# Spider webs as a source of eDNA

## LifeWatch ERIC worskop

*CIBIO InBIO Facilities, Vairão Campus, February 26-28, 2020*

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

Institute of Biology ZRC SAZU, Ljubljana, Slovenia

# Introduction: Institute of Biology ZRC SAZU

## Vegetation

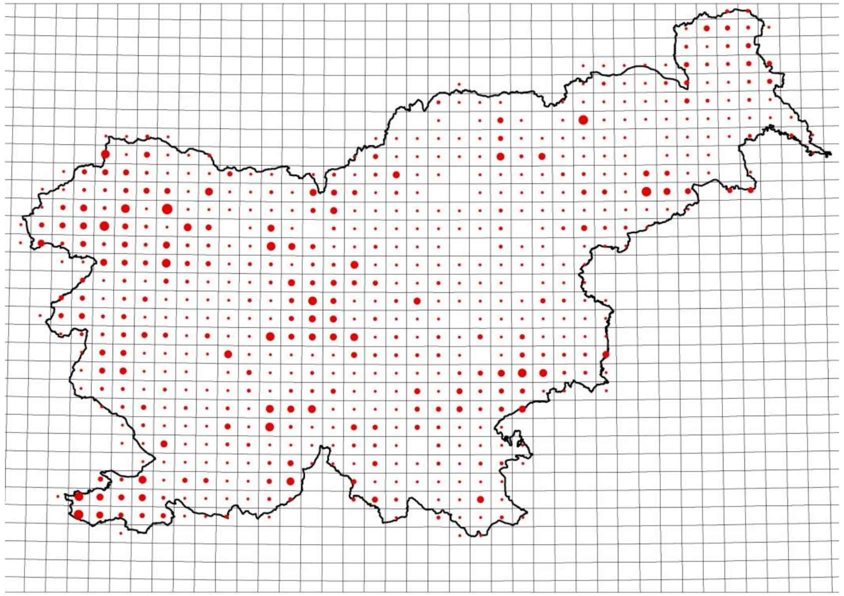
- Vegetation classification and community diversity
- Synsystematics
- Dynamics
- Macroecology
- Nature conservation (habitat types)
- Databases

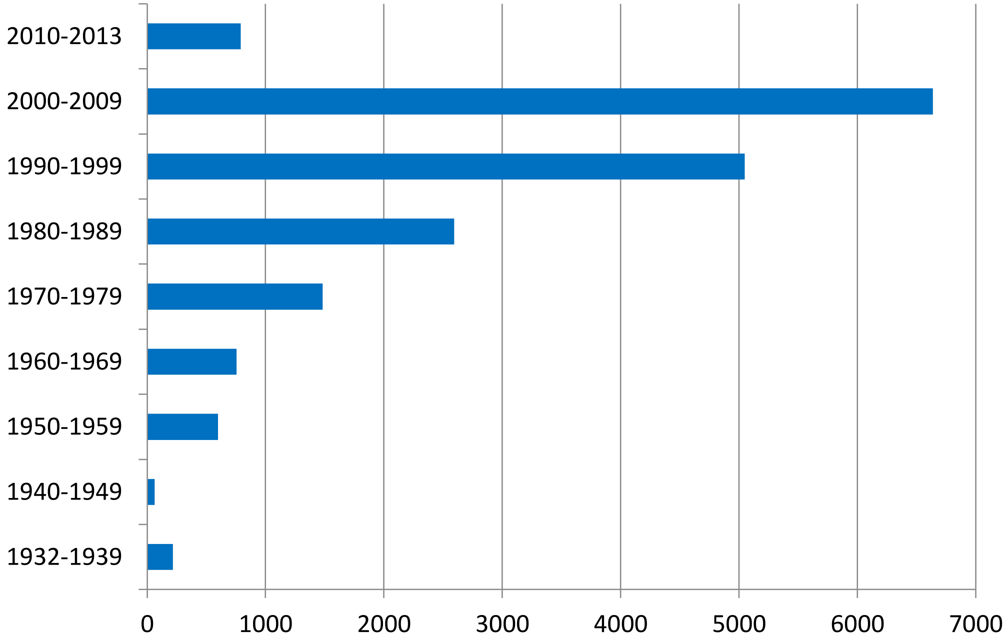
## Arachnology

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

# Vegetation: Slovenia

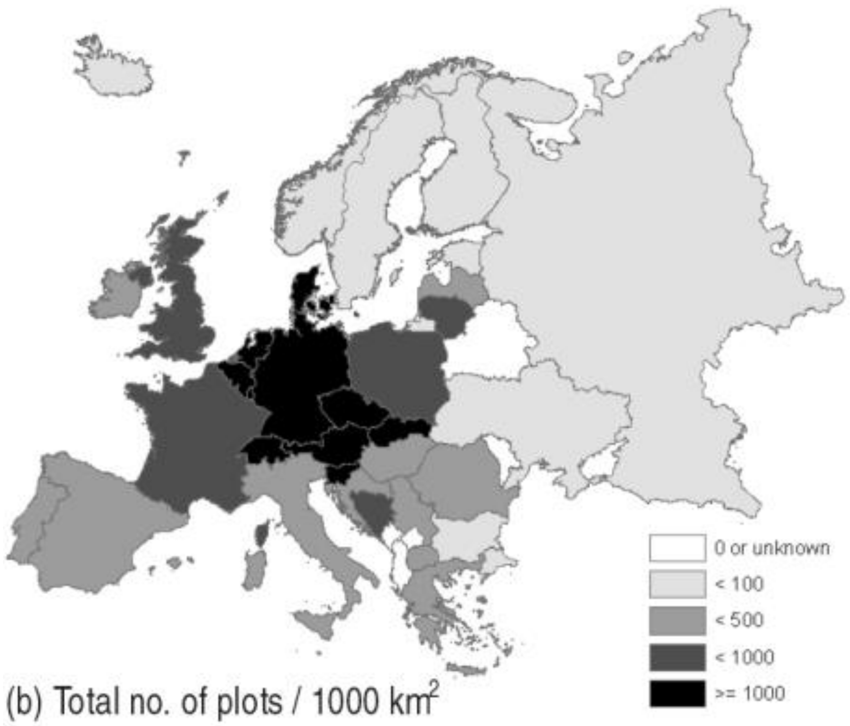
- Database – Institute of Biology ZRC SAZU
- Mostly Braun-Blanquet method
- Oldest relevé 1932
- 21.000 relevés





# Vegetation: European vegetation archive – EVA

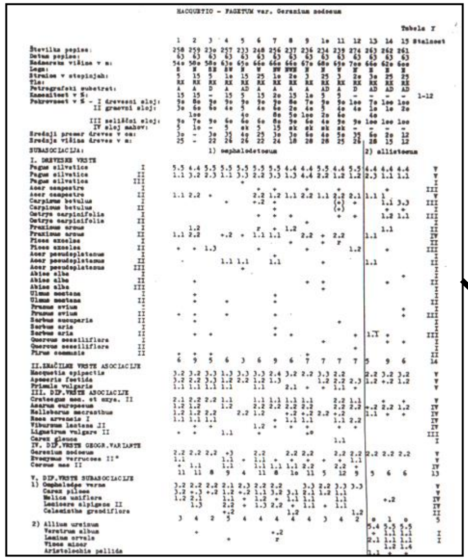
All relevés > 4.3 million

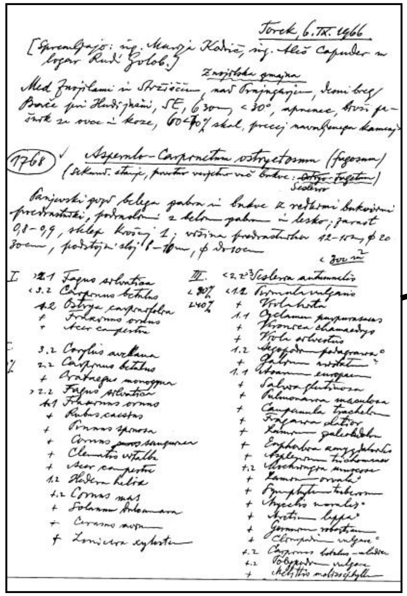



Nr relevés / 1000 km$^2$
(Schaminée et al. 2009)

International synergy

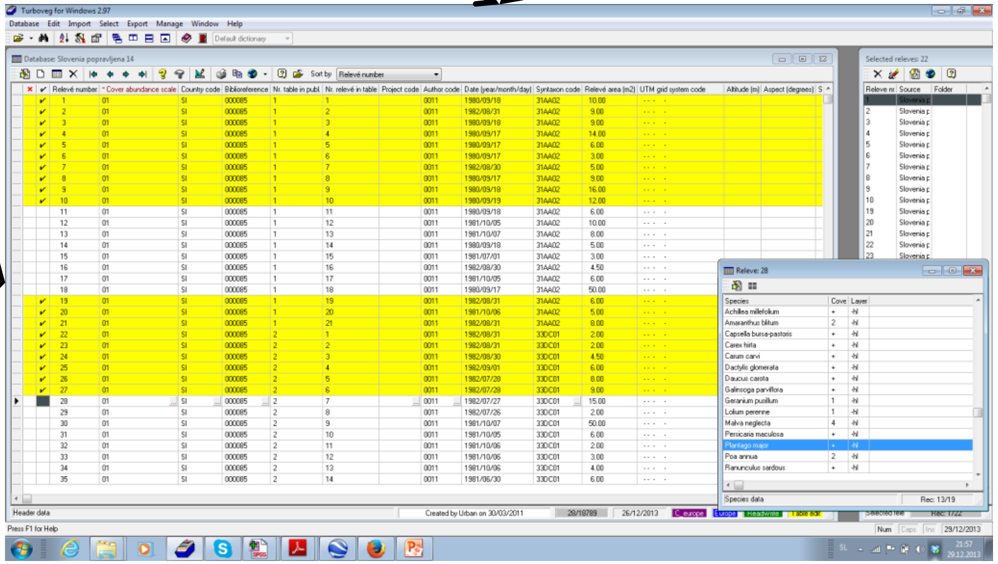
# Vegetation: Databases





Relevé head (altitude, aspect, author, syntaxa ...)

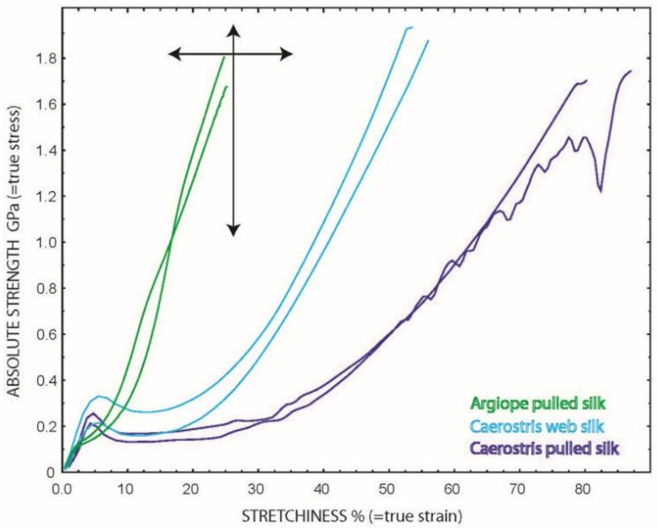
Species list



Turboveg software

# Arachnology: Web & silk evolution

- Web biology & evolution
- Silk – toughest biomaterial

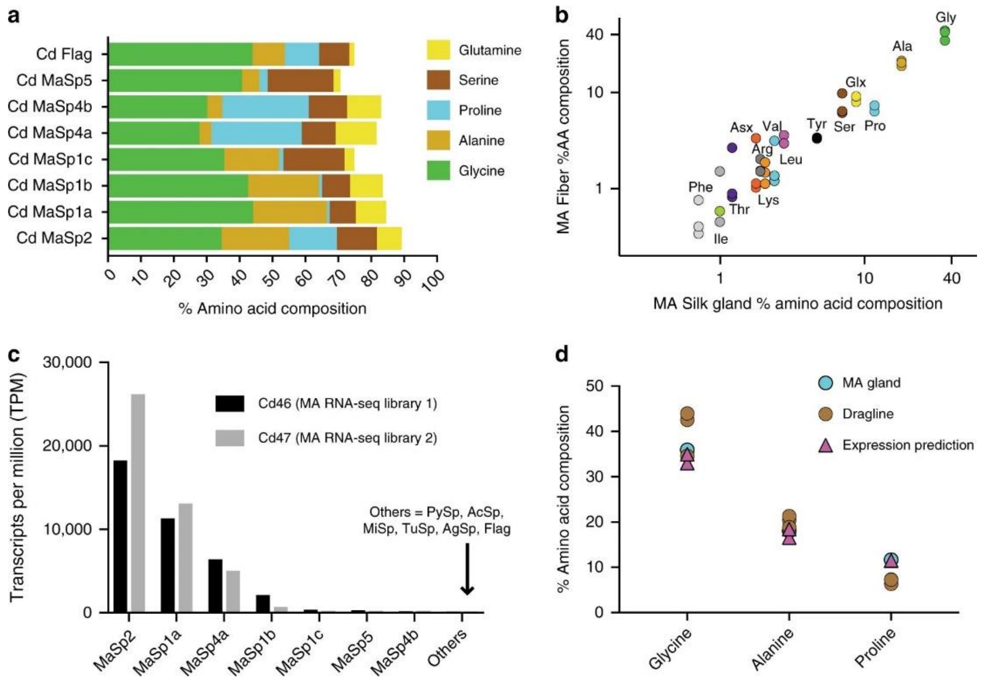


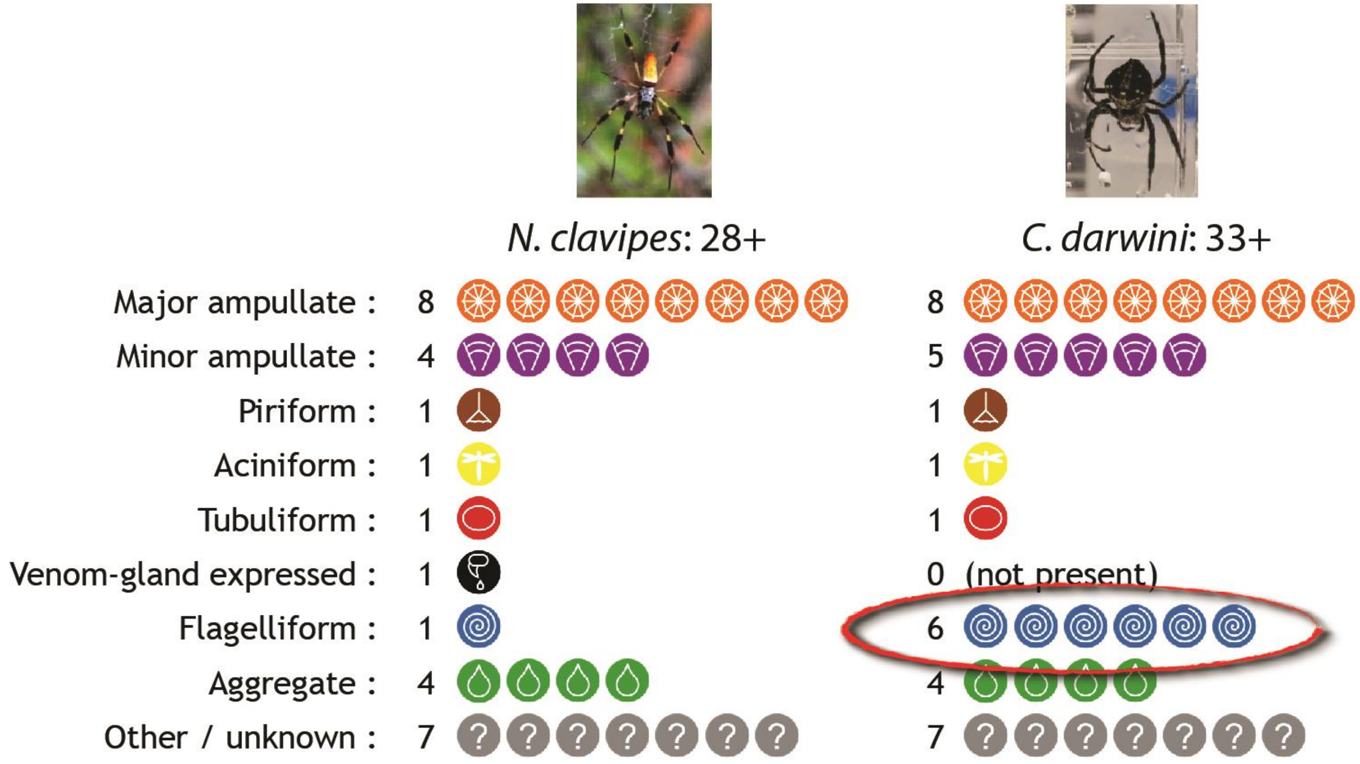

Agnarsson et al. 2010 PLoS ONE
Kuntner & Agnarsson 2010 J Arachnol
Gregorič et al. 2011 J Arachnol
Gregorič et al. 2011 PLoS ONE

# Arachnology: Web & silk evolution

- Web biology & evolution
- Silk – toughest biomaterial

- Comparative genomics
- Gland specific gene expression




Babb et al. 2017 Nature Genetics

Babb et al. In prep.

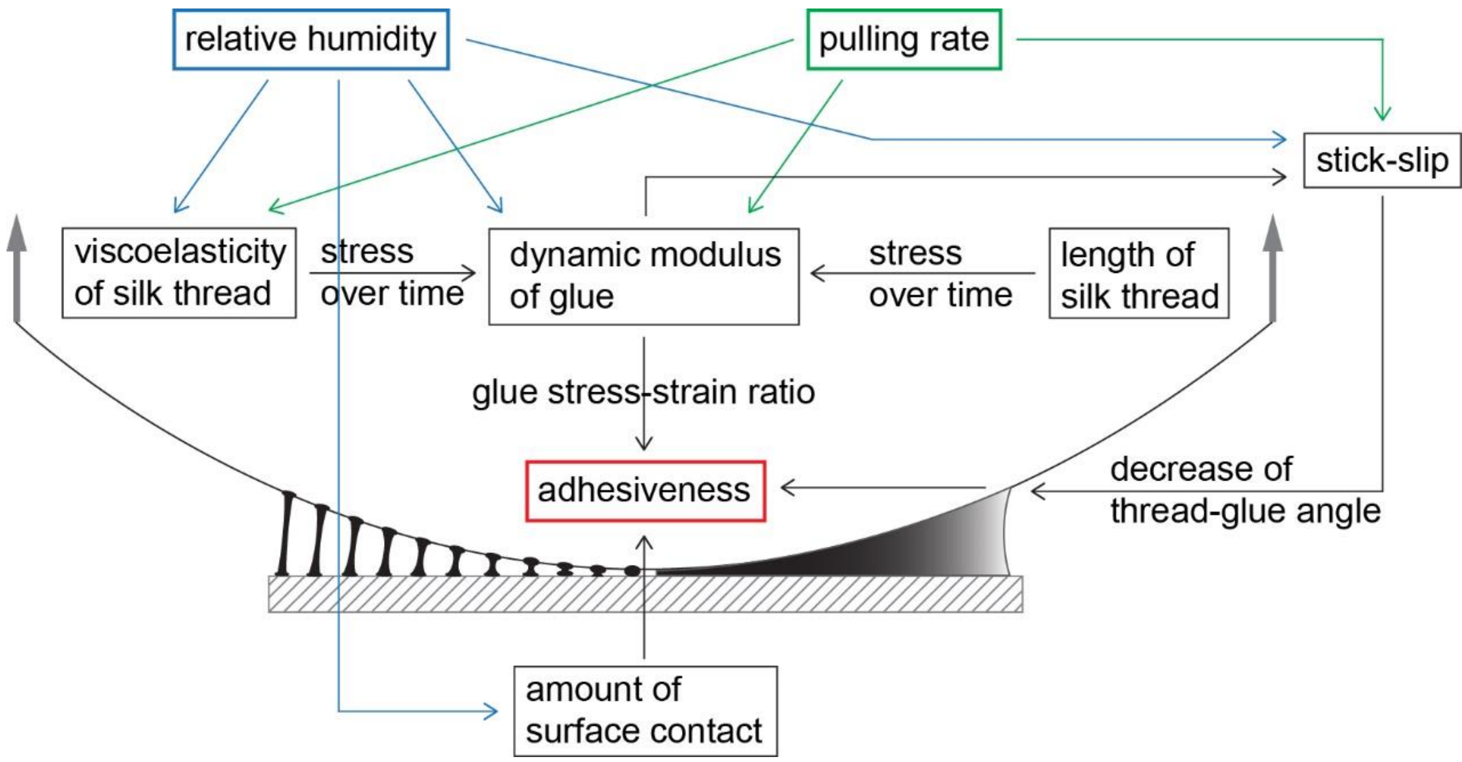
Garb et al. 2019 Communications Biology

# Arachnology: Web & silk evolution

- Web biology & evolution
- Silk – toughest biomaterial

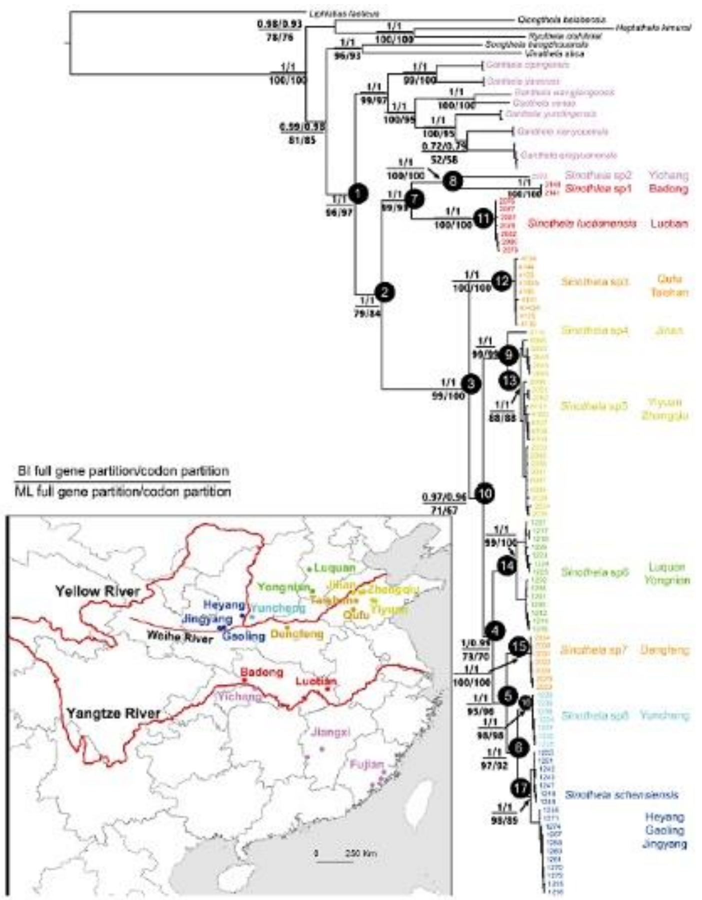
- Comparative genomics
- Gland specific gene expression

- Glue – responsive adhezive system

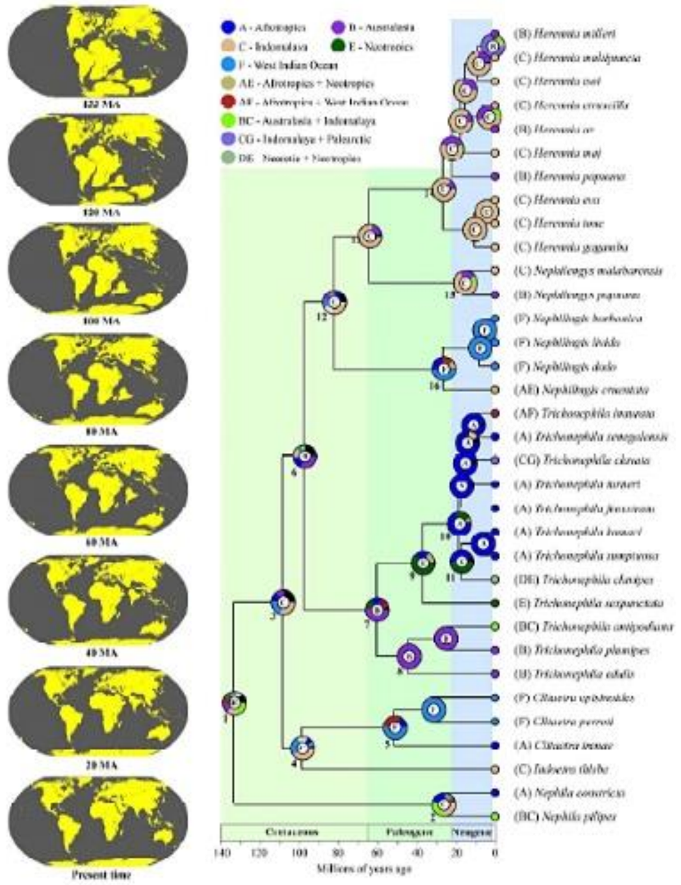



Gregorič et al. In prep.

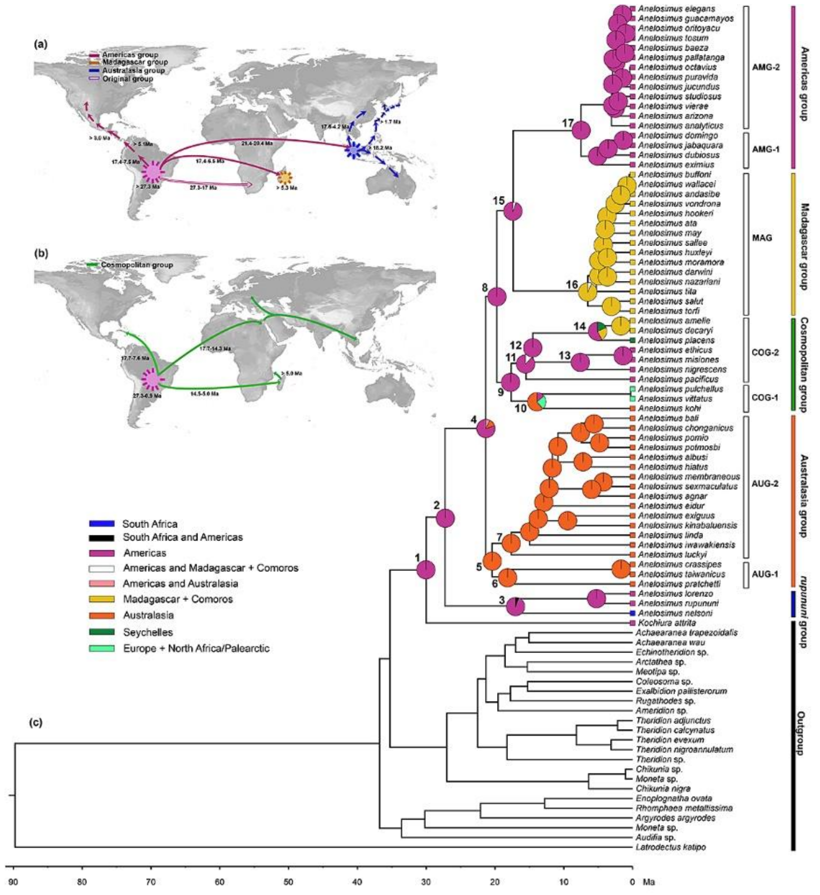
# Arachnology: Phylogeography



Xu et al. 2017 J Biogeography



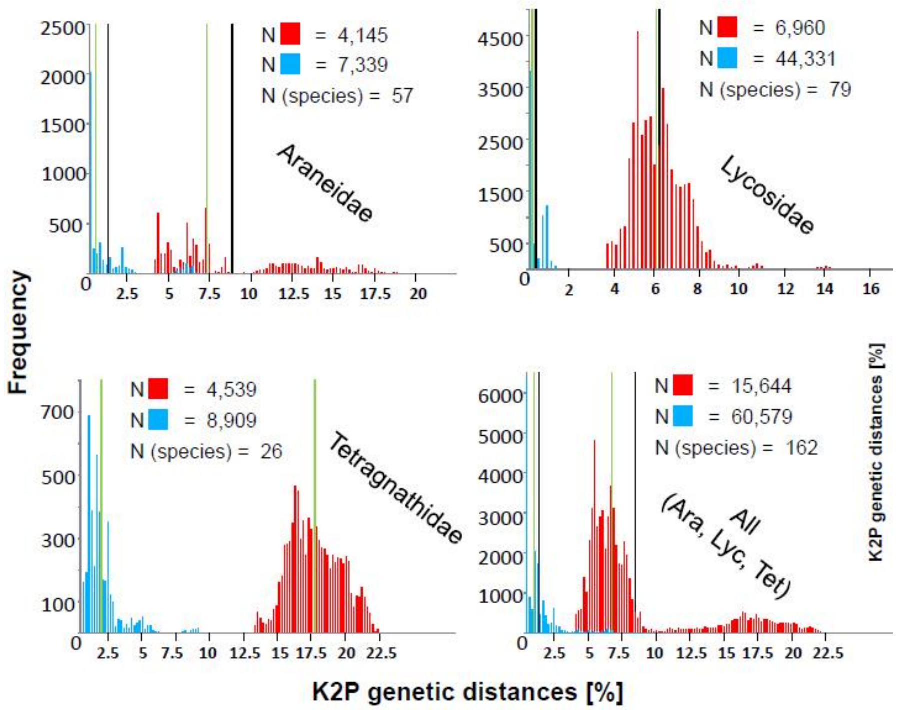
Turk et al. J Biogeography in press



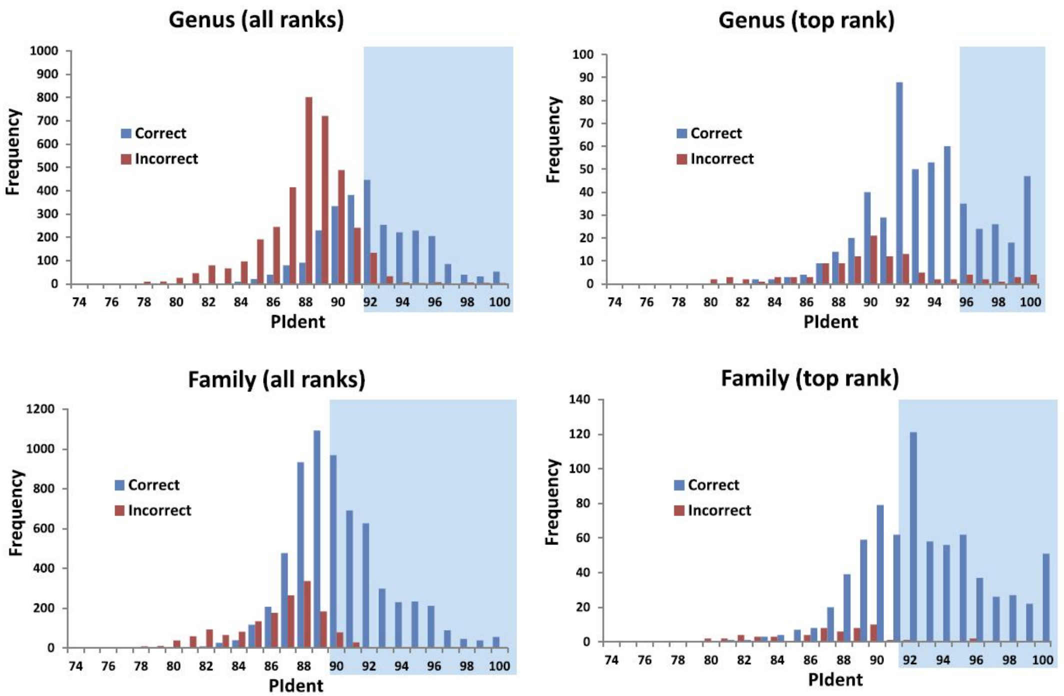
Luo et al. Systematic Biology in press

# Arachnology: DNA barcoding & presertvation

- 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

## Blake et al. 2016 Conservation Genet Resour

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

PLOS | ONE

RESEARCH ARTICLE

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

Charles C. Y. Xu[1¤a]*, Ivy J. Yen[1], Dean Bowman[2], Cameron R. Turner[1¤b]

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

CrossMark click for updates

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.



**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 Kuntner, Scientific Research Centre, Slovenian Academy of Sciences and Arts, SLOVENIA

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

CrossMark

TECHNICAL NOTE

### DNA extraction from spider webs

Max Blake[1] · Niall J. McKeown[1] · Mark L. T. Bushell[2] · Paul W. Shaw[1]

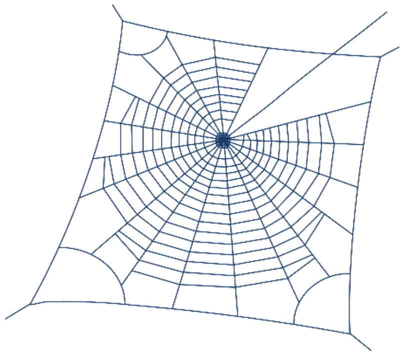
Received: 5 November 2015 / Accepted: 20 April 2016 / Published online: 26 April 2016


**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. obvs.*). *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

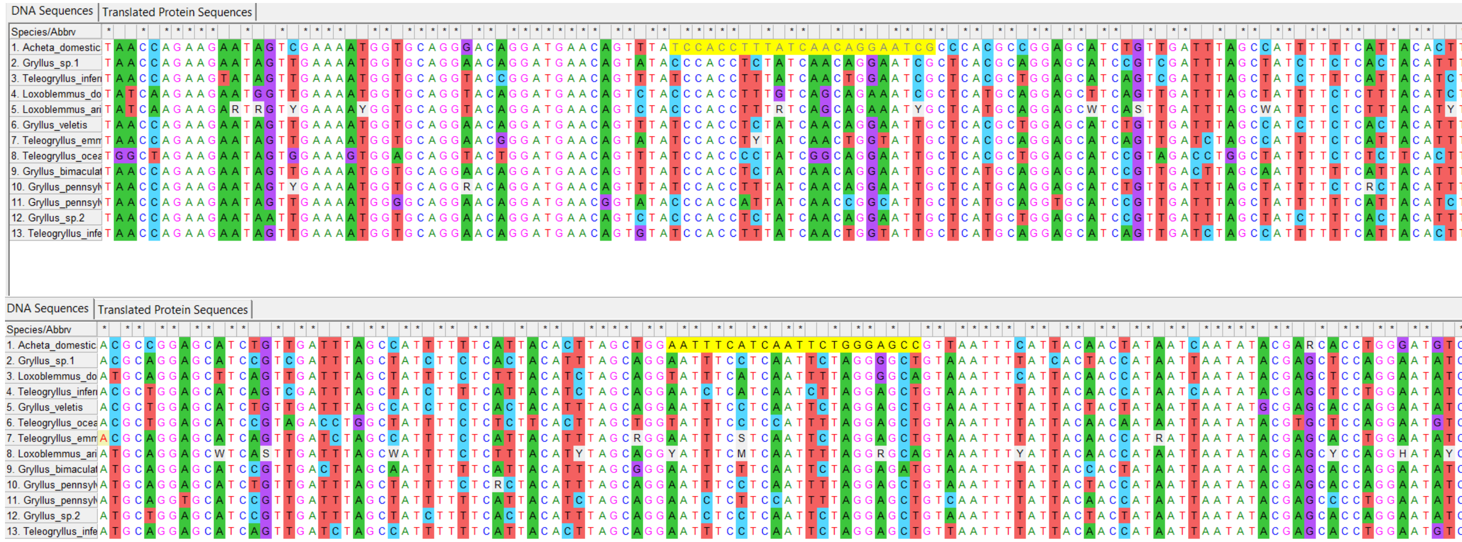
# Goal 1: Methods – prey

## *Acheta domestica*

- Used in *Xu et al. 2015*

## 2 COI markers

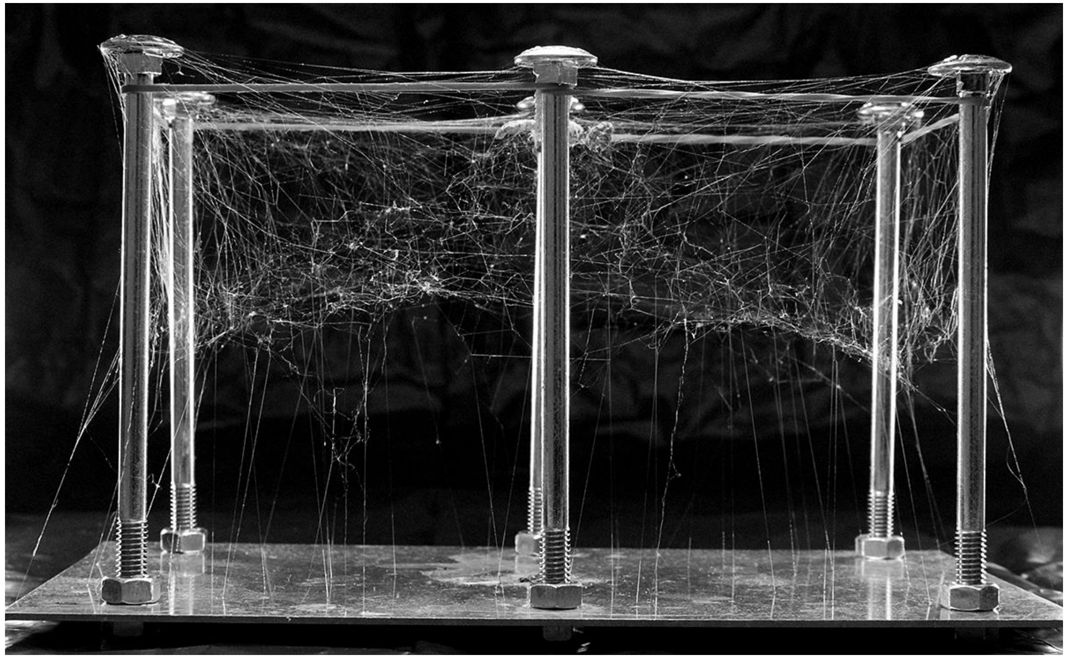
- Adom1
- Adom2



# Goal 1: Methods – webs

***Latrodectus tredecimguttatus***

- Cob web



***Nephilingis cruentata***

- Orb web

# Goal 1: Methods

## Isolation & amplification

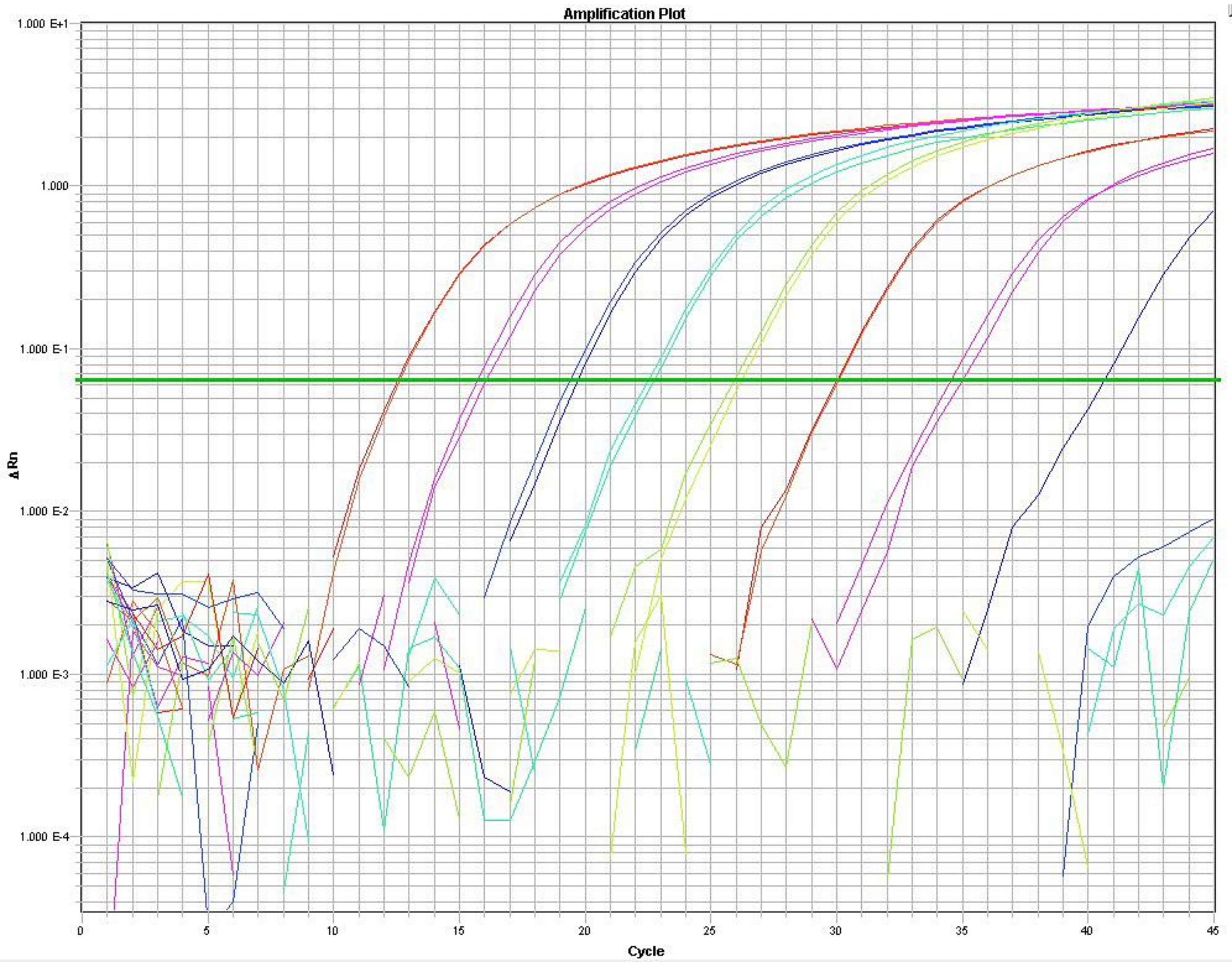
- Use of qPCR over conventional PCR
- Protocol controls
  - ✓ Negative isolation control
  - ✓ Positive amplification control
  - ✓ No template control
  - ✓ Internal quality control of process (18S)

Photo: OIS, Visualhunt.com

# Goal 1: Results

## Results

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



# Goal 2: Prey in nature

## Detection of *A. domestica*

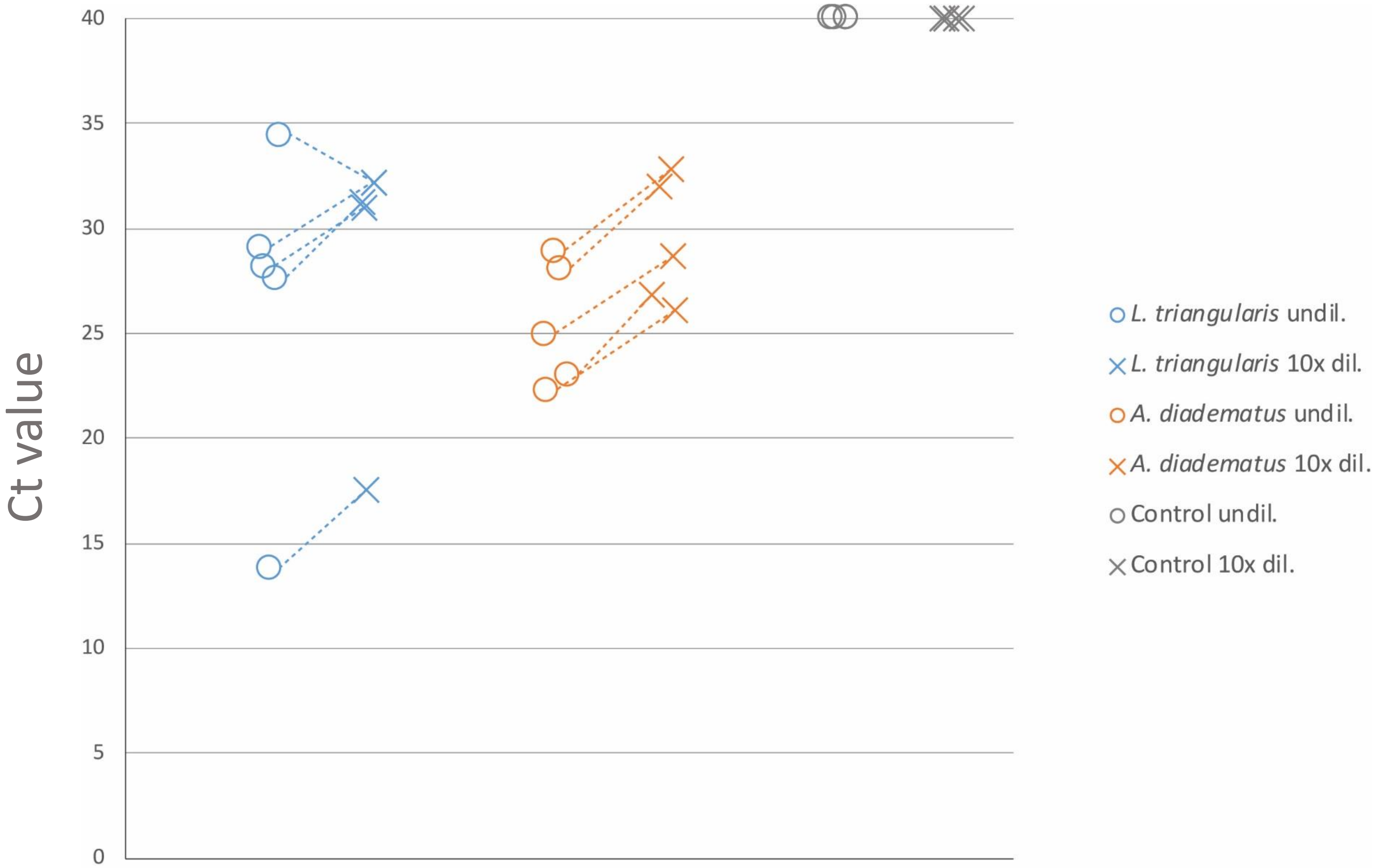
- 2 web types
  - *Araneus diadematus*
  - *Linyphia triangularis*
- 2 juv. crickets per web
- 5 webs of each type

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

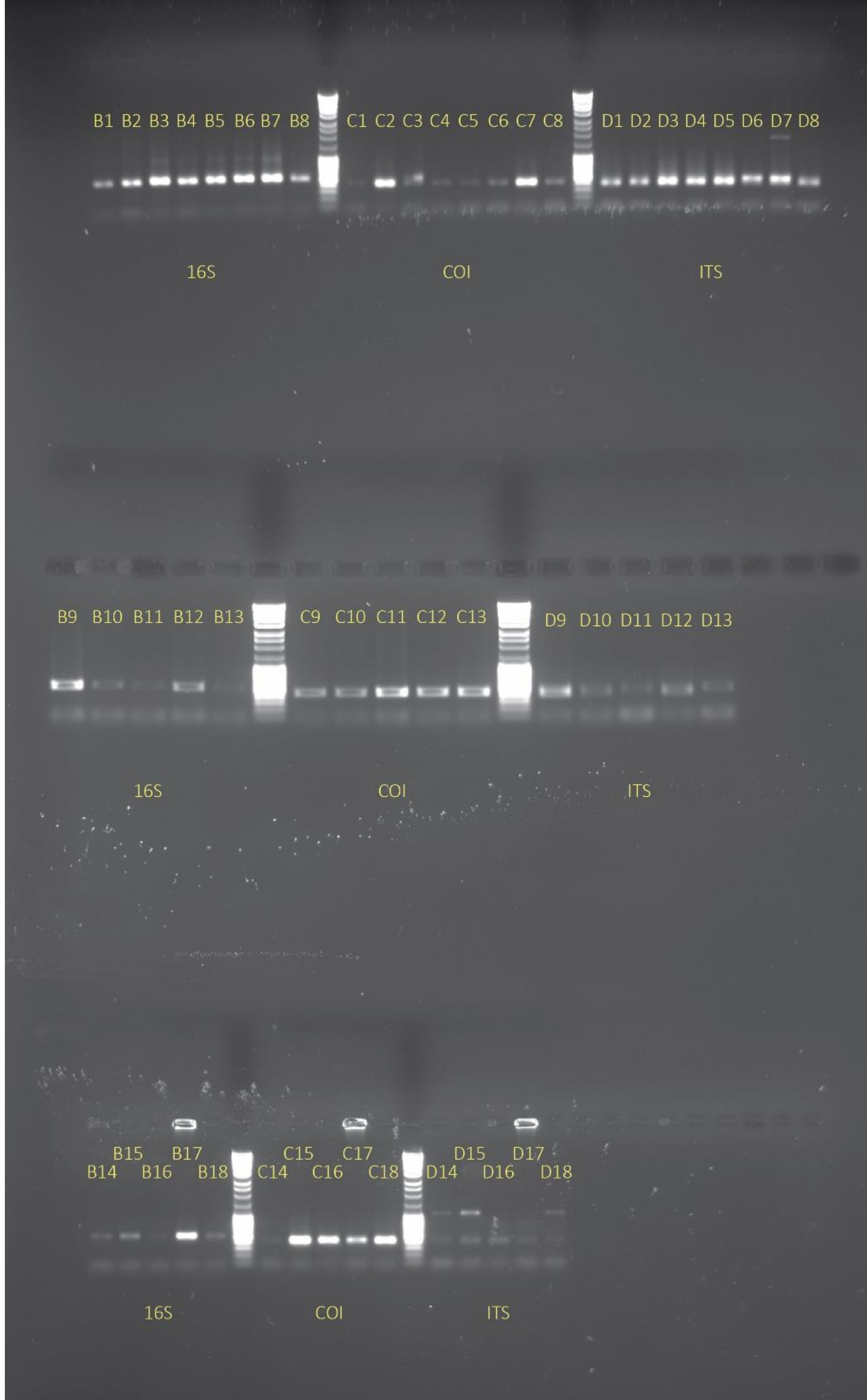
# Goal 3: eDNA metabarcoding

## Methods & results

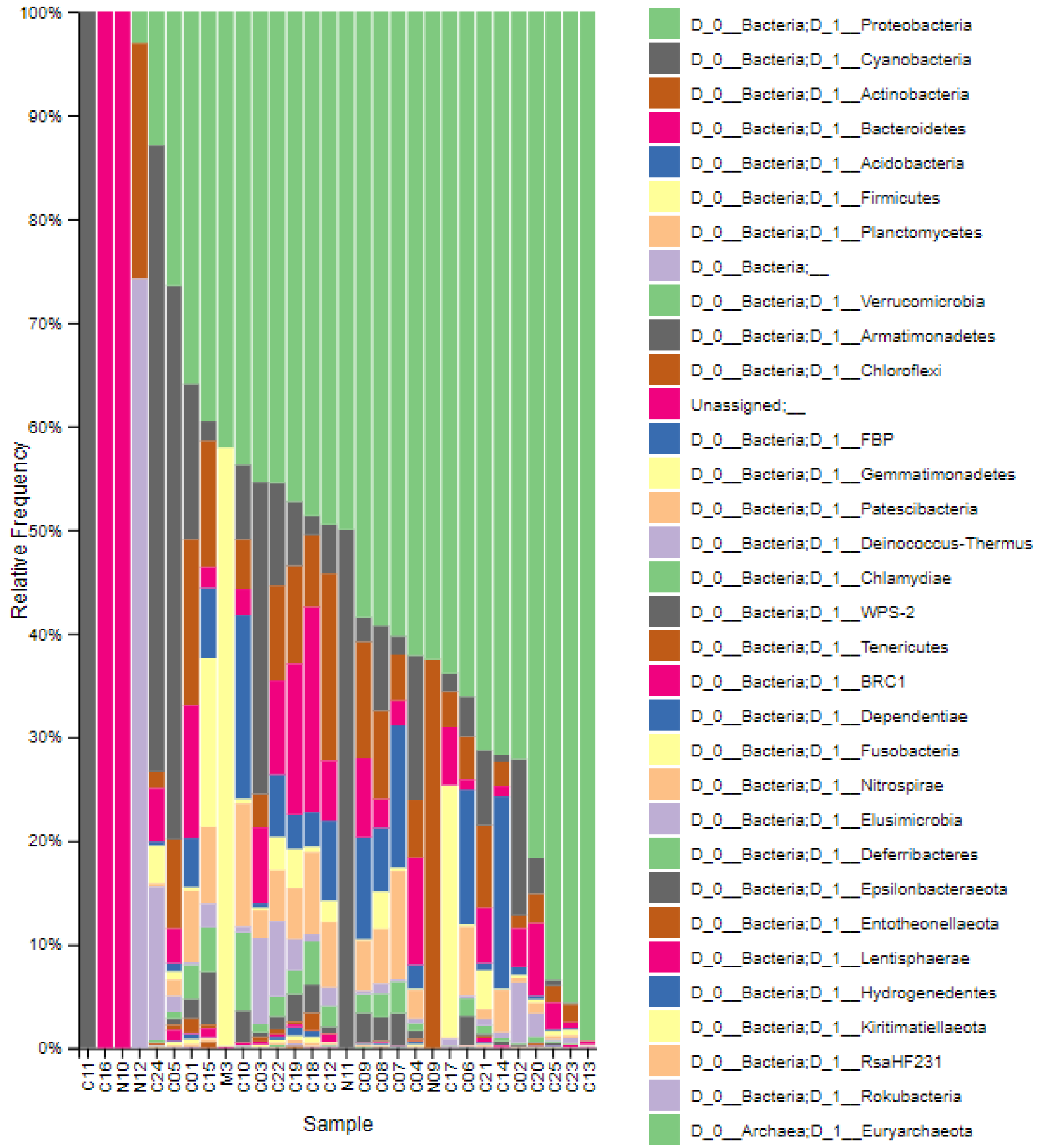
- Markers
  - ✓ Animals: COI, 313 bp
  - ✓ Fungi: ITS, 300-400 bp
  - ✓ Bacteria: 16S, 464 bp

- Illumina Mysec platform

- OTU across webs and habitats



# Goal 3: Results

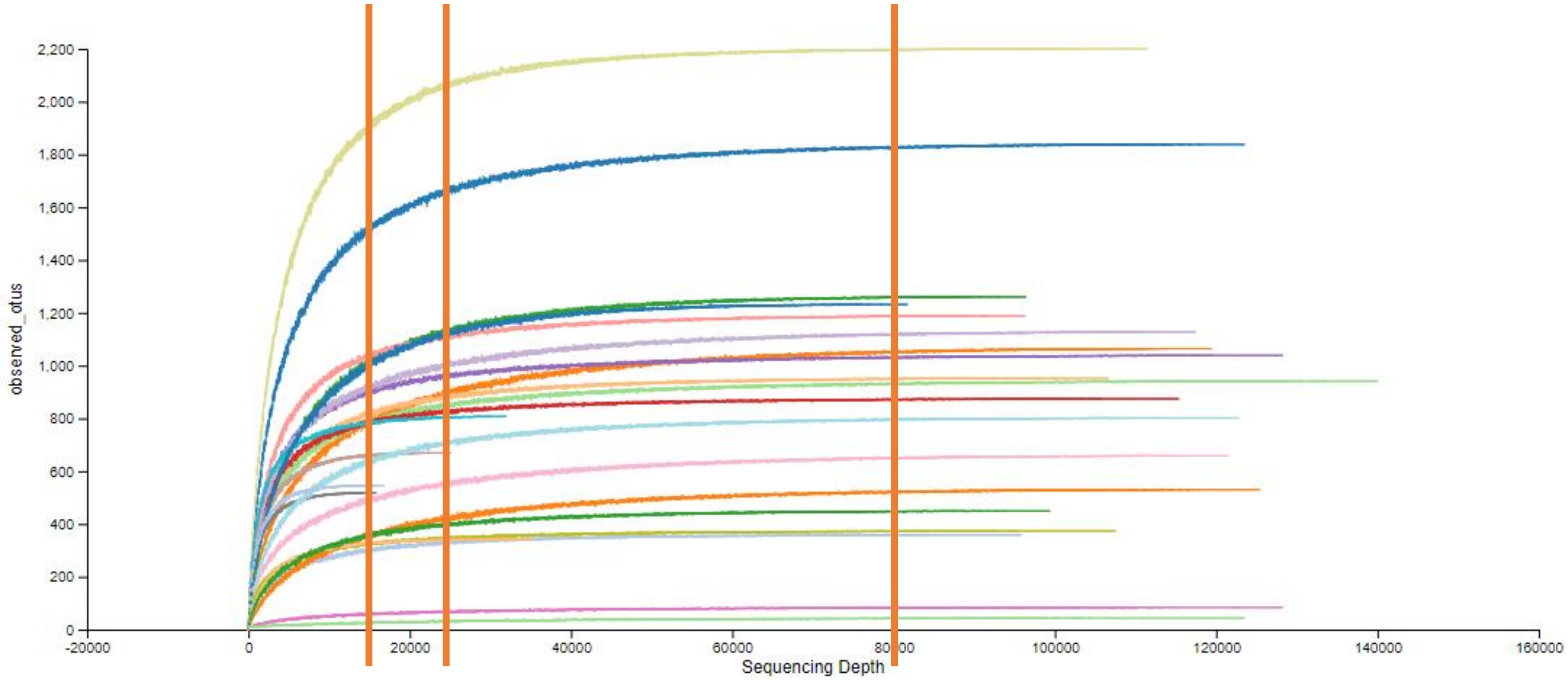



## Per-sample sequence counts

| 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

## Alpha rarefaction



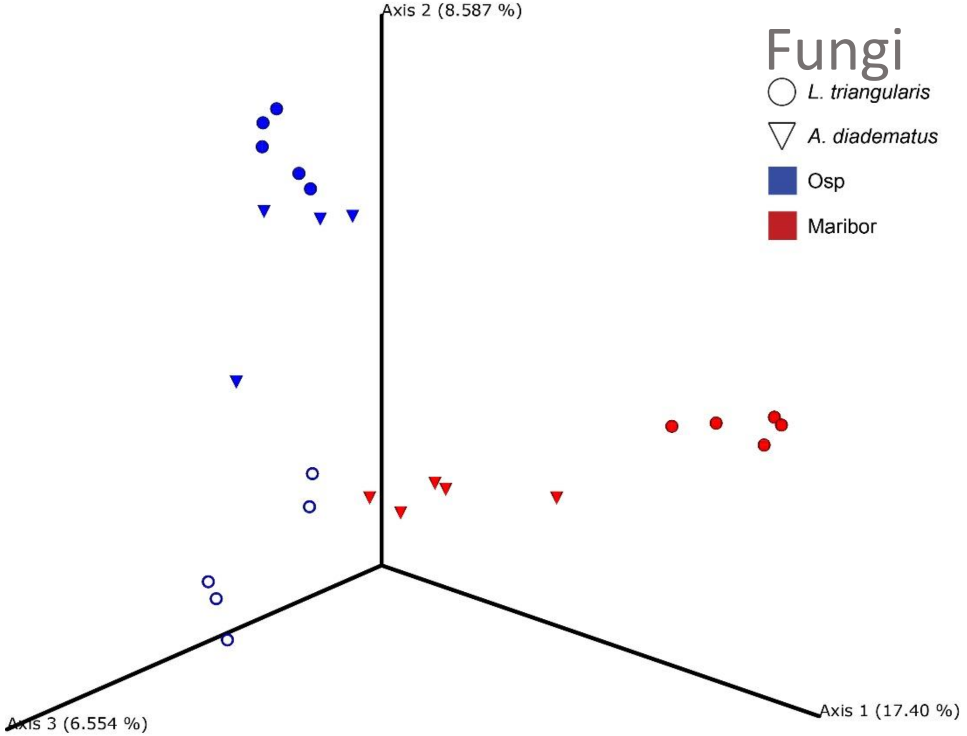

## Per-sample sequence counts

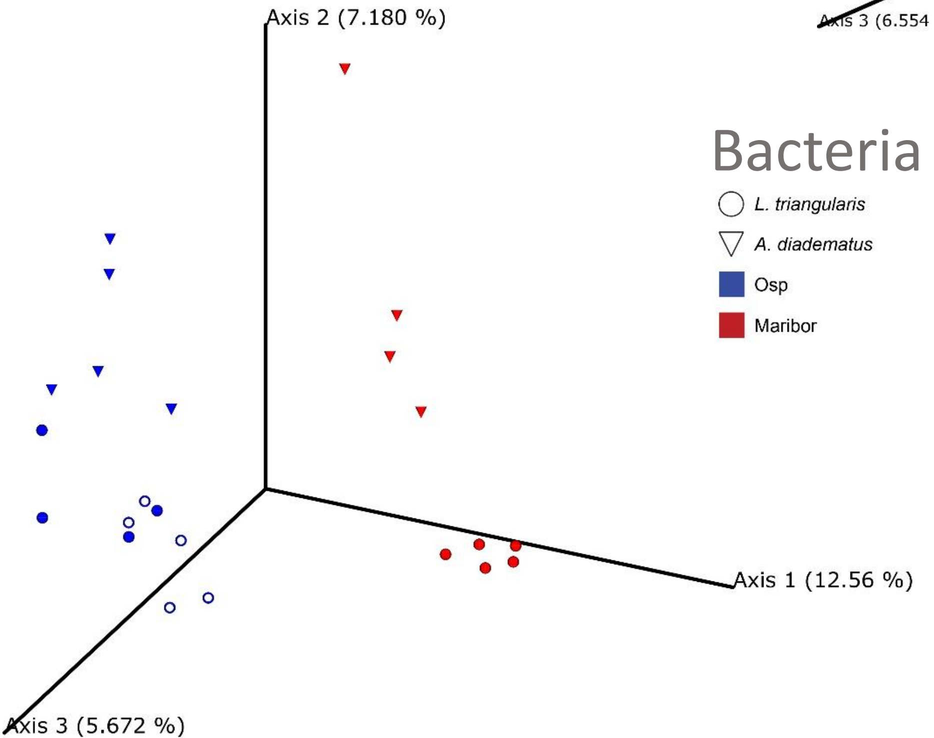
| Sample name | Sequence count |
|---|---|
| C06 | 162357 |
| C09 | 156301 |
| C01 | 147795 |
| C20 | 140901 |
| C03 | 140526 |
| C13 | 138495 |
| C10 | 138135 |
| C07 | 138083 |
| C17 | 137921 |
| C23 | 137651 |
| C14 | 136968 |
| M3 | 136459 |
| C18 | 136135 |
| C04 | 126656 |
| C08 | 112781 |
| C25 | 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 |

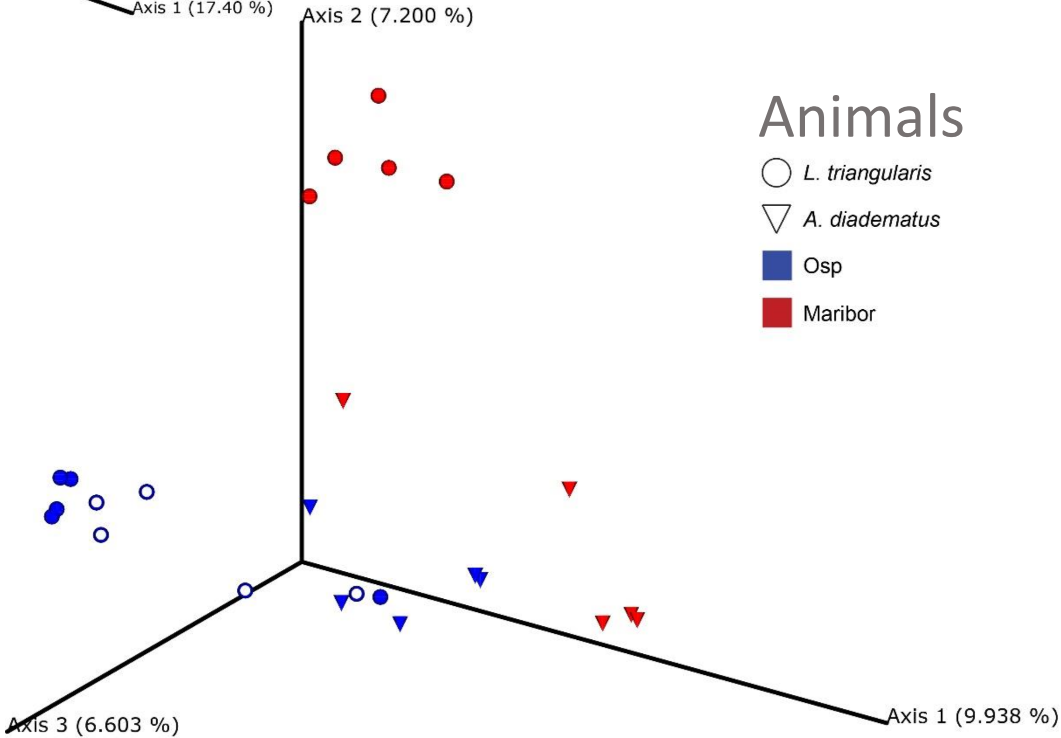
# Goal 3: Results

## Beta diversity

- Jaccard index



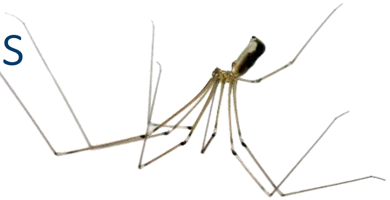
# Conclusions & future work

## 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

Biološki inštitut Jovana Hadžija ZRC SAZU

ZRC SAZU

## Follow EZ lab!

www http://ezlab.zrc-sazu.si/

Ez lab ZRC SAZU

@EZlabZRC

Ezlab ZRC SAZU