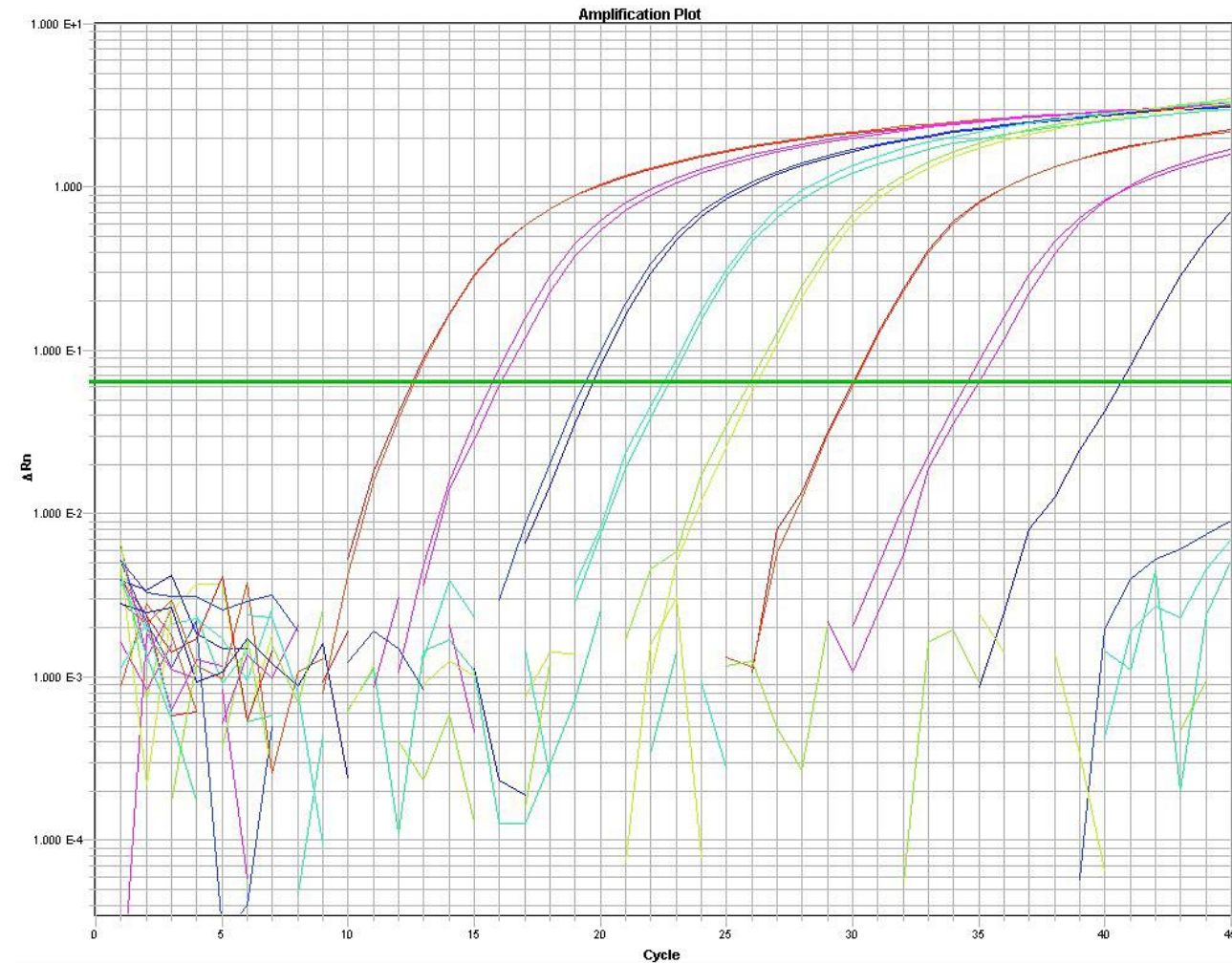
- Use of qPCR over conventional PCR
- Protocol controls
 - ✓ Negative isolation control
 - ✓ Positive amplification control
 - ✓ No template control
 - ✓ Internal quality control of process (18S)



Goal 1: Results

Results

- Protocol & sample controls as predicted
- Dilution series
- Adom1 outperformed Adom2



Goal 2: Prey in nature

Detection of *A. domestica*

- 2 web types
 - *Araneus diadematus*
 - *Linyphia triangularis*
- 2 juv. crickets per web
- 5 webs of each type



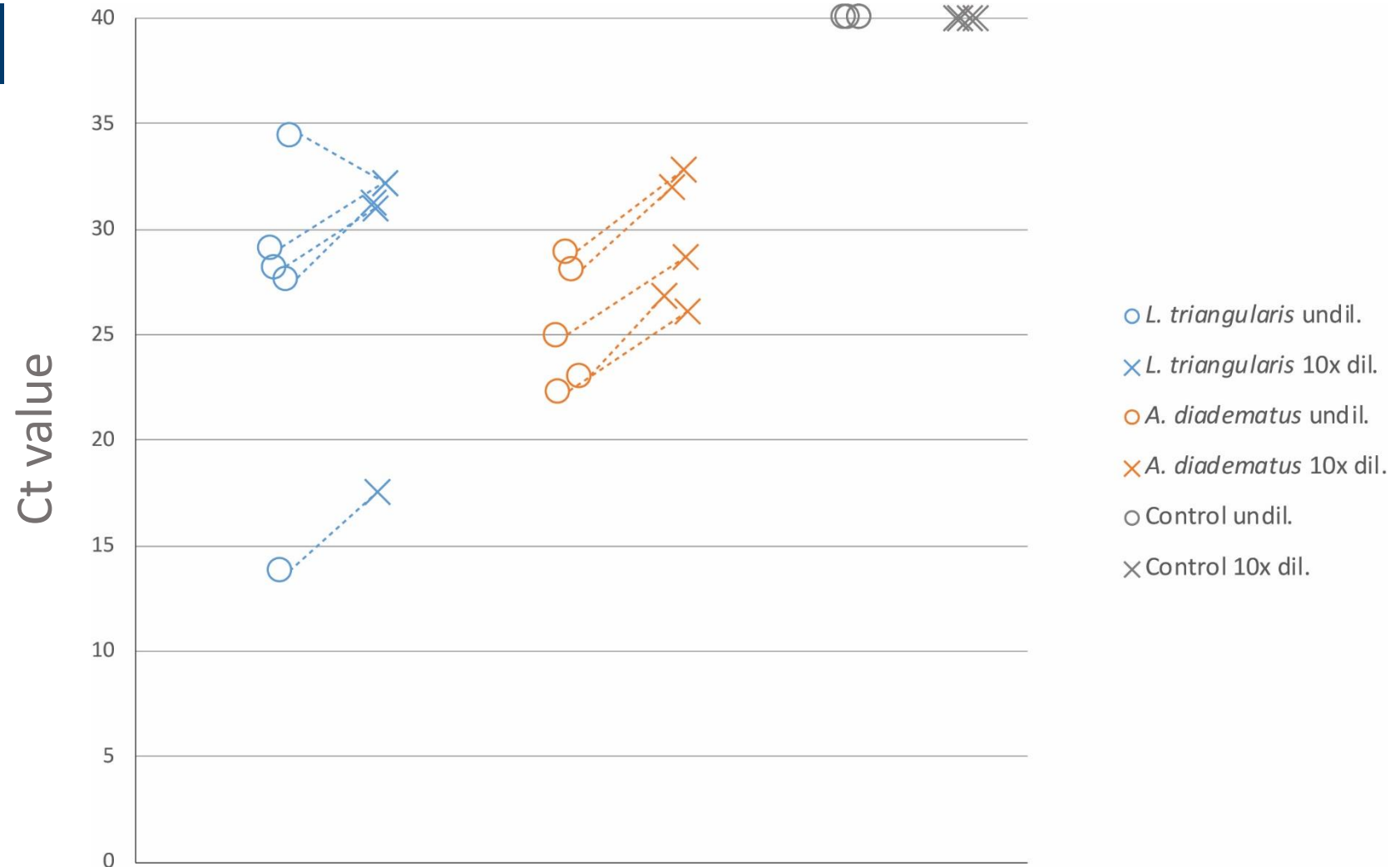
Photo: Peters & Lemke on Wikimedia Commons

Goal 2: Prey in nature

Detection of *A. domestica*

✓ *Linyphia triangularis*

✓ *Araneus diadematus*



Goal 3: eDNA metabarcoding

Sampling

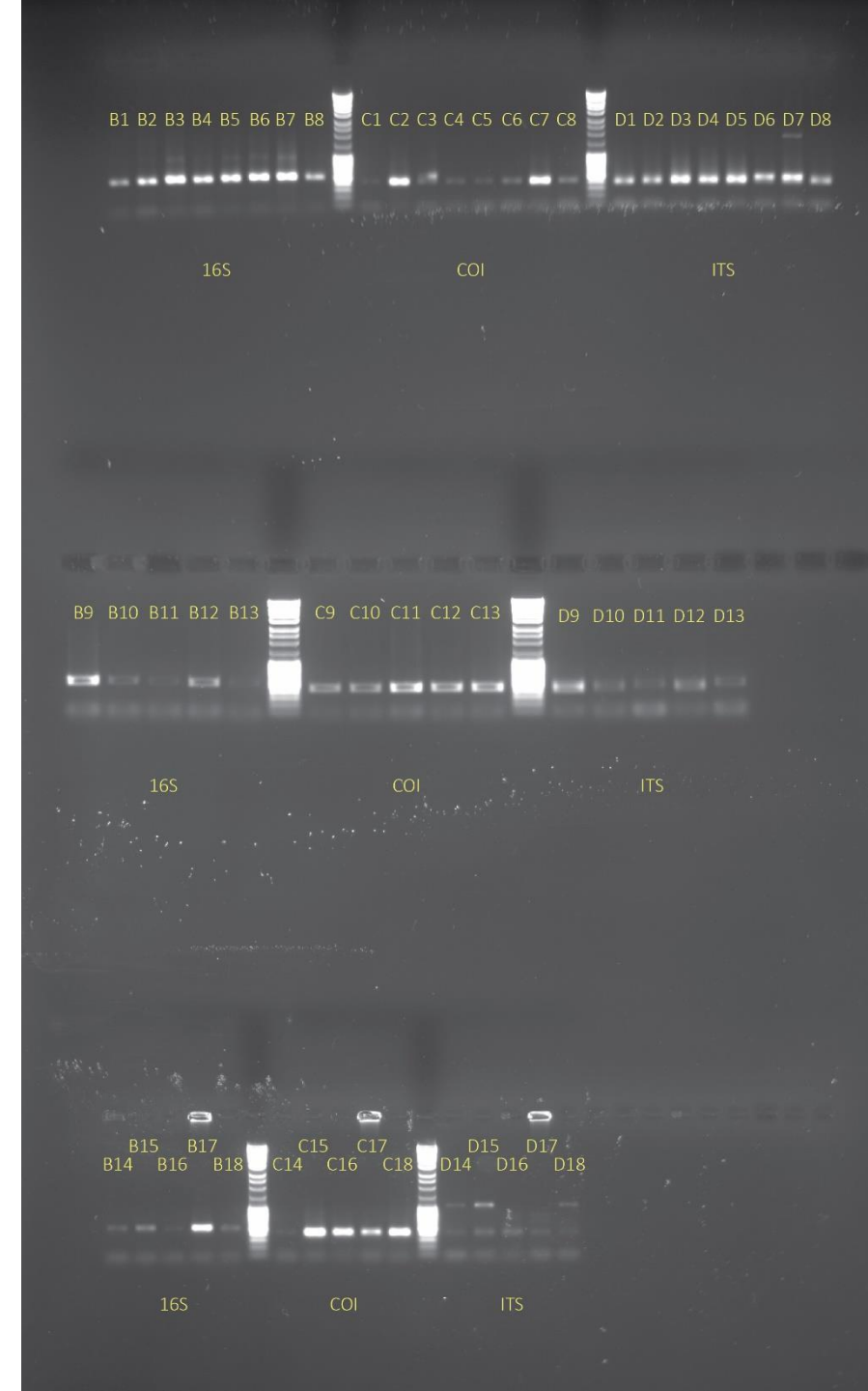
- 2 web types
- 5 webs per type per forest
- 2 forests
 - Continental & submediterranean
 - One sampled in 2 subsequent years



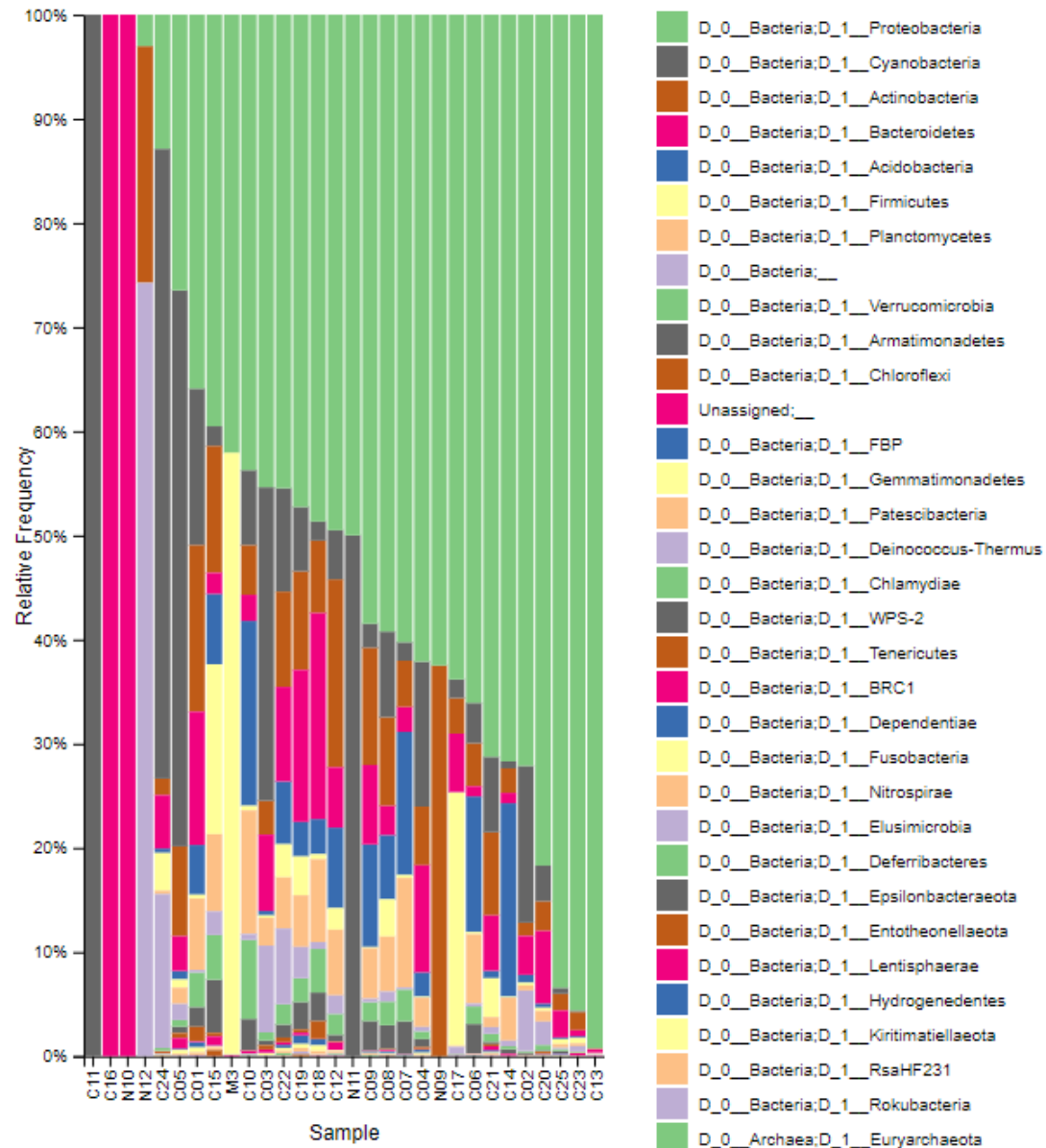
Goal 3: eDNA metabarcoding

Methods & results

- Markers
 - ✓ Animals: COI, 313 bp
 - ✓ Fungi: ITS, 300-400 bp
 - ✓ Bacteria: 16S, 464 bp
- Illumina Mysec platform
- OTU across webs and habitats



Goal 3: Results



Per-sample sequence counts

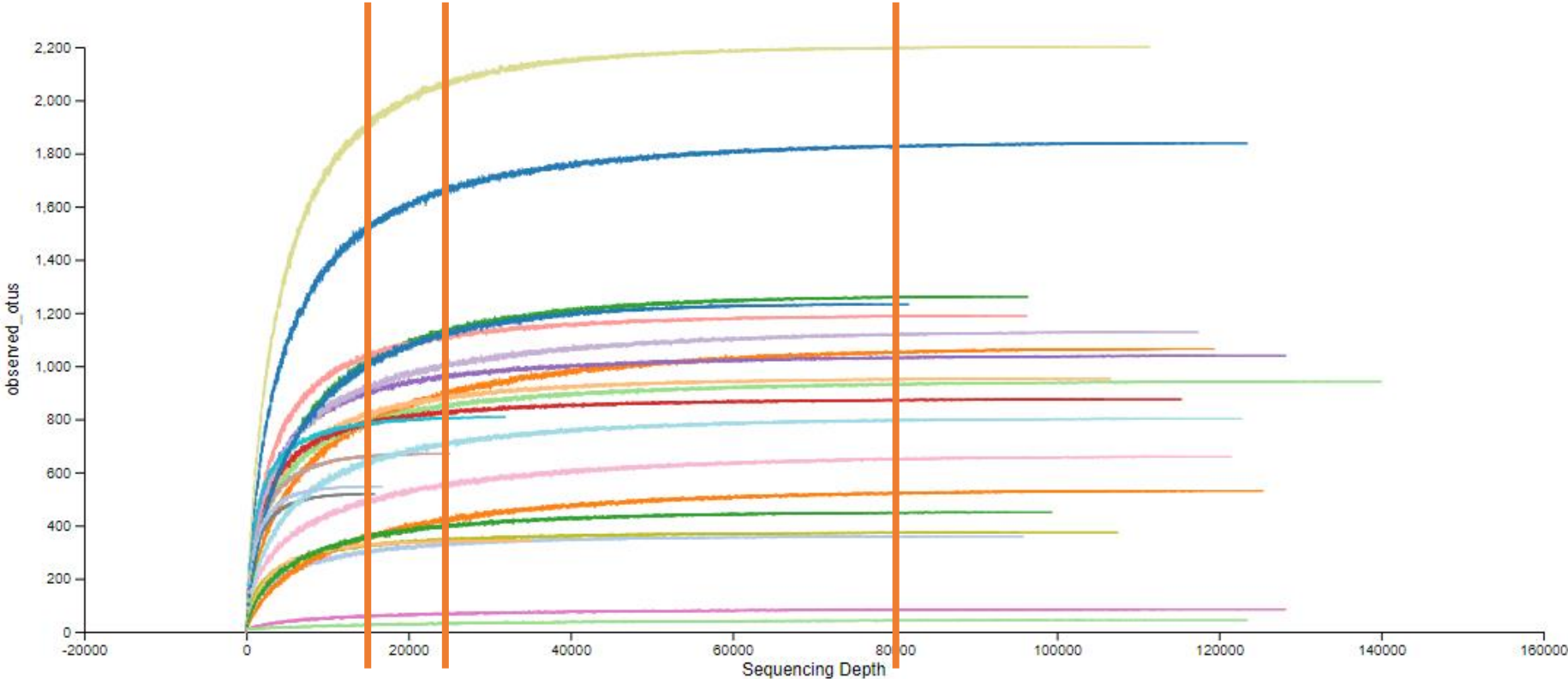
Sample name	Sequence count
C06	162357
C09	156301
C01	147795
C20	140901
C03	140526
C13	138495
C10	138135
C07	138083
C17	137921
C23	137651
C14	136968
M3	136459
C18	136135
C04	126656
C08	112781
C25	111884
C02	111673
C05	110223
C21	93764
C19	40559
C24	39294
C12	29827
C22	19952
C15	18865
N12	148
N10	87
C11	55
N11	52
N09	42
C16	32

Goal 3: Results

Per-sample sequence counts

Sample name	Sequence count
C06	162357
C09	156301
C01	147795
C20	140901
C03	140526
C13	138495
C10	138135
C07	138083
C17	137921
C23	137651
C14	136968
M3	136459
C18	136135
C04	126656
C08	112781
C25	111884
C02	111673
C05	110223
C21	93764
C19	40559
C24	39294
C12	29827
C22	19952
C15	18865
N12	148
N10	87
C11	55
N11	52
N09	42
C16	32

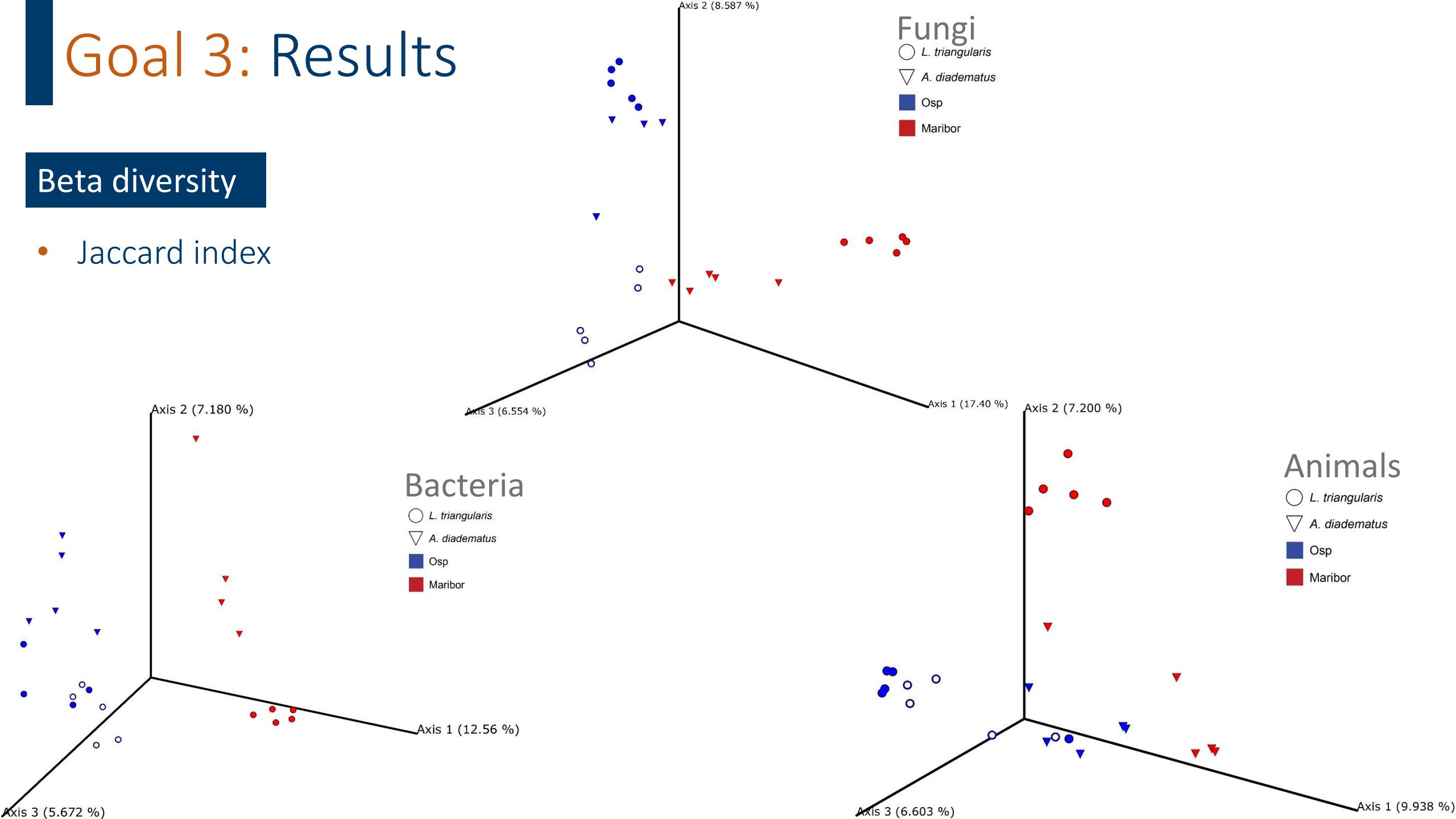
Alpha rarefaction



Goal 3: Results

Beta diversity

- Jaccard index



Conclusions & future work

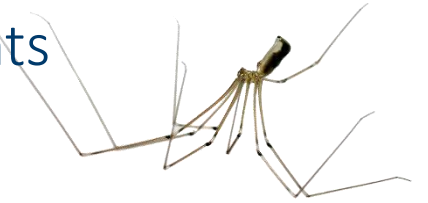
Conclusions

- Webs seem to be good at accumulating eDNA
- Likely useful as general aerial filters



Future work

- Spiders, plants
- Pollinators
- Invasive mosquitoes
- Aerial microbiota



Thank you for the attention!

Institute of Biology ZRC SAZU

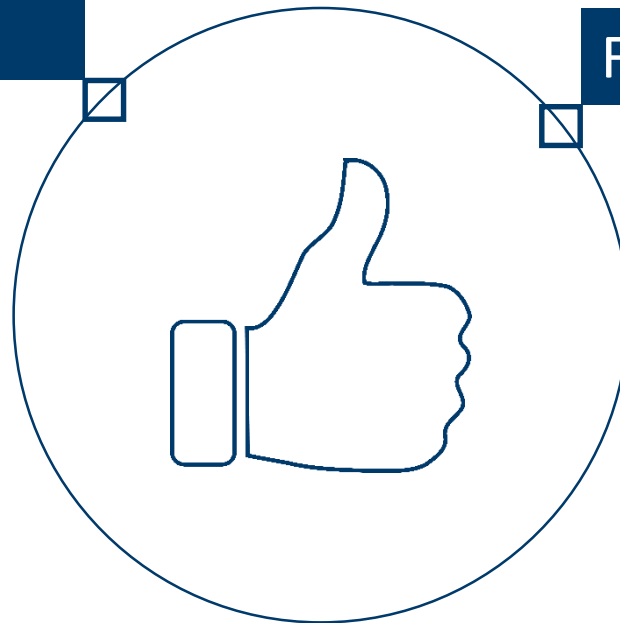
www <https://bijh.zrc-sazu.si/en/predstavitev#v>



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