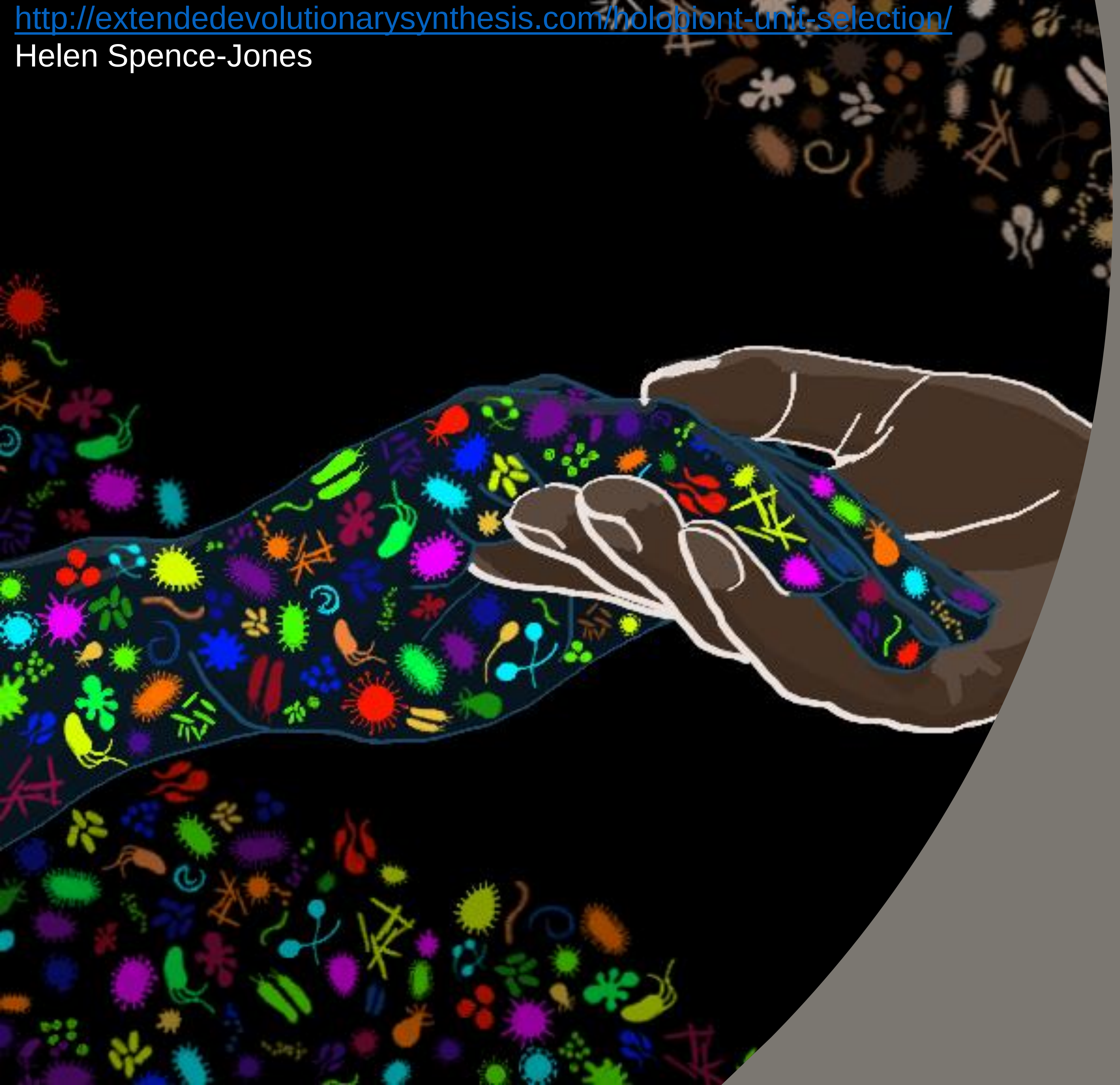
Unbiased metagenomic analysis of *Ixodes scapularis* microbiomes in the Kingston-Frontenac region

Amber Paulson, PhD

November 4, 2020

CLyDRN AGM



http://extendedevolutionarysynthesis.com/holobiont-unit-selection/
Helen Spence-Jones

The genetic diversity of the human microbiome is immense!

~46 million genes

Tierney, *et al*. (2019)

Tick microbiome is complex

Pathogens

- Enter midgut during blood meals;
- Colonize midgut;
- Survive moult; and
- Concentrate in salivary glands.

Ixodes scapularis - blacklegged tick

Tick microbiome is complex

Midgut microbiome

- Unstable;
- Modulated by pathogens and host; and
- Influences pathogen susceptibility.

Ross *et al.* (2018) Abraham *et al.* (2017)
Narasimhan *et al.* (2014, 2017)

Ixodes scapularis - blacklegged tick

Tick microbiome is complex

Endosymbiont ***Rickettsia buchneri***

- Maternally inherited;
- High abundances in reproductive tissues;
- Colonizes salivary glands; and
- Carries plasmids – one encodes entire biotin biosynthesis pathway.

Kurtti *et al.* (2015) Al-Khafaji *et al.* (2020)
Gillespie *et al.* (2012)

Ixodes scapularis - blacklegged tick

Tick microbiome is complex

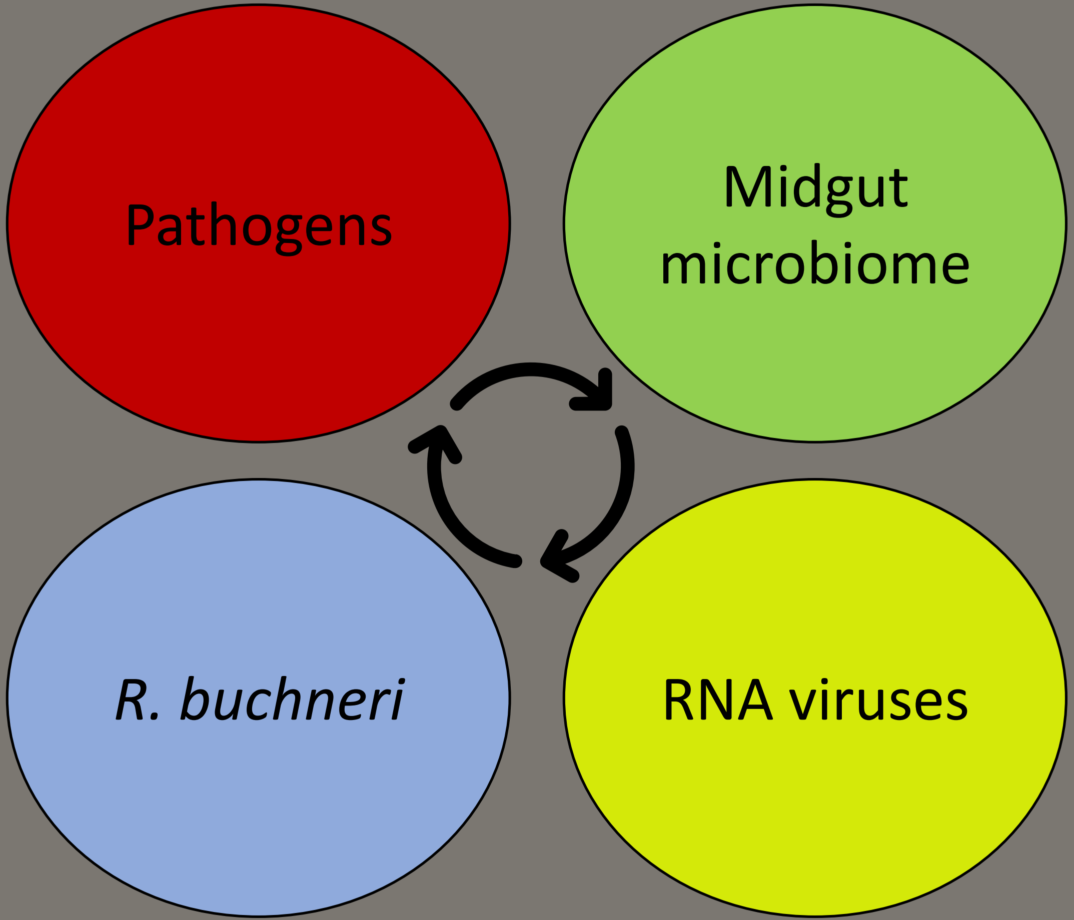
High diversity of RNA viruses

- *Bunyavirales* (-)ssRNA viruses; and
- Thought to be symbiotic as lacking glycoprotein-bearing "M segments".

Tokarz *et al.* (2014; 2018)

Ixodes scapularis - blacklegged tick

Tick microbiome is complex



Pathogens

Midgut microbiome

R. buchneri

RNA viruses

Ixodes scapularis - blacklegged tick

Metagenomics for microbiome surveillance

- High-throughput sequencing (HTS) is culture independent

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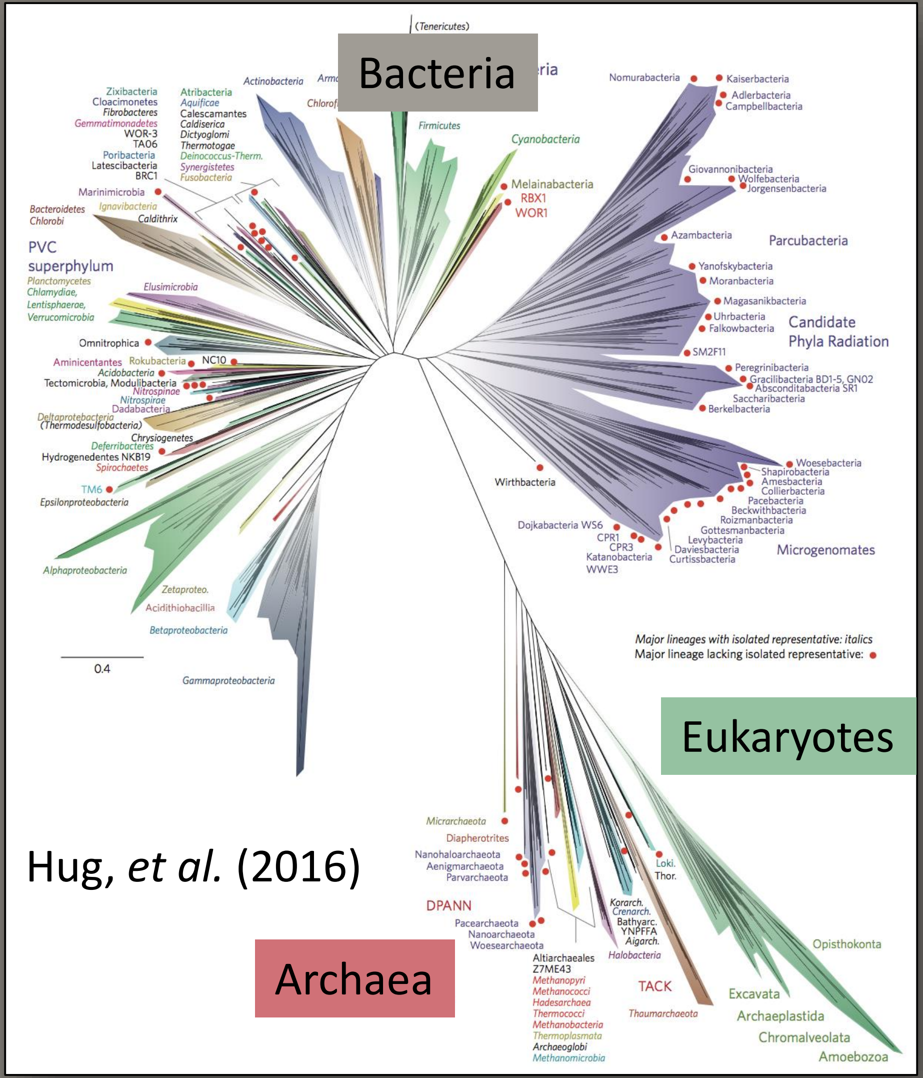

Hug, *et al.* (2016)

Metagenomics for microbiome surveillance

- High-throughput sequencing (HTS) is culture independent

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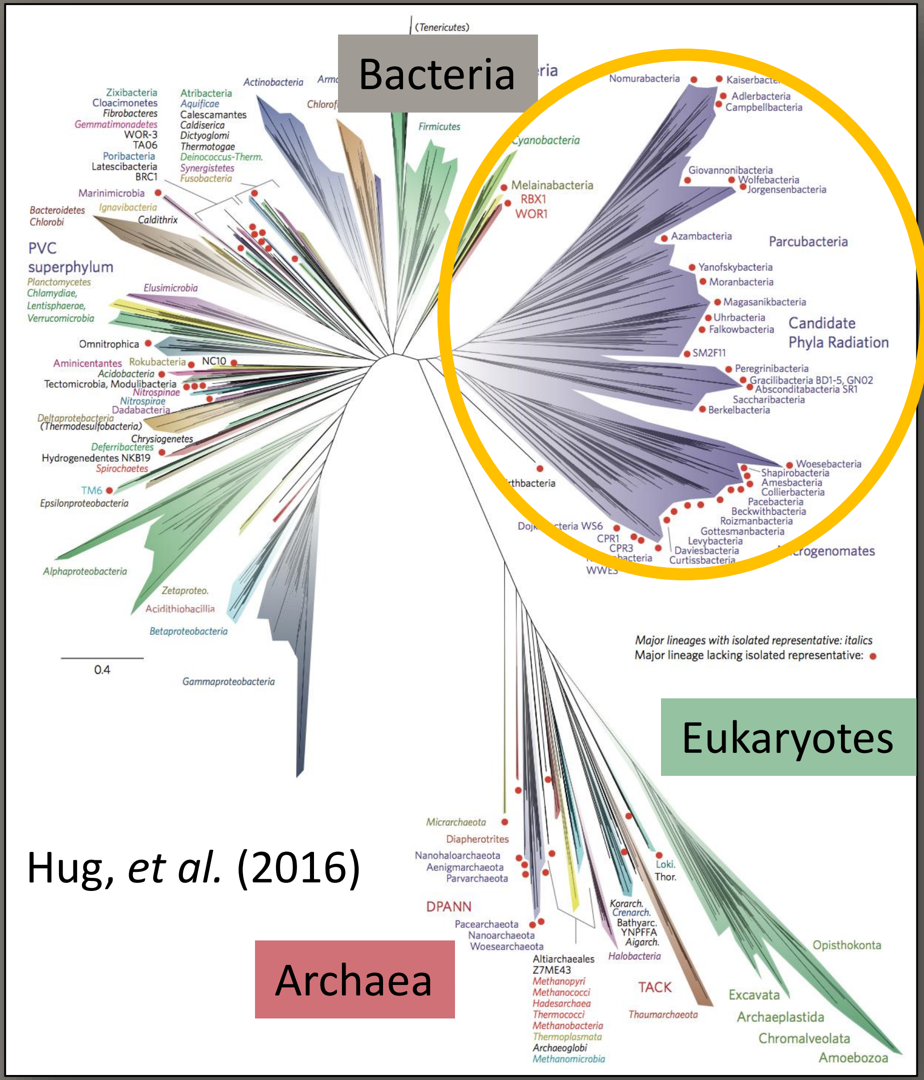


Hug, *et al.* (2016)

Candidate
Phyla
Radiation

Metagenomics for microbiome surveillance

- Novel organism and pathogen detection from ticks

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

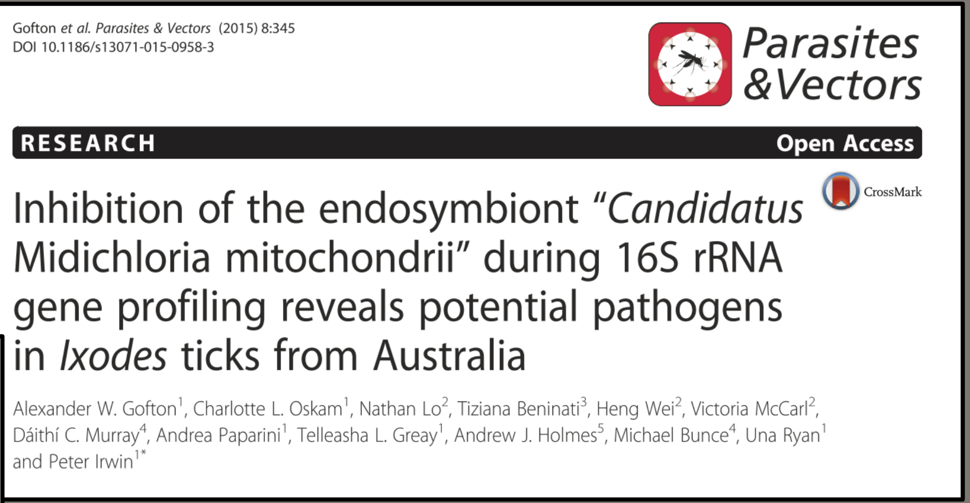
journal homepage: www.elsevier.com/locate/ttbdis

ELSEVIER

Original article

Bacterial community profiling highlights complex diversity and novel organisms in wildlife ticks

Siobhon L. Egan[a], Siew-May Loh[a], Peter B. Banks[b], Ambe
Peter J. Irwin[a], Charlotte L. Oskam[a,*]



Gofton *et al. Parasites & Vectors* (2015) 8:345
DOI 10.1186/s13071-015-0958-3

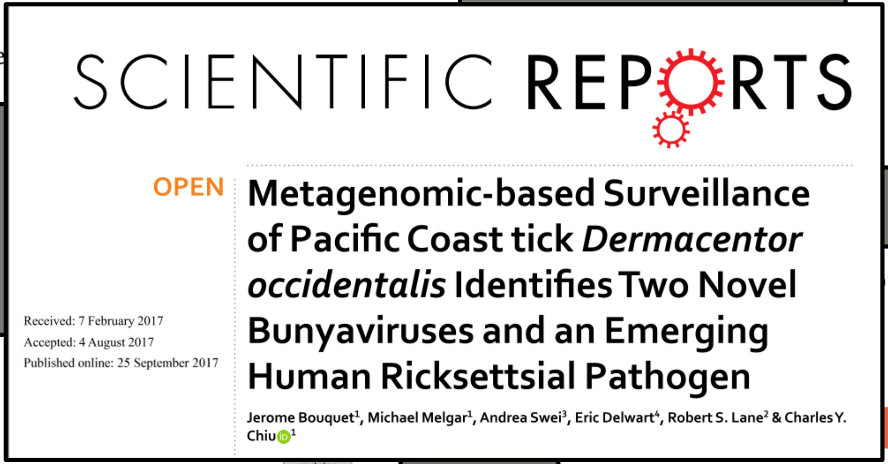
Parasites & Vectors

RESEARCH Open Access

CrossMark

Inhibition of the endosymbiont "*Candidatus* Midichloria mitochondrii" during 16S rRNA gene profiling reveals potential pathogens in *Ixodes* ticks from Australia

Alexander W. Gofton[1], Charlotte L. Oskam[1], Nathan Lo[2], Tiziana Beninati[3], Heng Wei[2], Victoria McCarl[2], Dáithí C. Murray[4], Andrea Paparini[1], Telleasha L. Greay[1], Andrew J. Holmes[5], Michael Bunce[4], Una Ryan[1] and Peter Irwin[1*]



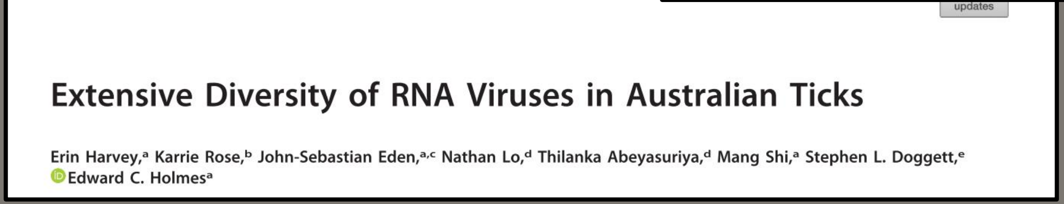
SCIENTIFIC REPORTS

OPEN

Metagenomic-based Surveillance of Pacific Coast tick *Dermacentor occidentalis* Identifies Two Novel Bunyaviruses and an Emerging Human Ricksettsial Pathogen

Received: 7 February 2017
Accepted: 4 August 2017
Published online: 25 September 2017

Jerome Bouquet[1], Michael Melgar[1], Andrea Swei[3], Eric Delwart[4], Robert S. Lane[2] & Charles Y. Chiu[1]

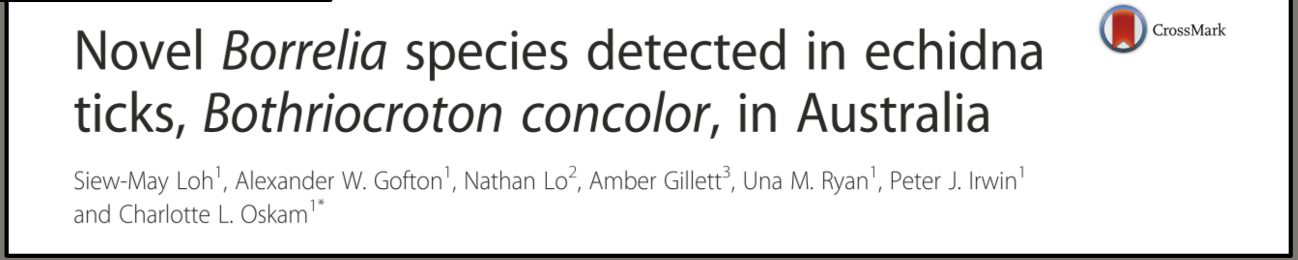


AMERICAN SOCIETY FOR MICROBIOLOGY Journal of Virology®

updates

Extensive Diversity of RNA Viruses in Australian Ticks

Erin Harvey,[a] Karrie Rose,[b] John-Sebastian Eden,[a,c] Nathan Lo,[d] Thilanka Abeyasuriya,[d] Mang Shi,[a] Stephen L. Doggett,[e] Edward C. Holmes[a]



16) 9:339

Parasites & Vectors

Open Access

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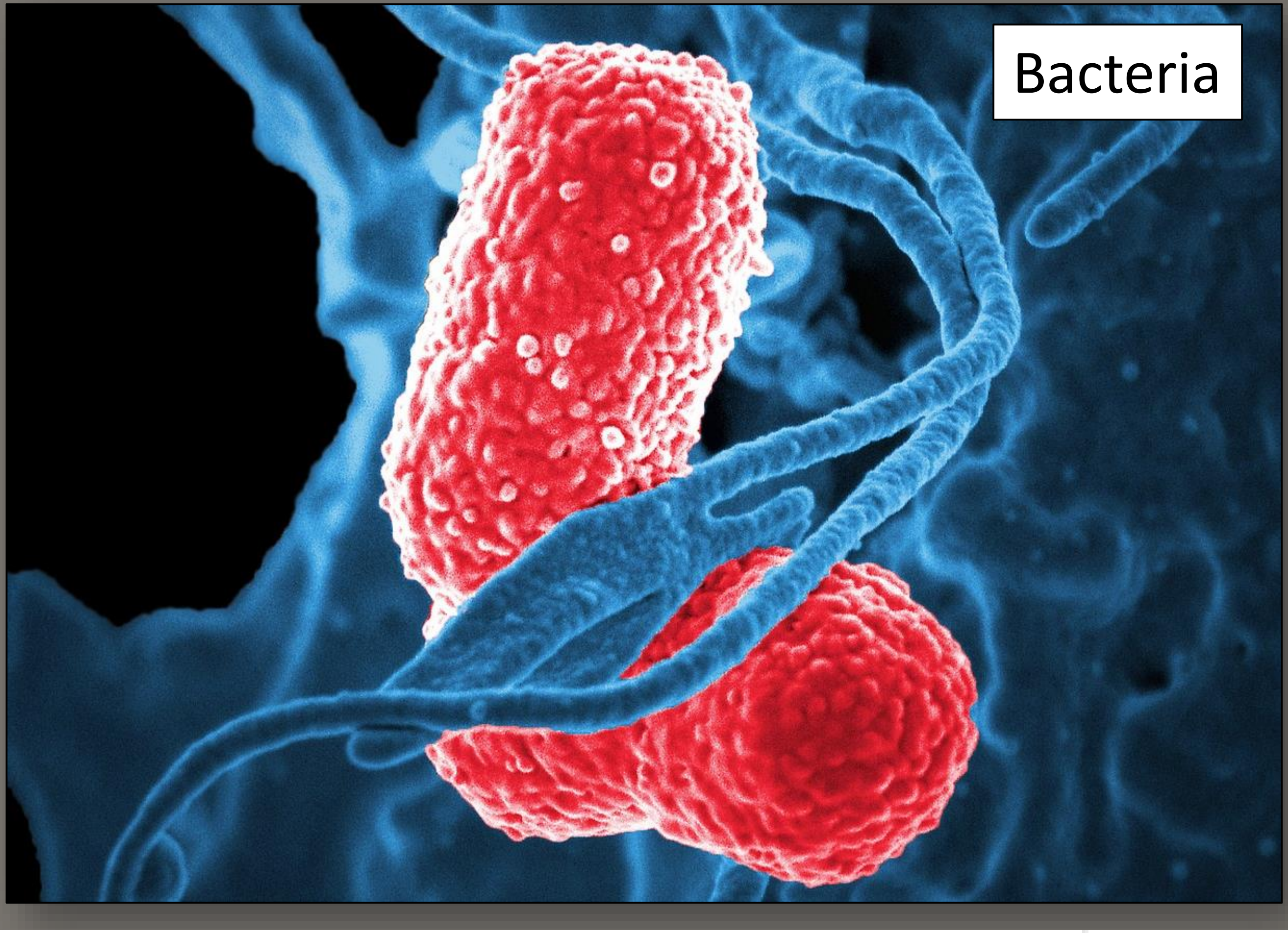
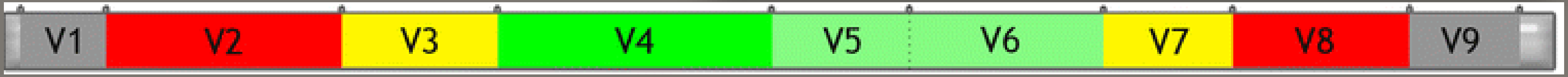
Novel *Borrelia* species detected in echidna ticks, *Bothriocroton concolor*, in Australia

Siew-May Loh[1], Alexander W. Gofton[1], Nathan Lo[2], Amber Gillett[3], Una M. Ryan[1], Peter J. Irwin[1] and Charlotte L. Oskam[1*]

Targeted metagenomics – 16S rRNA sequencing

Variable regions of the 16S rRNA gene used to determine bacterial communities



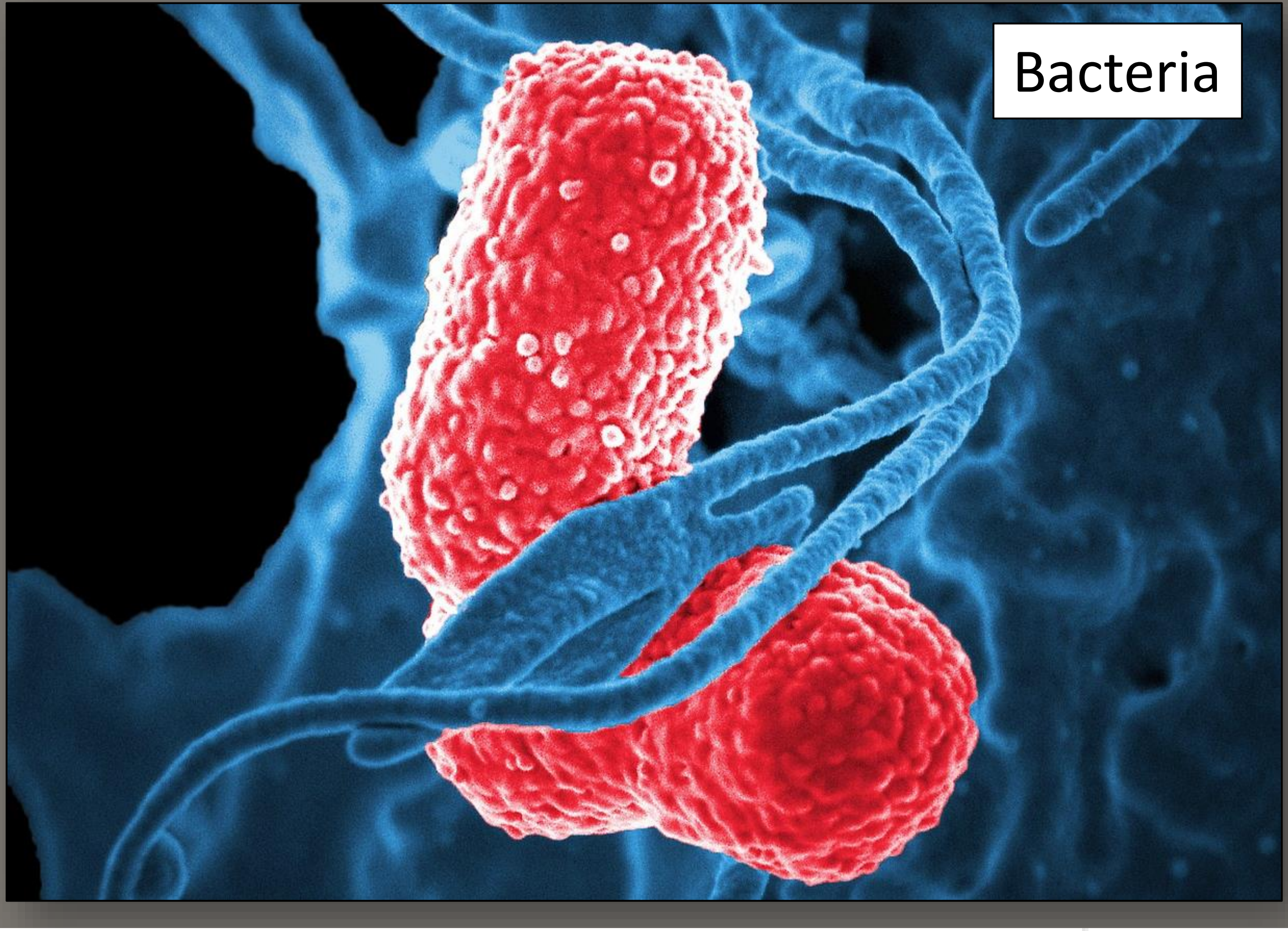


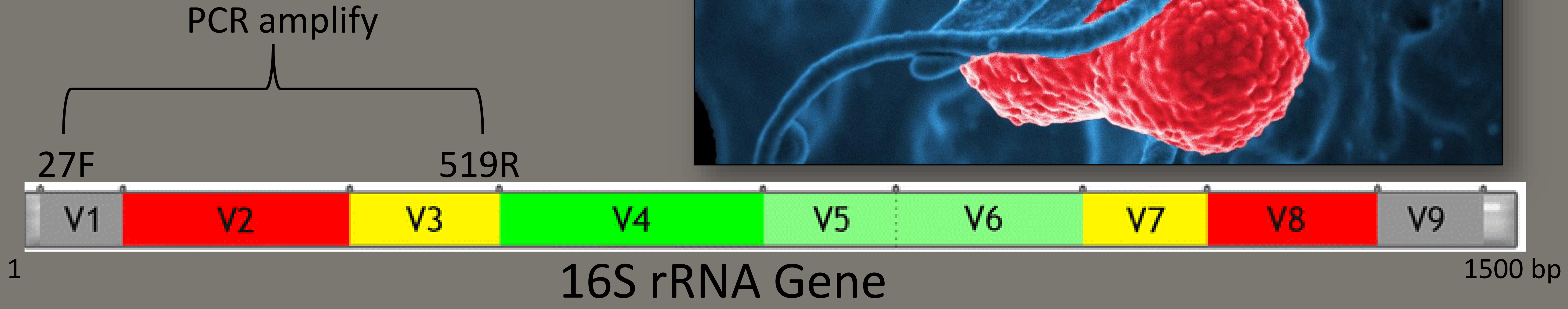
16S rRNA Gene

Modified from: Yang, *et al.* (2016)

Targeted metagenomics – 16S rRNA sequencing

Variable regions of the 16S rRNA gene used to determine bacterial communities





16S rRNA Gene

Modified from: Yang, *et al.* (2016)

Meta-transcriptomics

Snapshot of all microbial gene expression in a moment in time

Start with **RNA**

↓

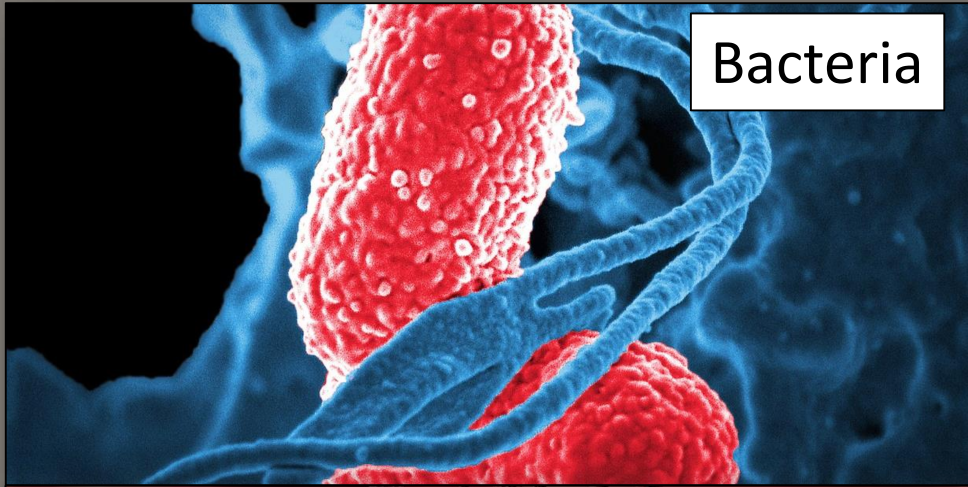
Dual rRNA depletion

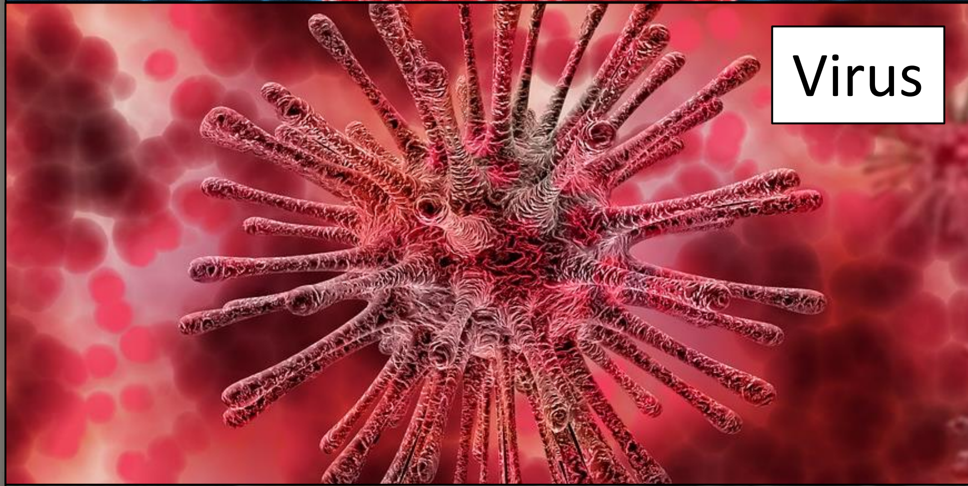
↓

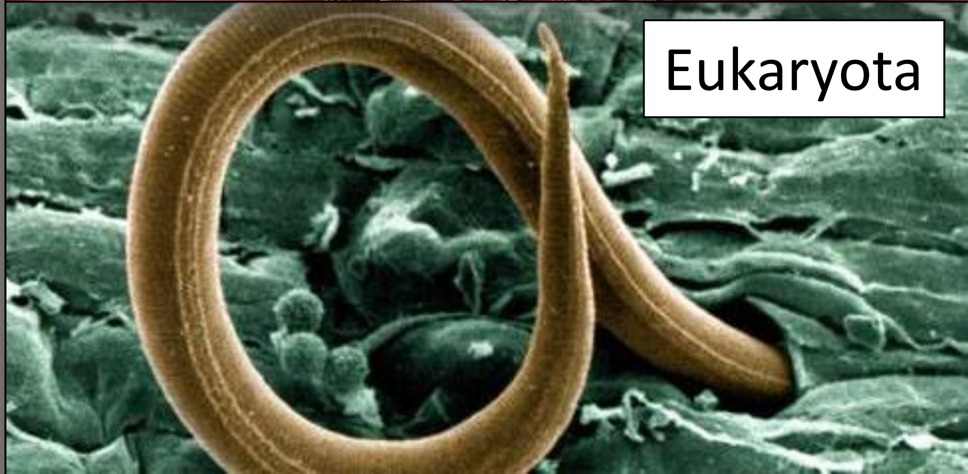
Convert cDNA

↓

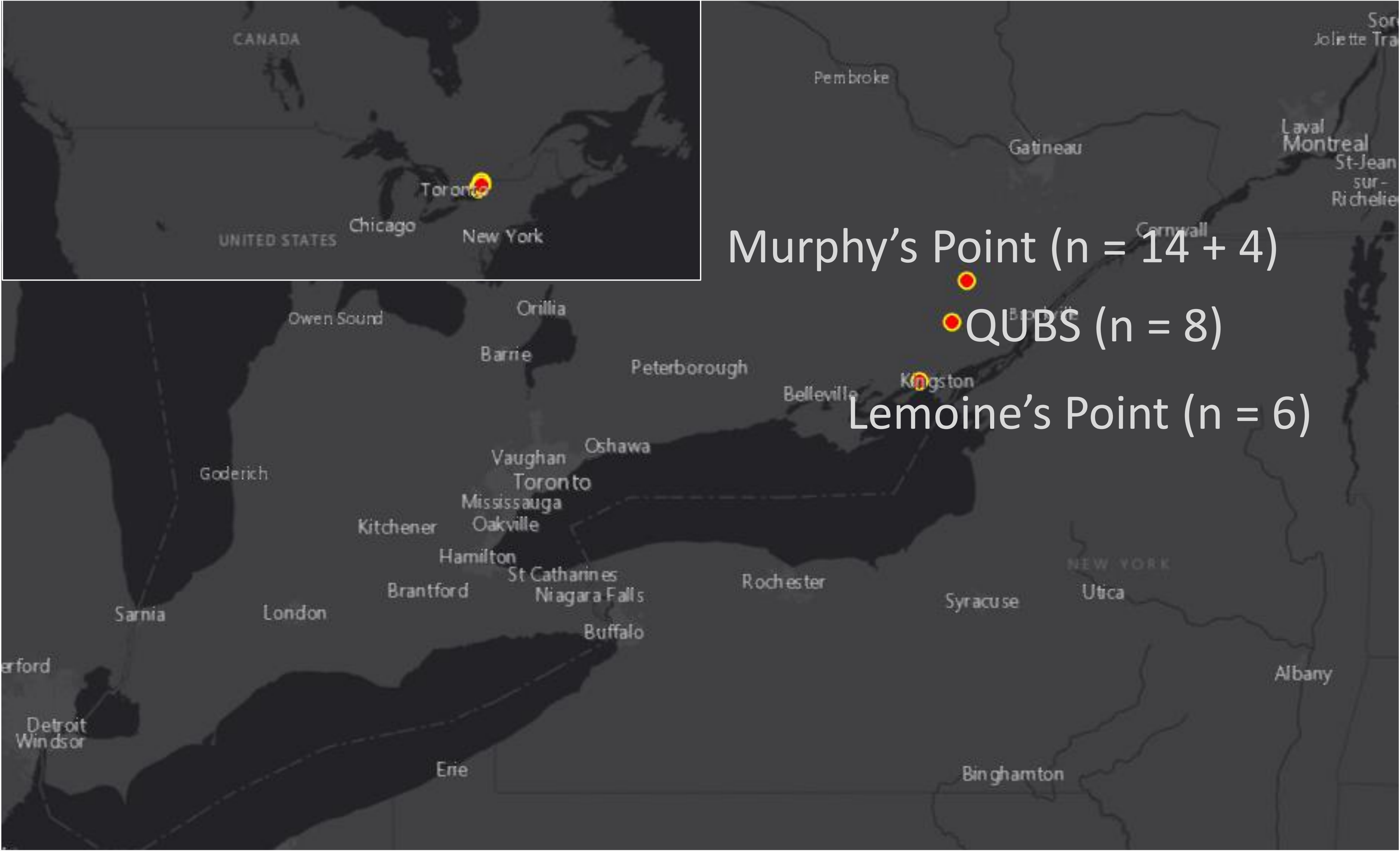
Sequence libraries


Bacteria


Virus


Eukaryota

Ixodes scapularis from KFL&A Health Region



Murphy's Point (n = 14 + 4)

QUBS (n = 8)

Lemoine's Point (n = 6)

Objectives

1. Compare **bacterial** communities of *I. scapularis* from three sites within new Lyme disease risk area;

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1. Compare **bacterial** communities of *I. scapularis* from three sites within new Lyme disease risk area;
2. Compare **bacterial** communities in salivary gland, midgut and whole ticks; and
3. Apply metatranscriptomics to capture the **whole** microbiome of *I. scapularis*.

16S rRNA sequencing analysis pipeline

- Goal – promote open science through creation of a *reproducible research* workflow specific for tick microbiome

Denoise & Amplicon Sequence variants (ASV)

Decontaminate

Analyze

16S rRNA sequencing analysis pipeline

- Goal – promote open science through creation of a *reproducible research* workflow specific for tick microbiome
    - Open-source R packages: *Dada2* & *Phyloseq ;*

Denoise & Amplicon Sequence variants (ASV)

Decontaminate

Analysis

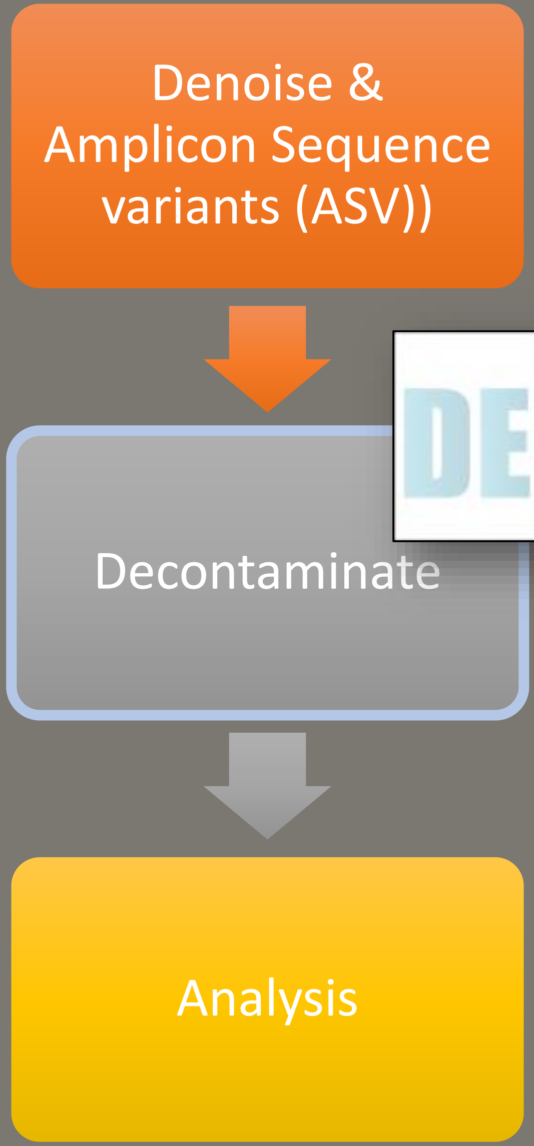
Callahan *et al.* (2016)

McMurdie & Holmes. (2013)

16S rRNA sequencing analysis pipeline

- Goal – promote open science through creation of a *reproducible research* workflow specific for tick microbiome
  - Open-source R packages: *Dada2* & *PhyloseqR ;*

- Important to remove contaminants from tick microbiome data



Denoise & Amplicon Sequence variants (ASV))

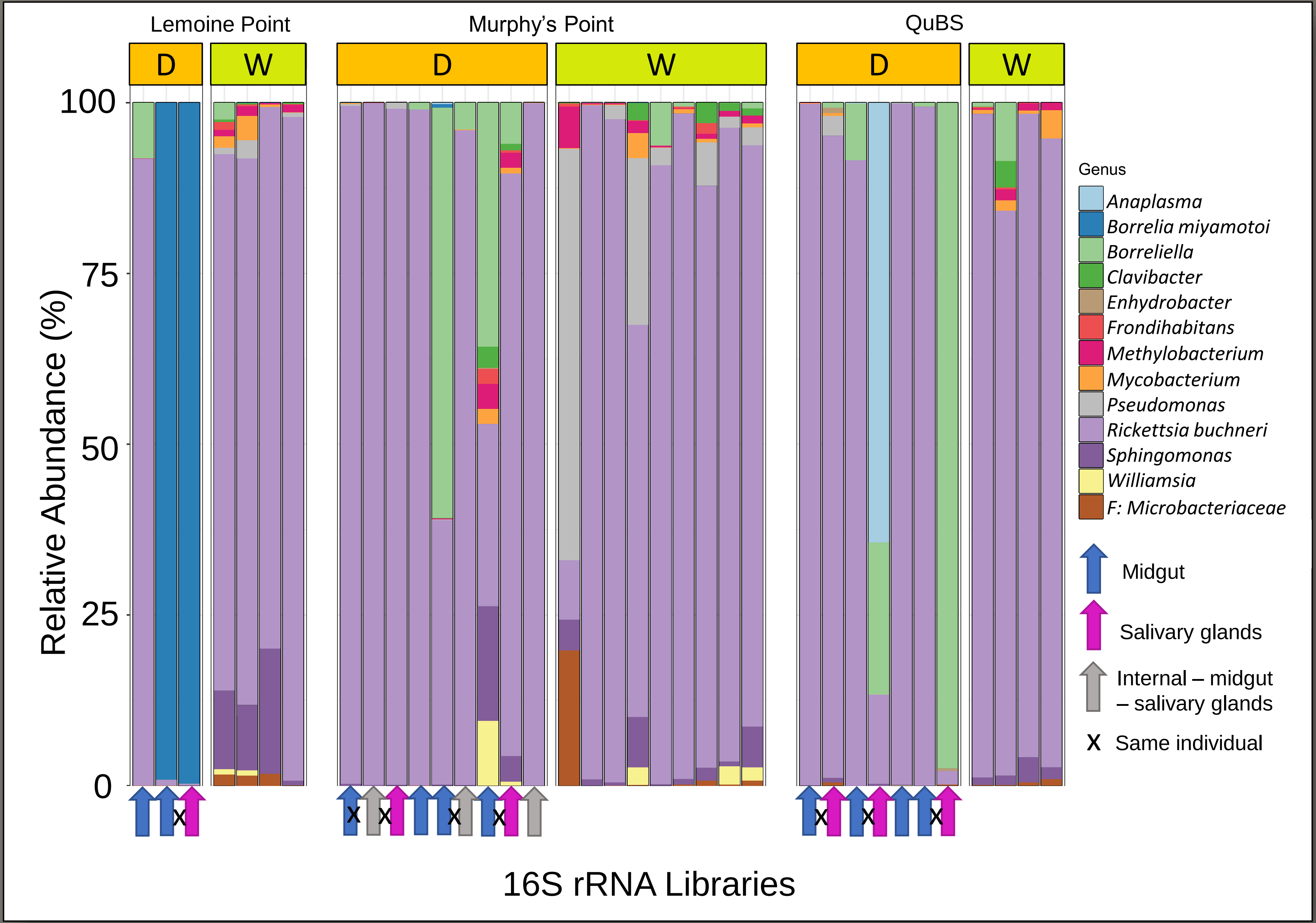
Decontaminate

Analysis

DECONTAM

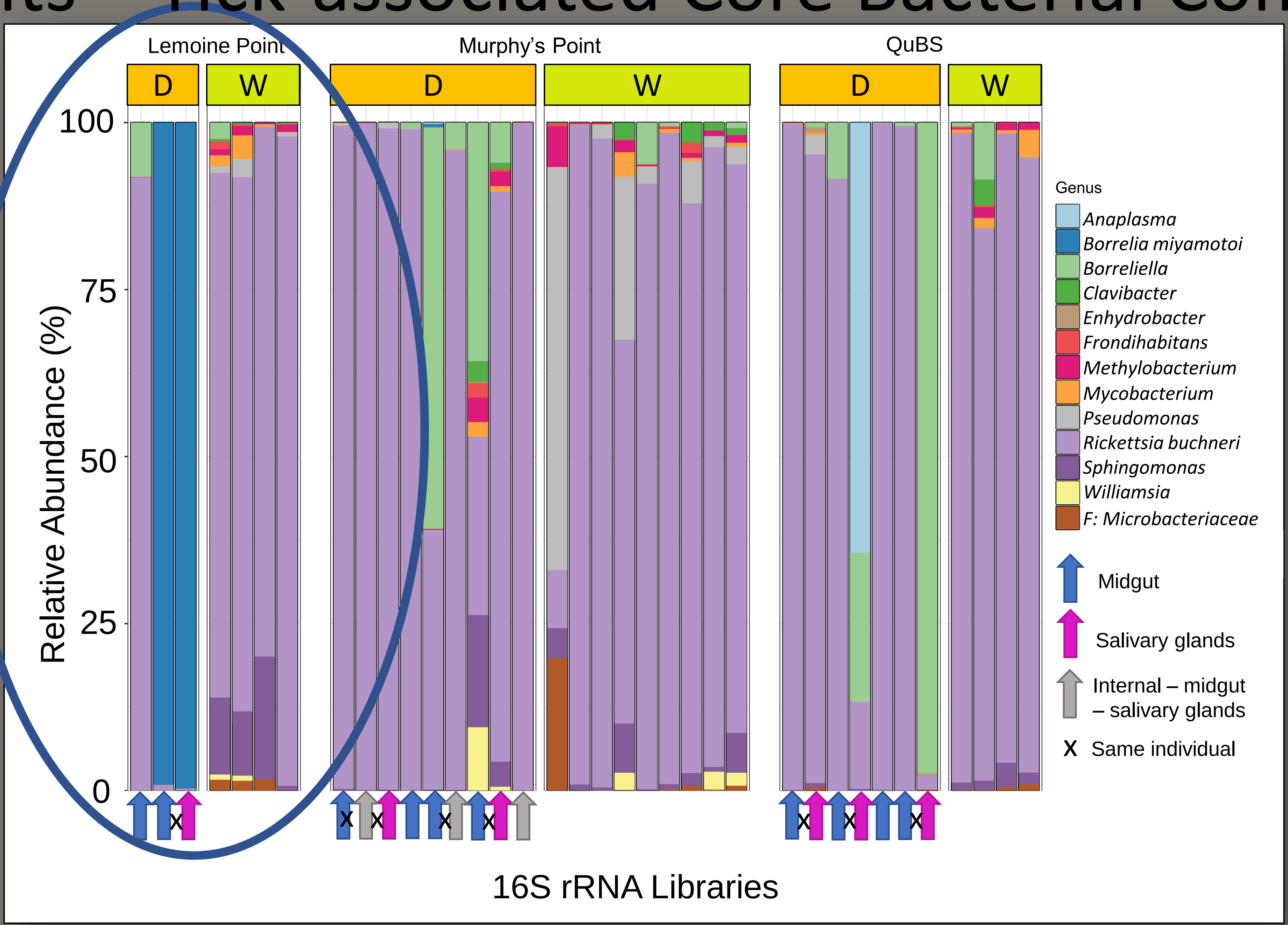
Davis *et al.* (2018)

Results – Tick-associated Core Bacterial Community



Paulson *et al.* In prep

Results – Tick-associated Core Bacterial Community



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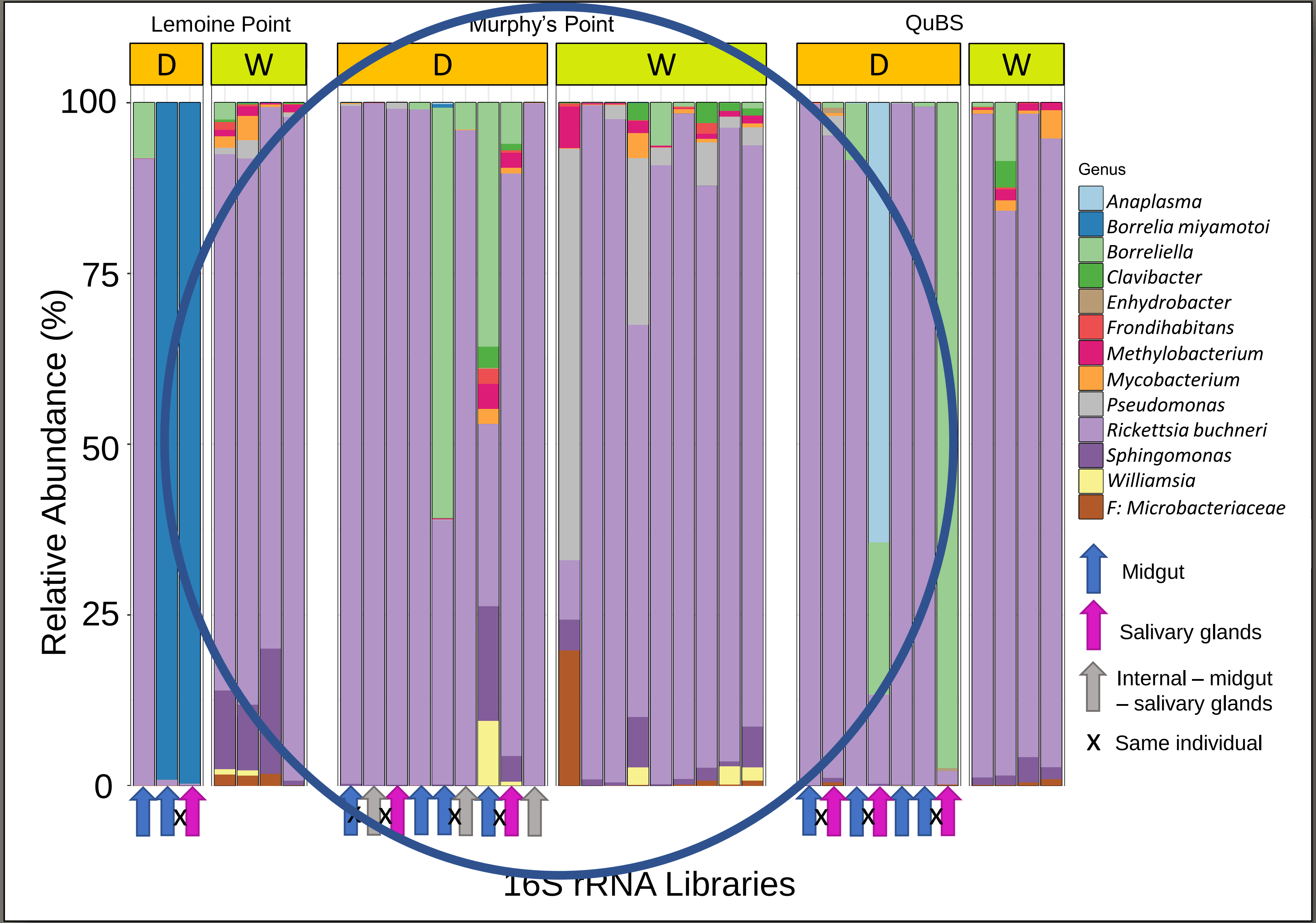
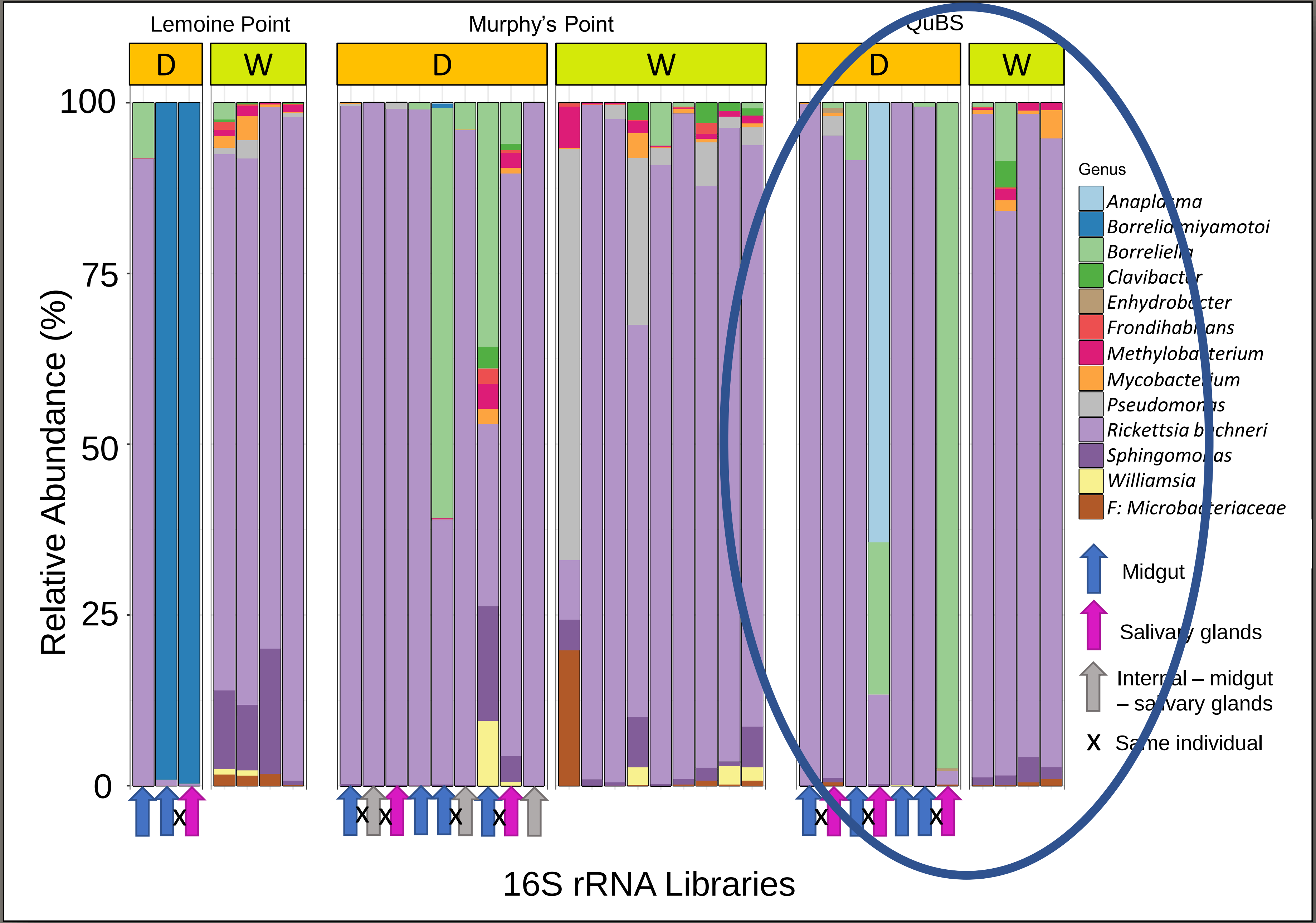


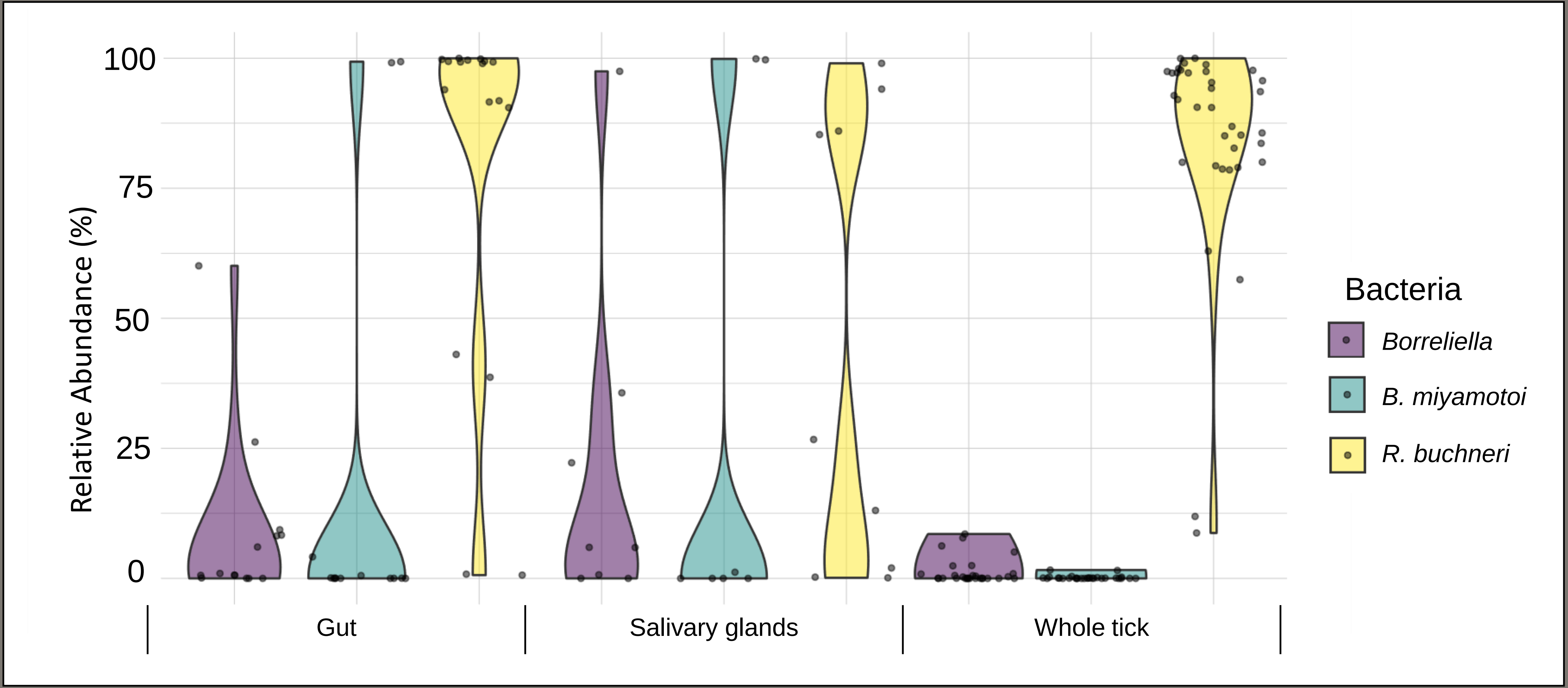
Paulson *et al.* In prep

Results – Tick-associated Core Bacterial Community

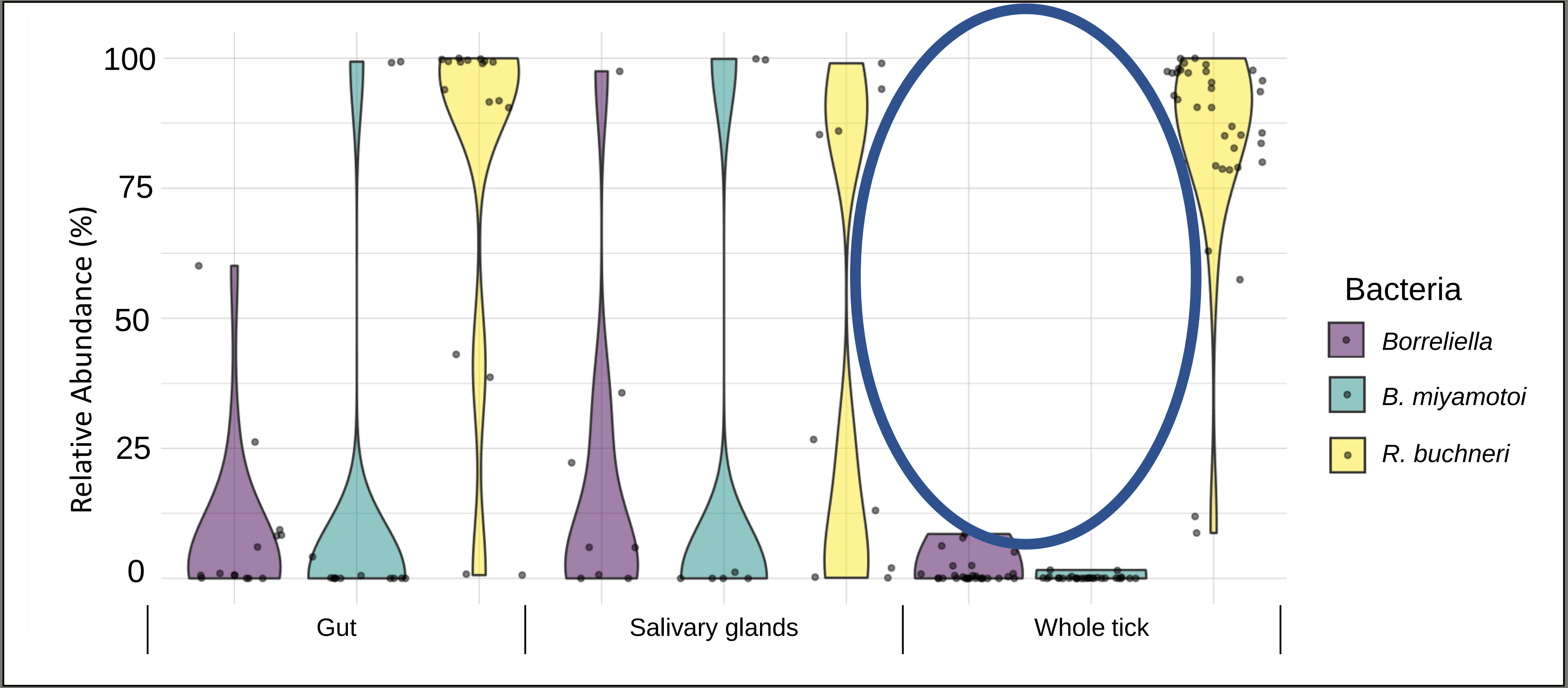


Paulson *et al.* In prep

Pathogens are more abundant in dissected tissues than whole ticks

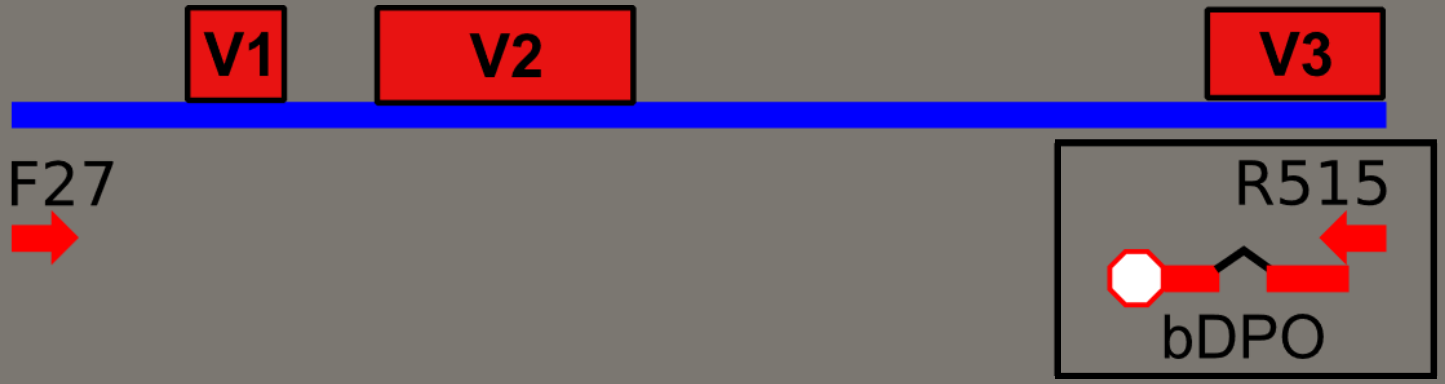


Pathogens are more abundant in dissected tissues than whole ticks



Next Steps

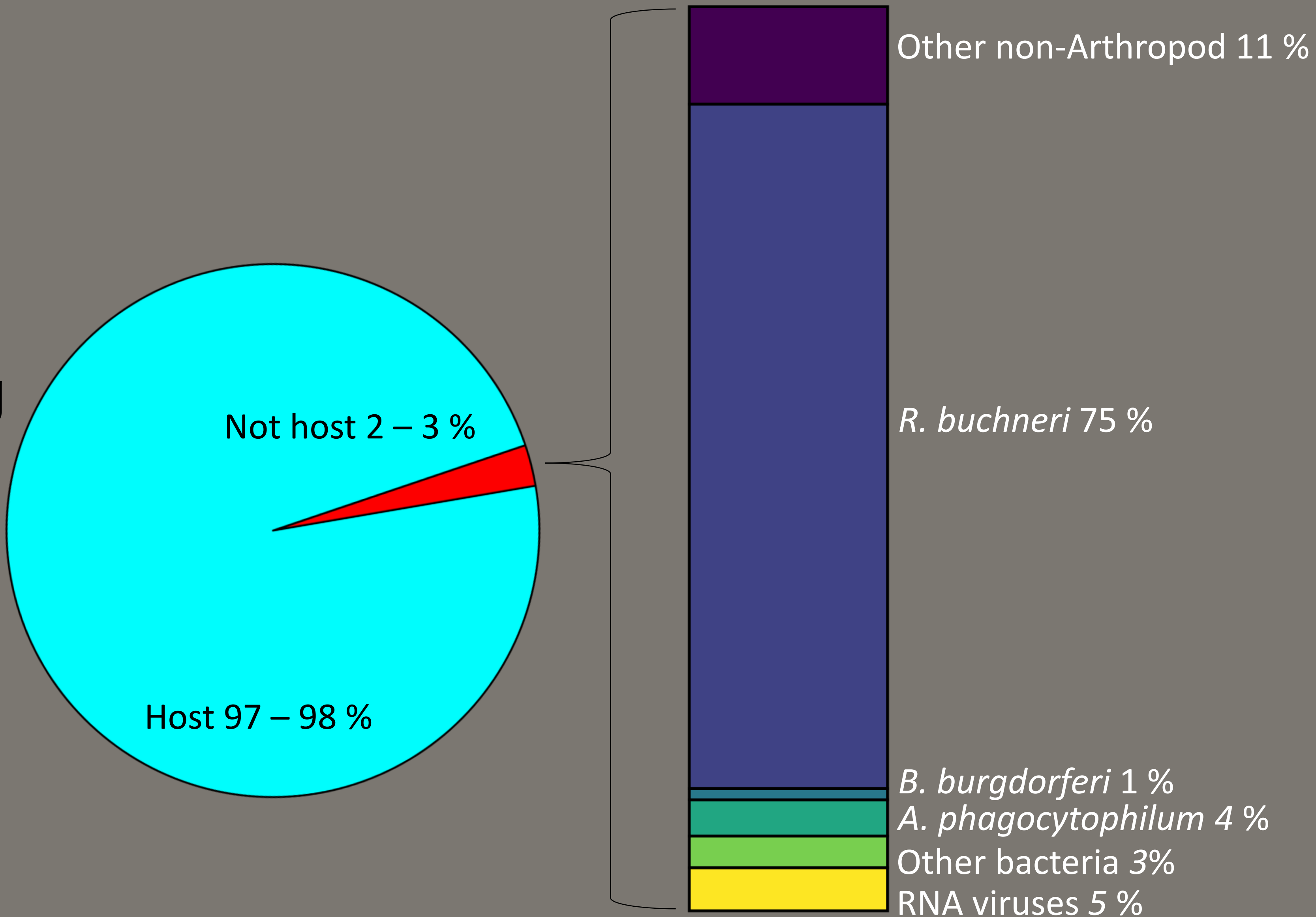
- 16S rRNA blocking primers for *Rickettsia* –
  - tested > 70 % *R. buchneri* reduction



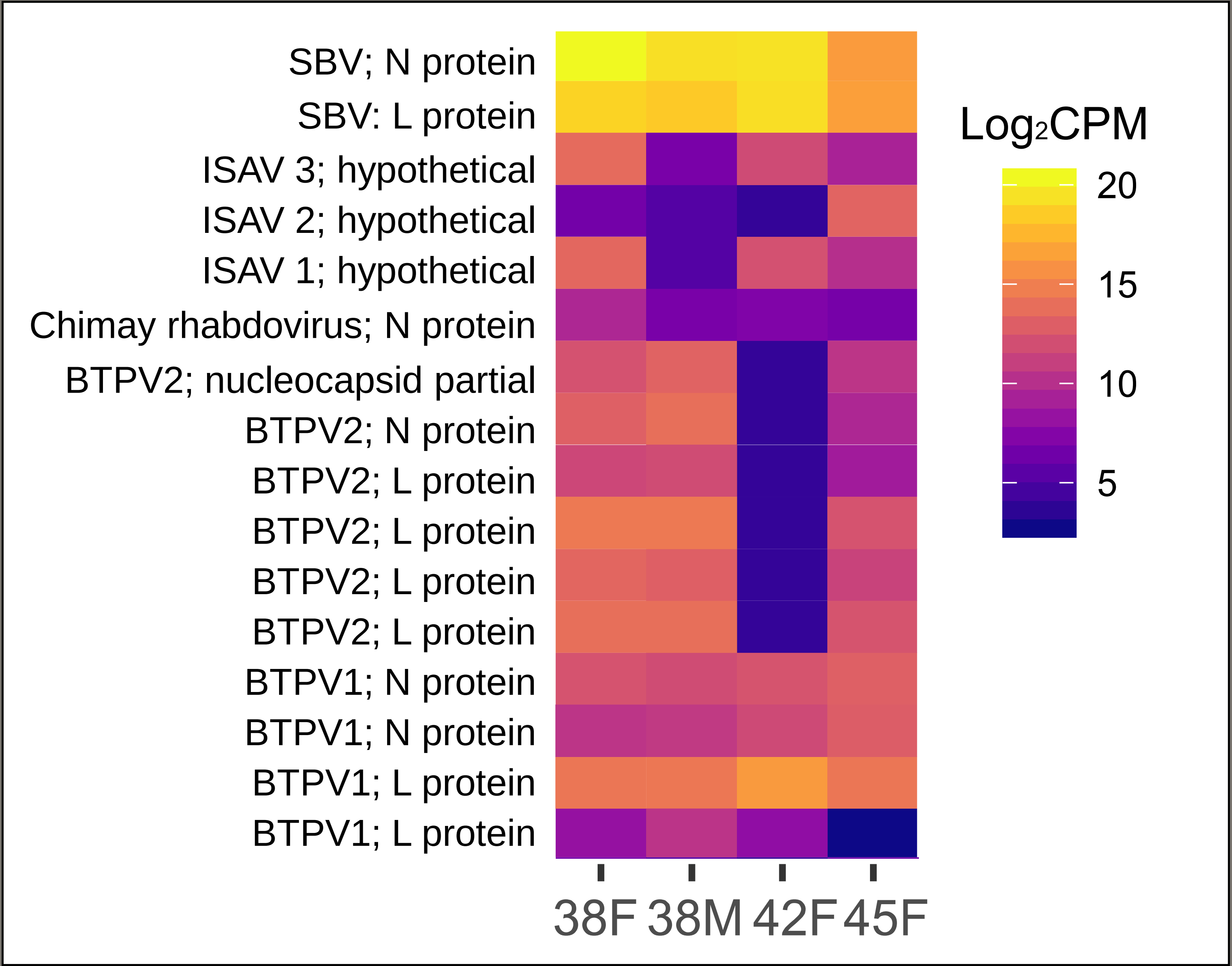
Santos-Garcia *et al.* (2020)

Meta-transcriptome Results: Taxonomy

Sequencing data:



Meta-transcriptome: Abundant RNA Viruses



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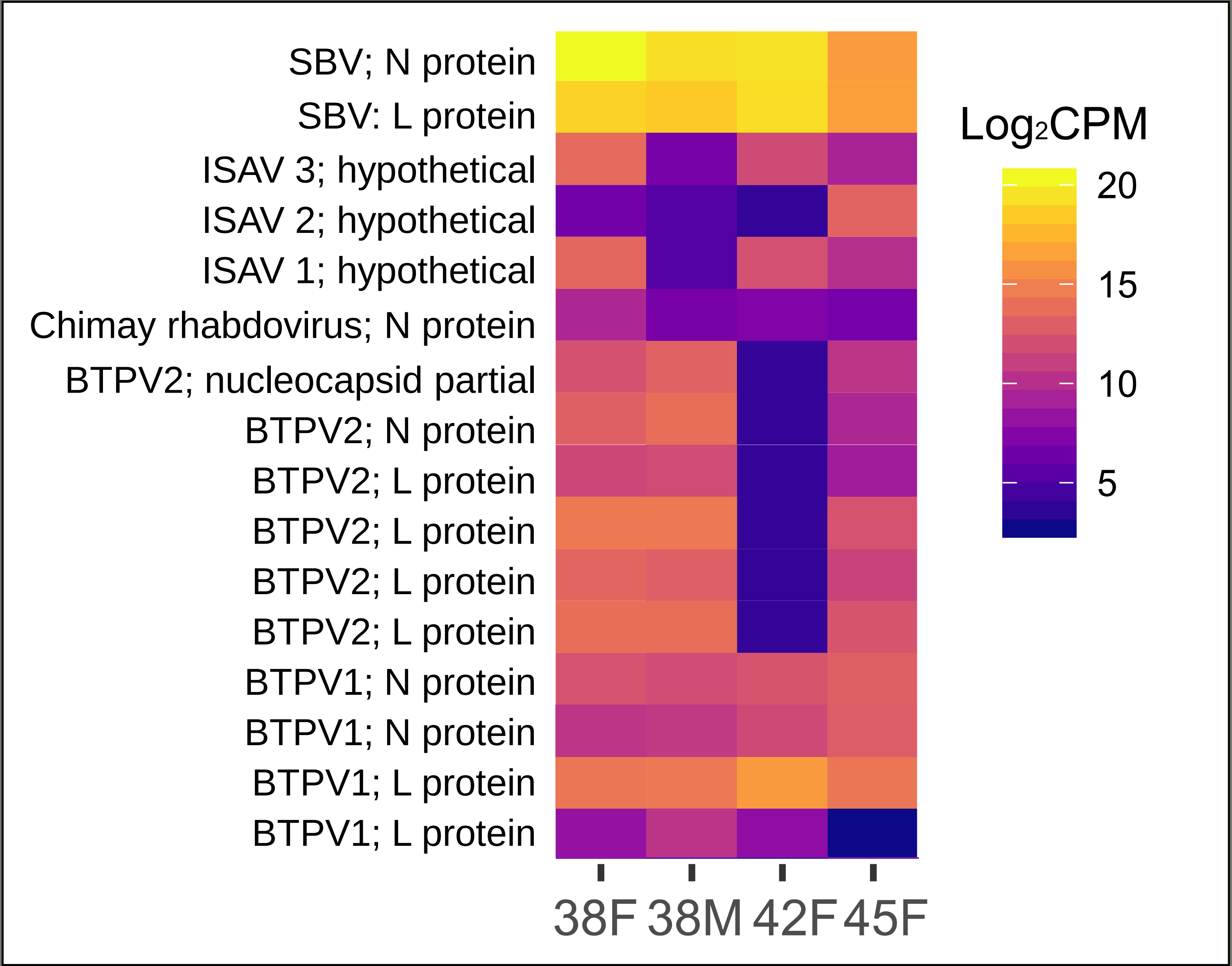
Meta-transcriptome: Abundant RNA Viruses



Next Steps:

Tissue-specific meta-transcriptomics

Paulson *et al.* In prep

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- Emilie Norris-Roozmon

Park's Ontario
Cataraqui Reserve Conservation Association
QUBS staff
Curtis Russell – trainer
Tick Photos: @mikullashbee

GenomeQuébec

Centre for Advanced Computing

THANK YOU

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Bacteria: https://www.pxfuel.com/en/free-photo-jjixb

Virus: https://www.pxfuel.com/en/free-photo-jyixo

Nematode Photo by William Wergin and Richard Sayre. Colorized by Stephen Ausmus. U.S. Department of Agriculture

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