Thesis progression title:

Viral Diversity and Phylogenetic Characterization of Mammalian-Associated Viruses in Thai Rodents

Thesis title:

Diversity of zoonotic viruses and Cross-species Transmission Potential of Candidate Virus in Rodents in Chanthaburi and Chiang Rai, Thailand

Presented by :

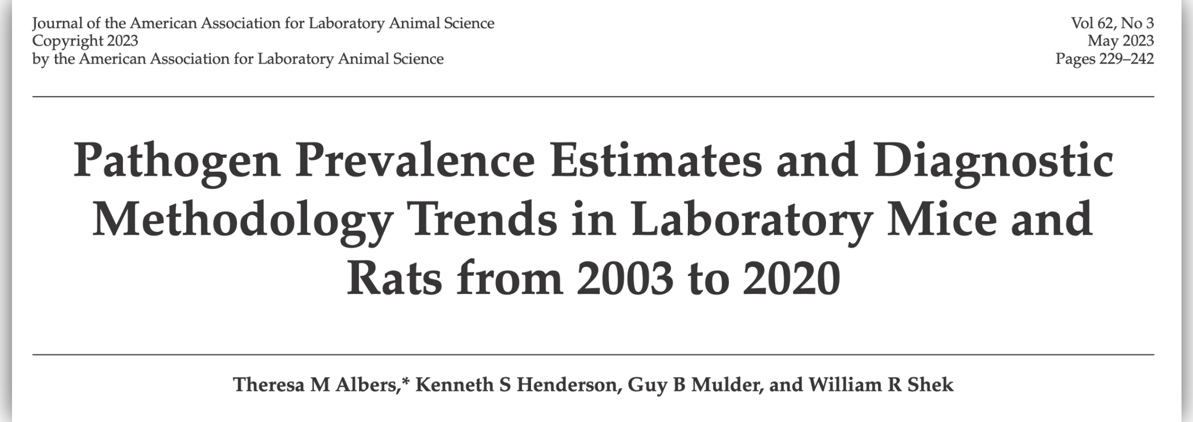
Sutharini Rakkiattikhun

Advisor :

Asst. Prof. Dr. Sirinart Aromseree

Introduction

Types of pathogen in rodents



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Pathogen Prevalence Estimates and Diagnostic Methodology Trends in Laboratory Mice and Rats from 2003 to 2020

Theresa M Albers,* Kenneth S Henderson, Guy B Mulder, and William R Shek

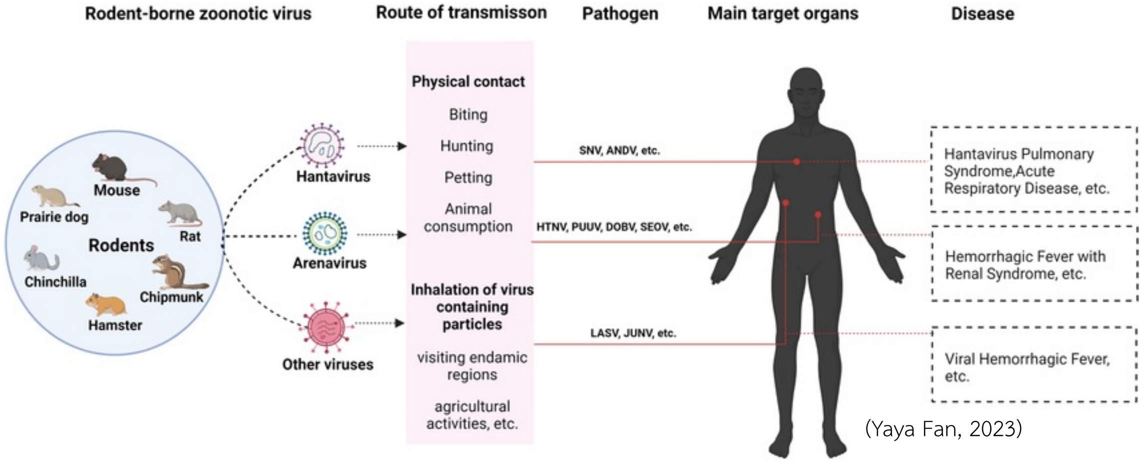
(Albers, T. M., et al., 2023)

52%
Virus

33%
Bacteria

15%
Parasite

Rodent-borne viral diseases



(Yaya Fan, 2023)

Type of viral genome	Viral families	Prevalence
DNA viruses	*Herpesviridae*	0.70%
RNA viruses	*Arenaviridae*	53.10%
	Coronaviridae	1.25%
	Flaviviridae	5.78%
	Hantaviridae	22.83%
	Hepeviridae	0.07%
	Paramyxoviridae	0.38%
	Picorbirnaviridae	1.70%
	Picornaviridae	0.33%
	Reoviridae	0.14%
Other		13.72%

(Wu et al., 2021)

Hypothesis

1. The diversity and prevalence of bacterial pathogens in rodents from different habitats are different.
2. A candidate virus circulating in rodents has the potential of cross-species transmission.

Objectives

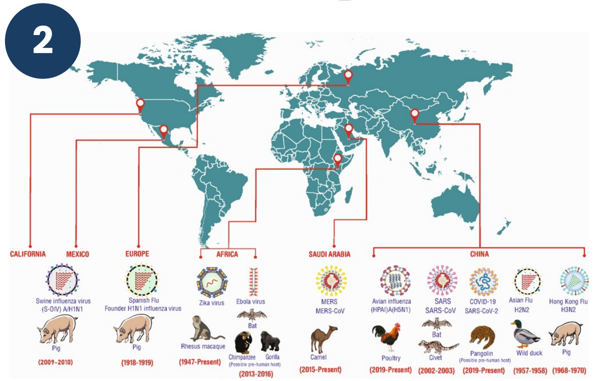
- To investigate the diversity and prevalence of bacterial pathogens in rodents from different habitats
- To determine the distribution of viruses belonging to common zoonotic viral families in rodents from different habitats
- To determine the cross-species transmission potential of a candidate virus in rodents in Chanthaburi and Chiang Rai, Thailand

Conceptual framework

Problem

1

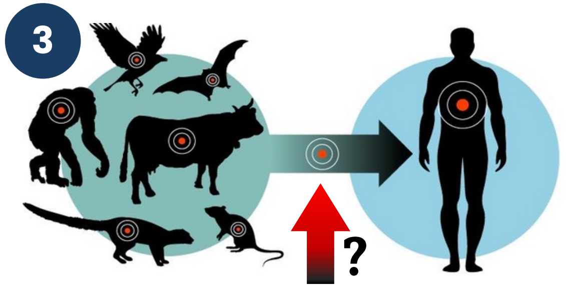
2



They comprise: **60%** of all infectious diseases in humans **75%** of all emerging infectious diseases

Source: *UNEP Frontiers 2016 Report*

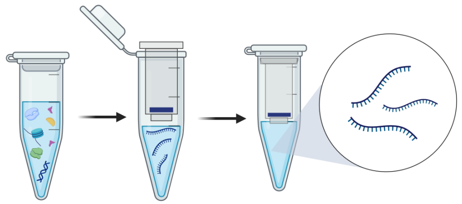
3



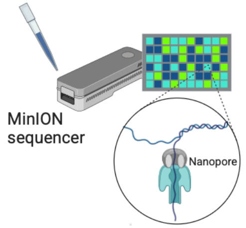
Spillover factors

Process

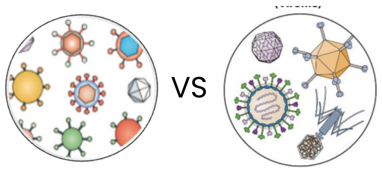
1) Sample collection from different habitats and preparation for sequencing



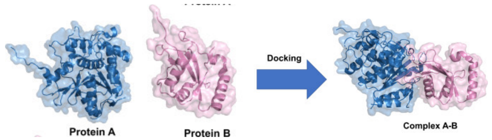
2) Investigation of diversity of bacterial pathogen in rodents



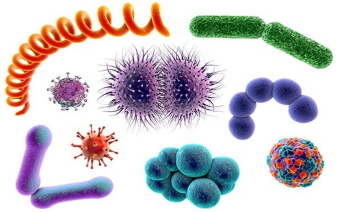
3) Determination of distribution of viruses belonging to common zoonotic viral families



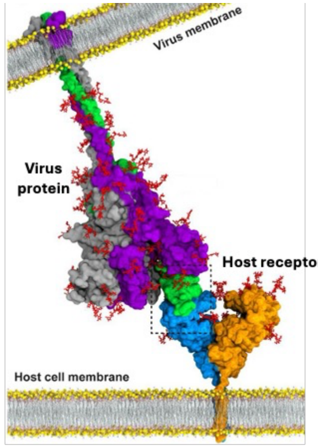
4) Determination of candidate virus with cross-species transmission potential



Outputs



- Diversity and prevalence of bacterial pathogens
- Distribution of viruses in the common zoonotic viral families
- Phylogenetic tree of common zoonotic viral families



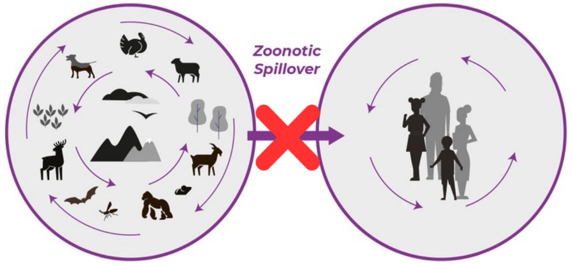
Virus-Host receptor as high-risk for cross-species transmission

Outcomes

Information

- for analysis factor to zoonotic spillover
- for characterization the cross-species transmission potential *in vitro* and *in vivo*
- for design tools to control diseases

Impacts



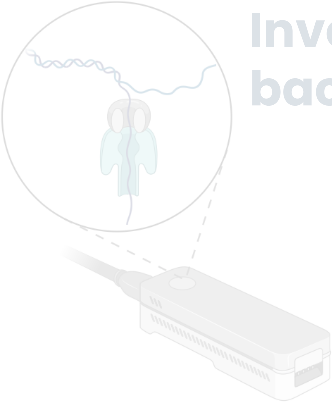
Risk of pathogen spillover ↓

Previous progression

Part 1

Sample collection from different habitats and preparation for sequencing

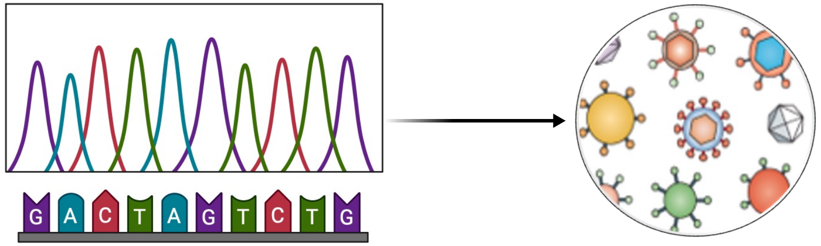
Part 2



Investigation of the diversity of bacterial pathogens in rodents

Part 3

Determination of the distribution of viruses belonging to common zoonotic viral families and construction of phylogenetic trees in rodents



Part 4

Determination of candidate virus with cross-species transmission potential

Previous progression

Part 3 Distribution of common zoonotic viral families in rodents

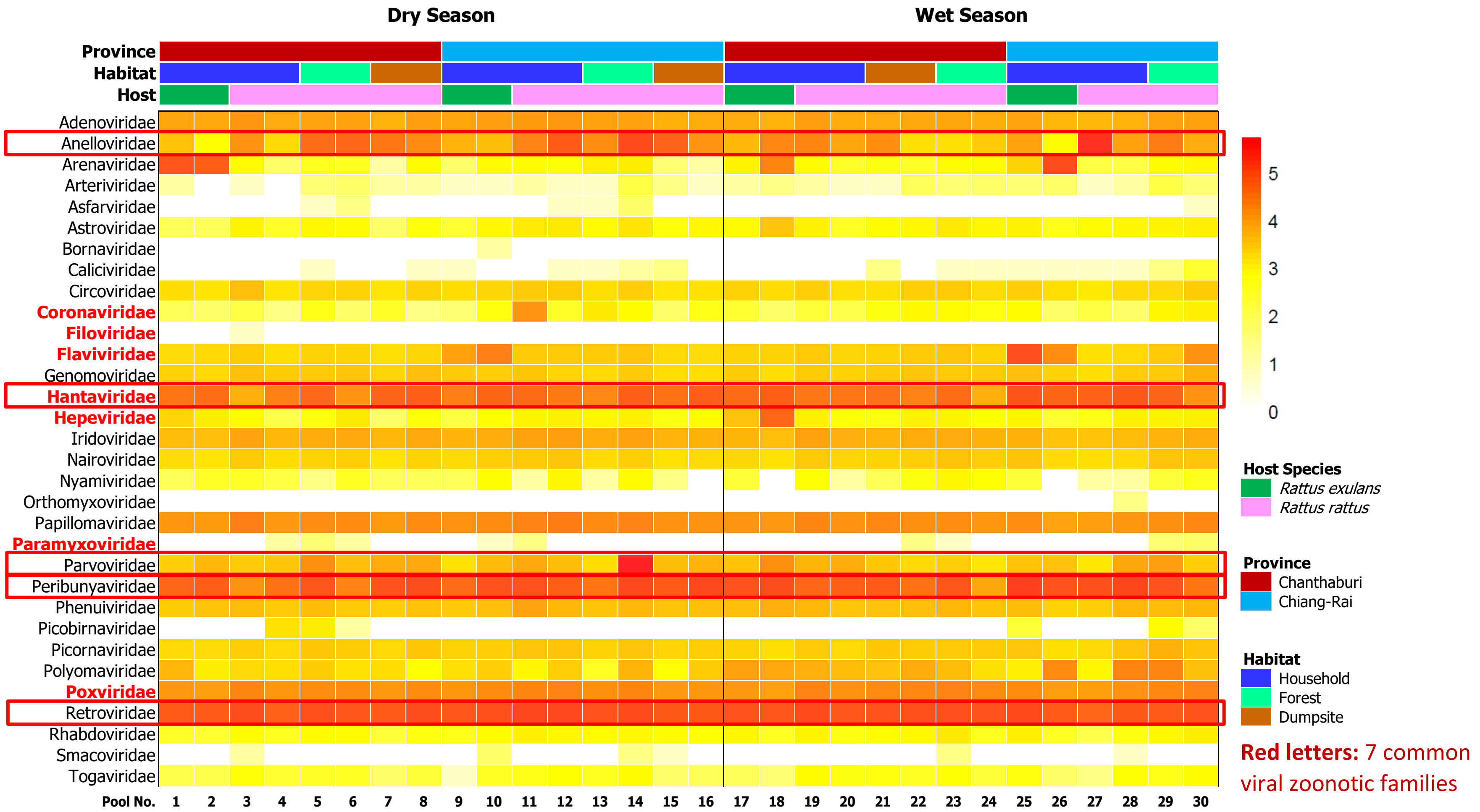


Figure 1 Relative abundance of the zoonotic viral family in rodent in each pool

Top 5 viral families with high abundance: *Retroviridae, Peribunyaviridae, Hantaviridae, Anelloviridae,* and *Parvoviridae*.

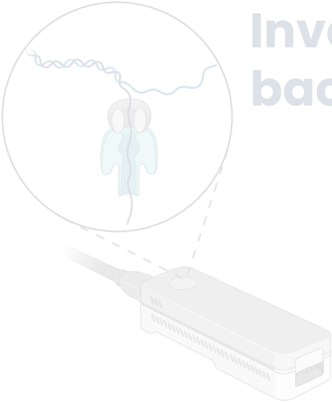
Current progression

Part 1

Sample collection from different habitats and preparation for sequencing

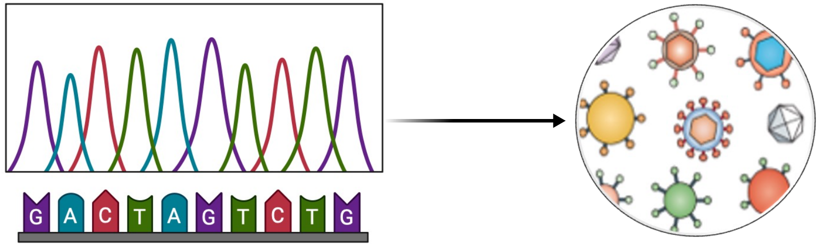
Part 2

Investigation of the diversity of bacterial pathogens in rodents



Part 3

Determination of the distribution of viruses belonging to common zoonotic viral families and construction of phylogenetic trees in rodents



Part 4

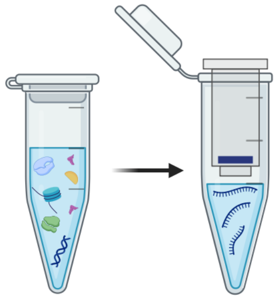
Determination of candidate virus with cross-species transmission potential

Part 3- Distribution of common zoonotic viruses

Pool samples

- ➢ Oral swab
- ➢ Rectal swab
- ➢ Blood

Nucleic acid extraction

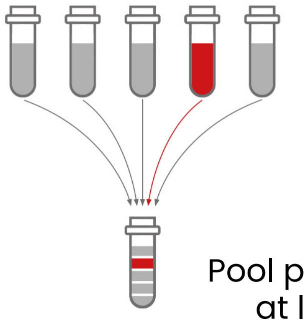


QIAamp Viral RNA Mini Kit

Pan- viral PCR

1. *Coronaviridae*
2. *Flaviviridae*
3. *Filoviridae*
4. *Hepeviridae*
5. *Hantaviridae*
6. *Paramyxoviridae*
7. *Poxviridae*

Pool sample for sequencing

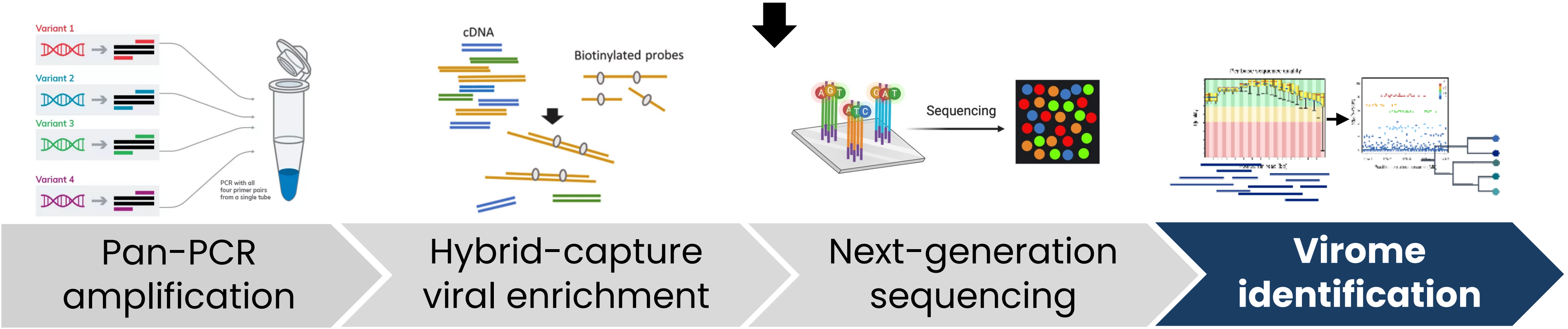


- ➢ Species
- ➢ Habitats
- ➢ Season
- ➢ Provinces

Pool positive Pan-viral PCR
at least 1 viral family.

Part 3– Distribution of common zoonotic viruses

To determine the **distribution of viruses** belonging to common zoonotic viruses in rodents from different habitats



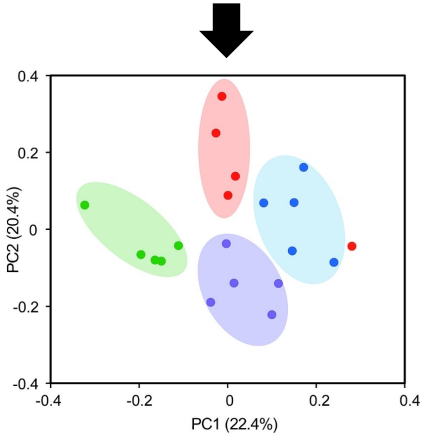
Pan-PCR amplification

Hybrid-capture viral enrichment

Next-generation sequencing

Virome identification

To **evaluate viral community** composition in rodents from different habitats and species



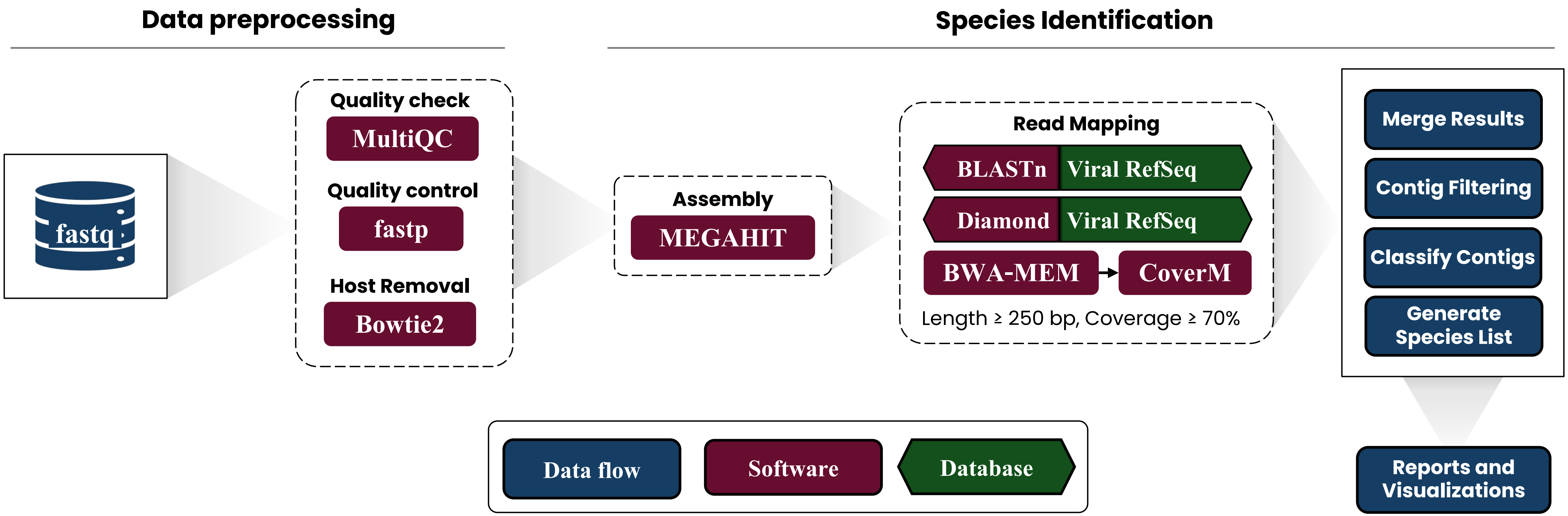
Principal coordinate analysis

Part 3– Distribution of common zoonotic viruses

Virome Identification

To determine the distribution of viruses belonging to common zoonotic viruses in rodents

Viral Metagenomic Analysis Workflow



Part 3- Distribution of common zoonotic viruses

Virome Identification

To determine the distribution of viruses belonging to common zoonotic viruses in rodents

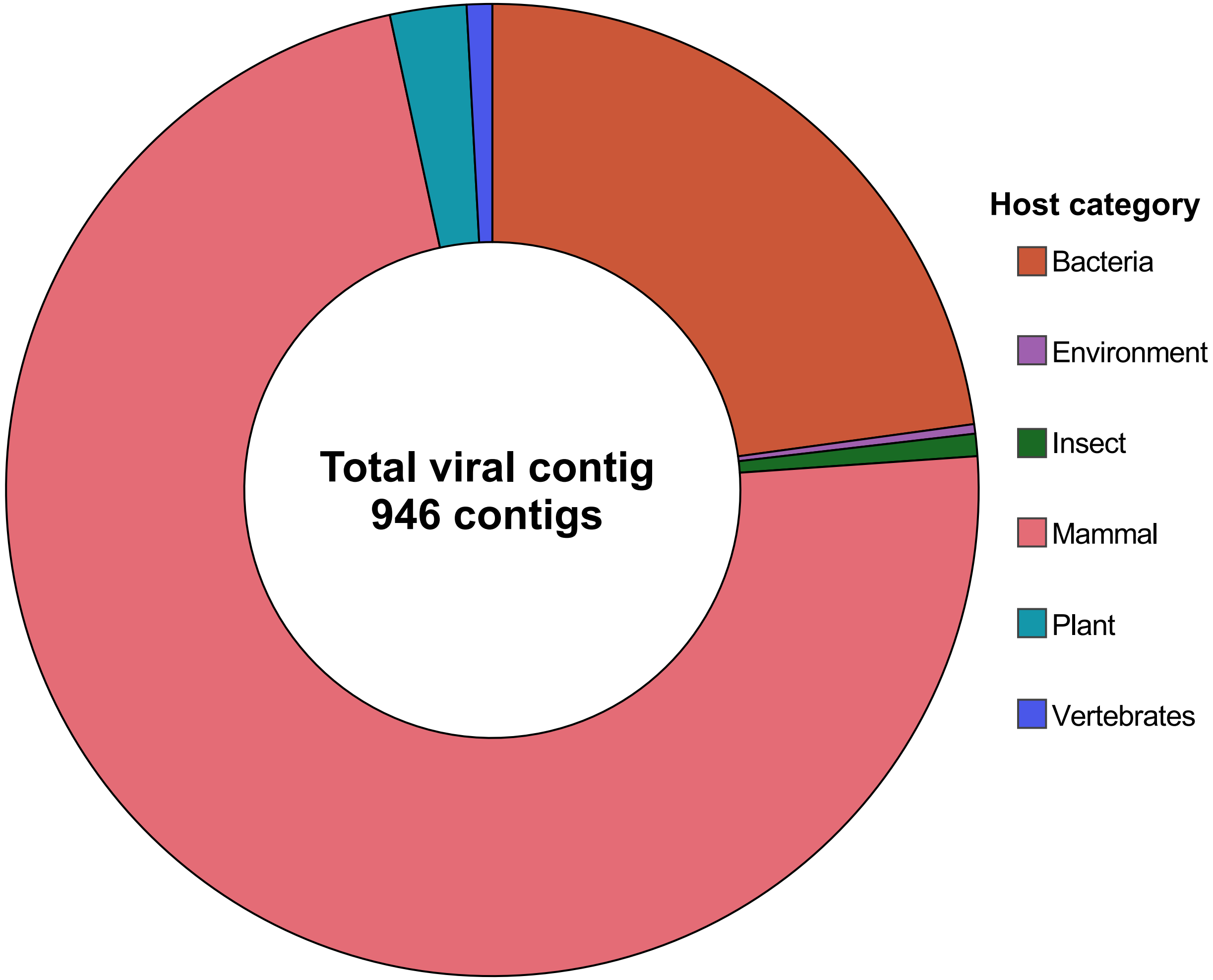


Figure 2 Proportional distribution of viral contig associated with host range

72.73% Mammalian viruses

22.83% Bacteriophages

2.54% Plant viruses

0.85% Vertebrates viruses

0.74% Insect viruses

0.32% Environmental viruses

Part 3– Distribution of common zoonotic viruses

Virome Identification

To determine the distribution of viruses belonging to common zoonotic viruses in rodents

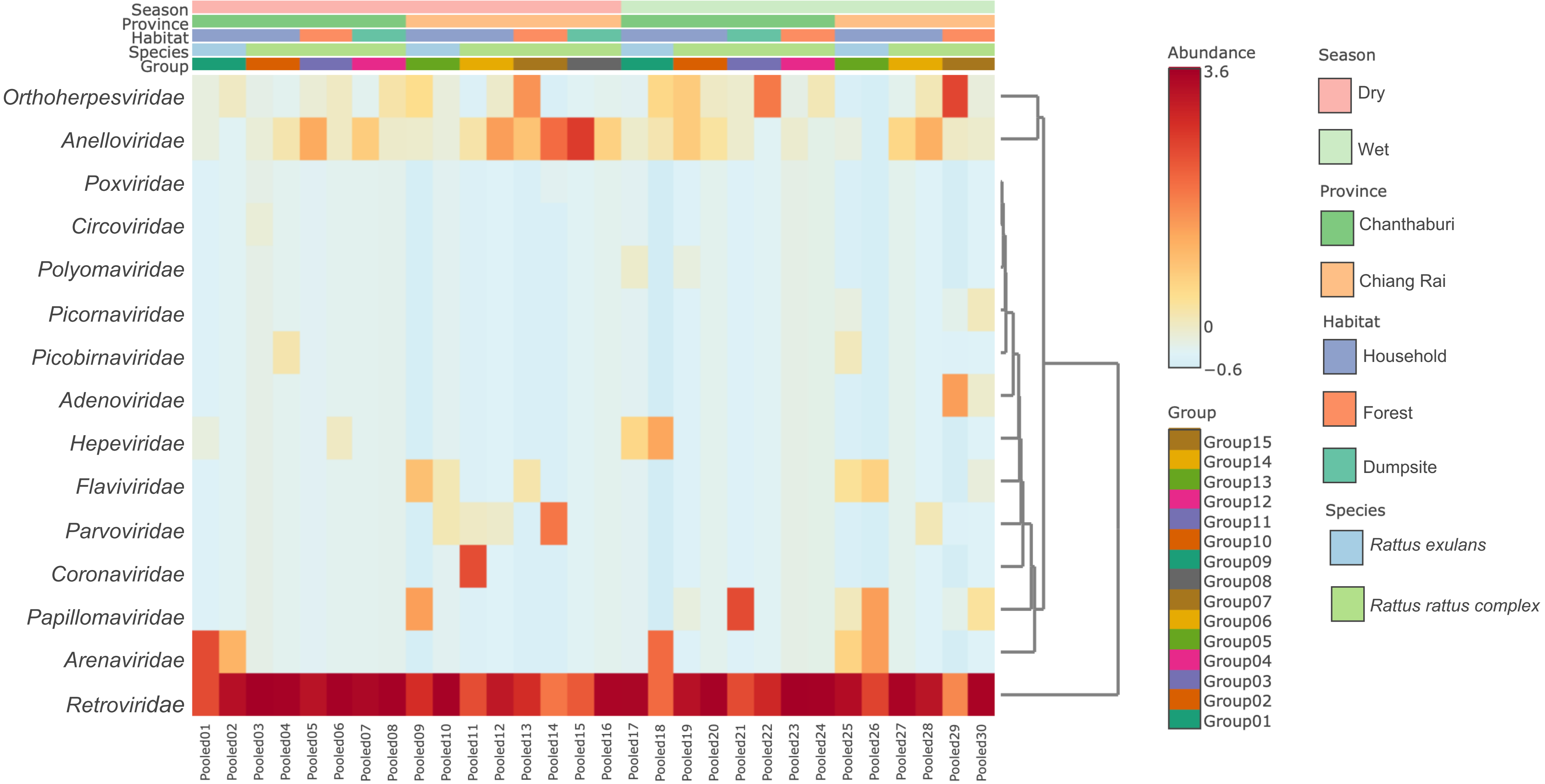


Figure 3 Relative abundance of the viral families in rodents in each pool.

Anelloviridae and *Orthoherpesviridae* showing a broad distribution pattern with varying levels of abundance.

Part 3– Distribution of common zoonotic viruses

Alpha diversity

To evaluate viral community composition between rodent species and habitats

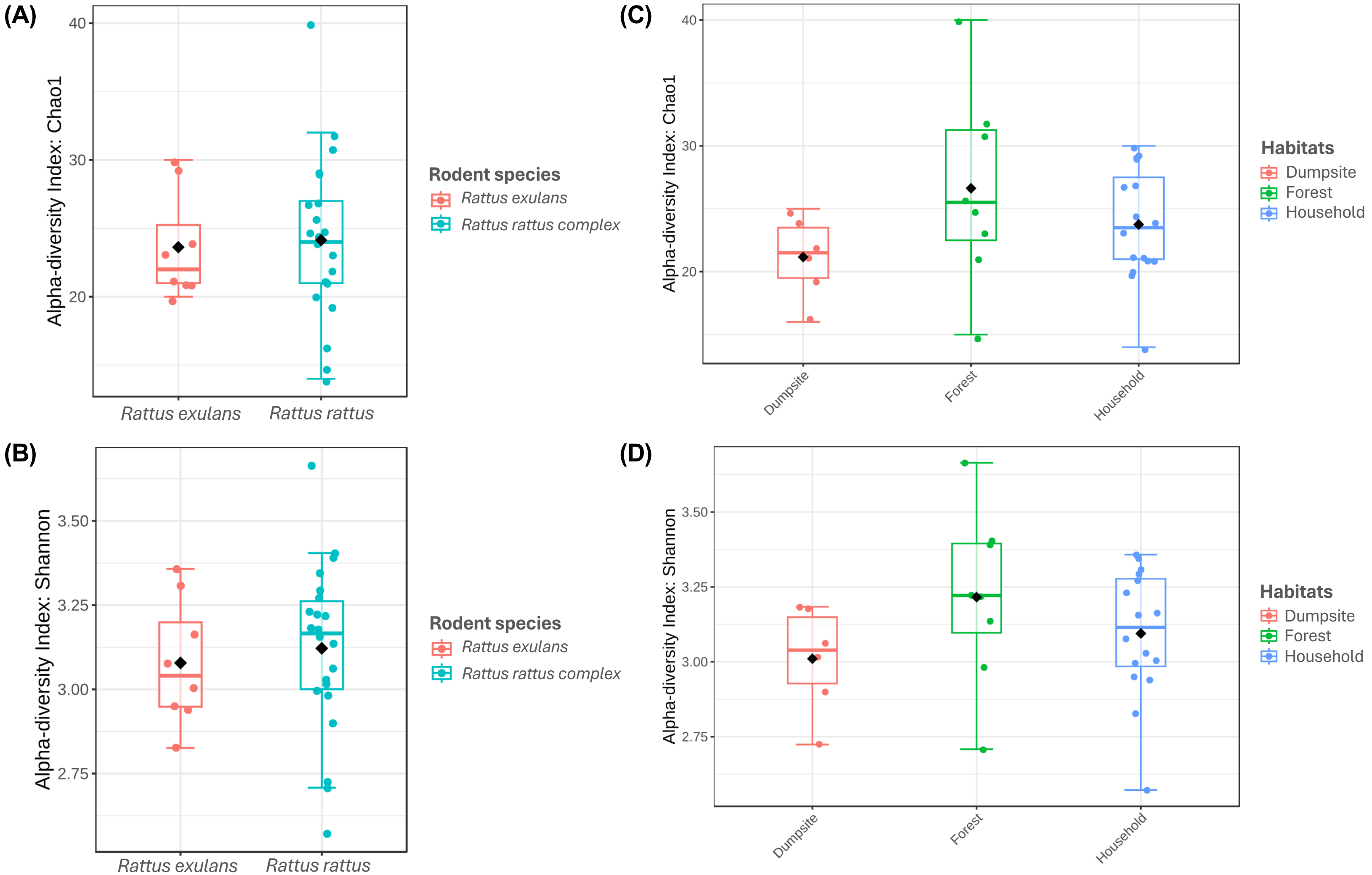


Figure 4 Alpha diversity of viral communities of Rodent species ((A) Chao1 index, (B) Shannon index) and Habitats ((C)Chao1 index, (D) Shannon index). Statistic significant differences in the diversity indices (Kruskal-Wallis, p-value ≤ 0.05).

Diversity of viral species were not significant differences among species or habitats ($p > 0.05$).

Part 3– Distribution of common zoonotic viruses

Principal coordinate analysis To evaluate viral community composition between rodent species and habitats

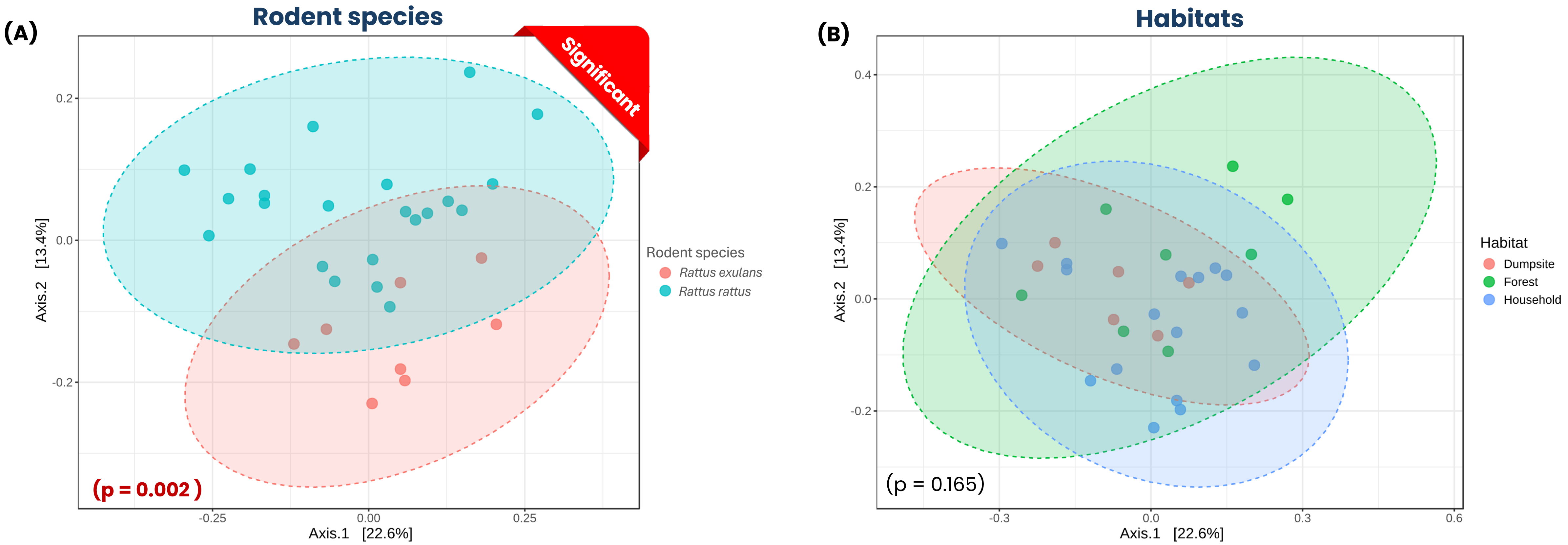


Figure 5 Principal coordinate analysis (PCoA) of viral family composition based on the Bray–Curtis dissimilarity, p-value ≤ 0.05, visualized by (A) Rodent species and (B) Habitats.

The composition of the viral community showed **significant** variation at the species level **among 2 rodent species**, but not significant among 3 habitats.

Part 3- Distribution of common zoonotic viruses

Protein annotation To determine the cross-species transmission potential of a candidate virus in rodents

Protein annotation

Annotation viral genomes

↓

Multiple sequence alignment

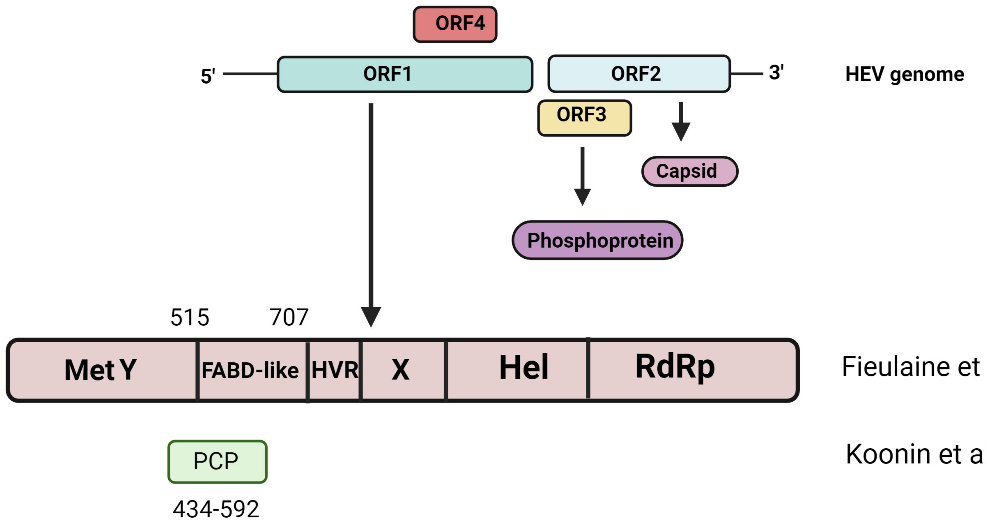
↓

- Bootstrap 1,000 replicates
- ModelFinder

Phylogenetic analyses by IQ-TREE

↓

Visualization using iTOL



Fieulaine et al., 2023

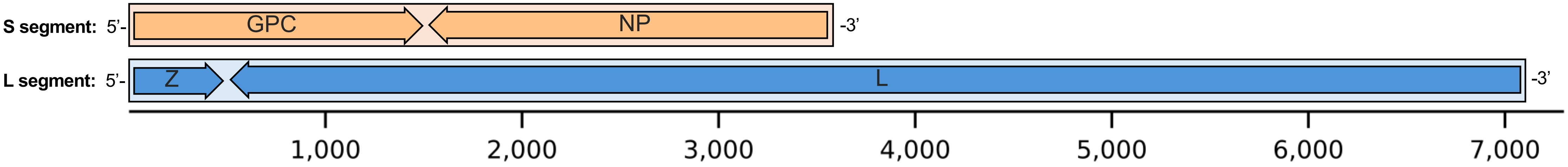
Koonin et al., 1992

- *Adenoviridae*
- *Arenaviridae*
- *Coronaviridae*
- *Flaviviridae*
- *Hepeviridae*
- *Orthroherpesviridae*
- *Papillomaviridae*
- *Parvoviridae*
- *Picorbirnaviridae*

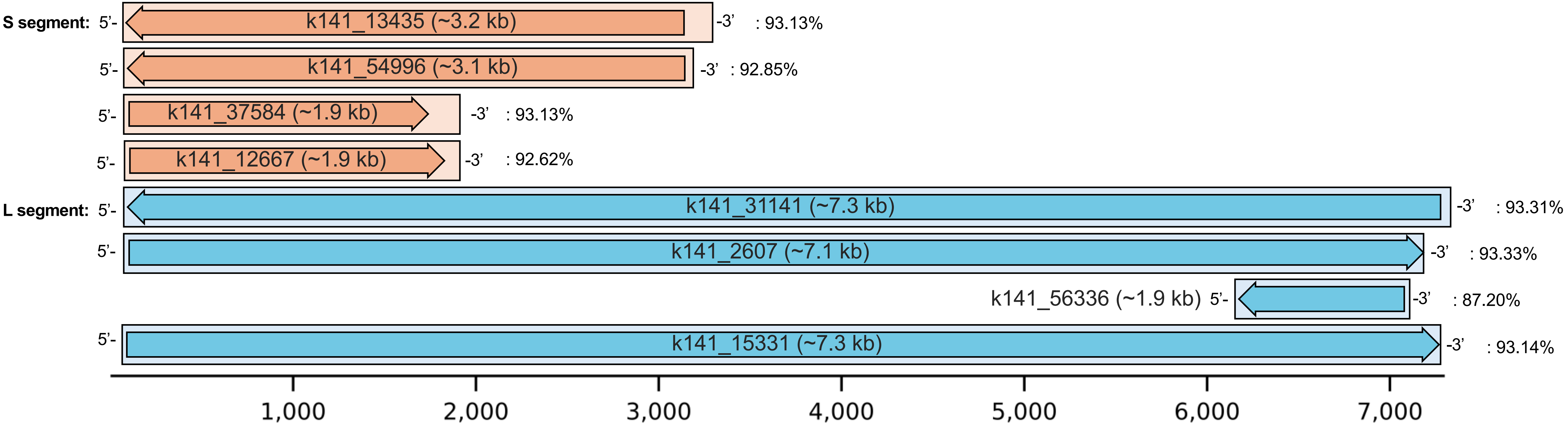
Part 3- Phylogenetic tree analysis

Arenaviridae To determine the cross-species transmission potential of a candidate virus in rodents

NC_038365.1 Loie River virus (~7.2 kb)



Contig in this study

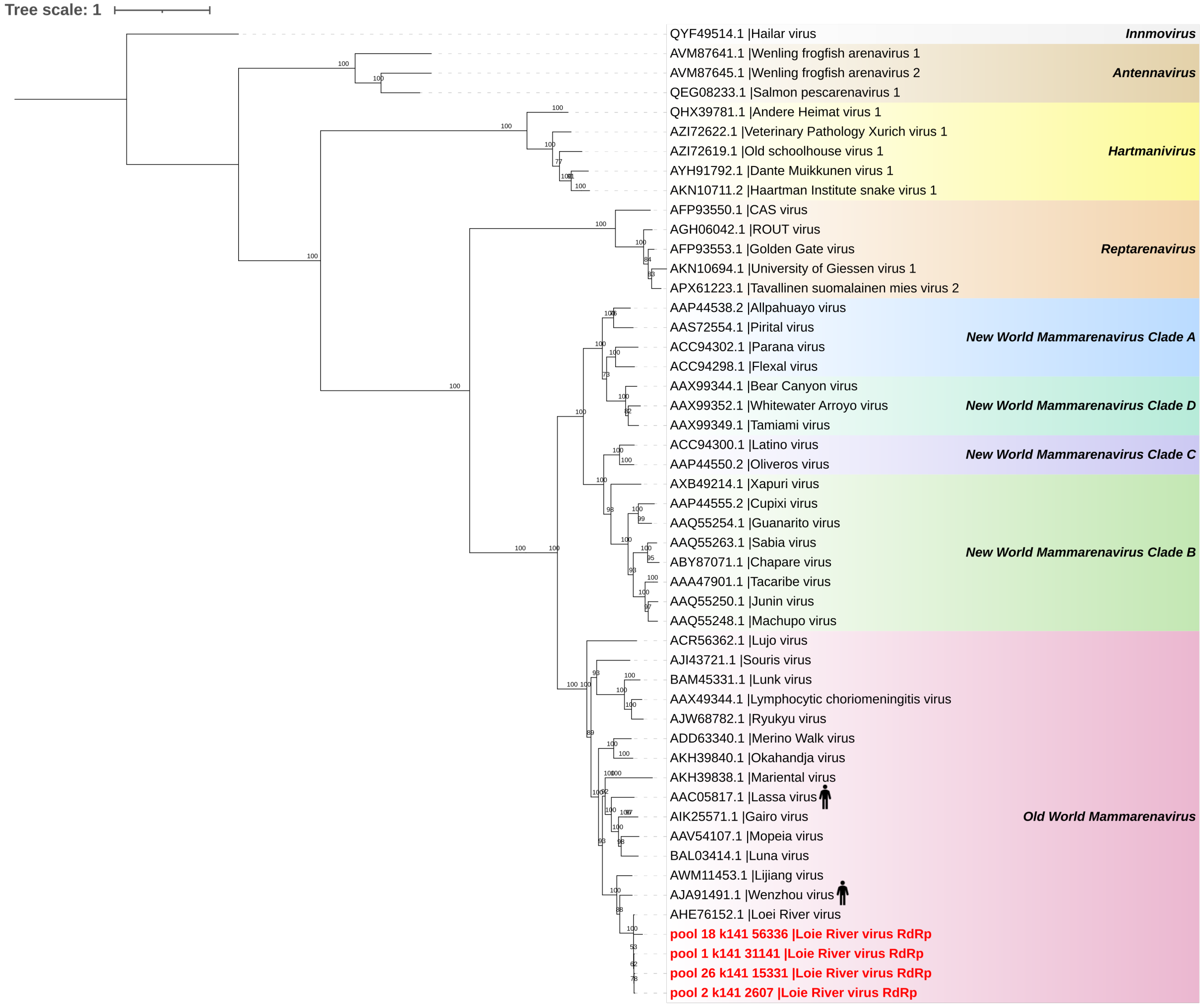


Part 3- Phylogenetic tree analysis

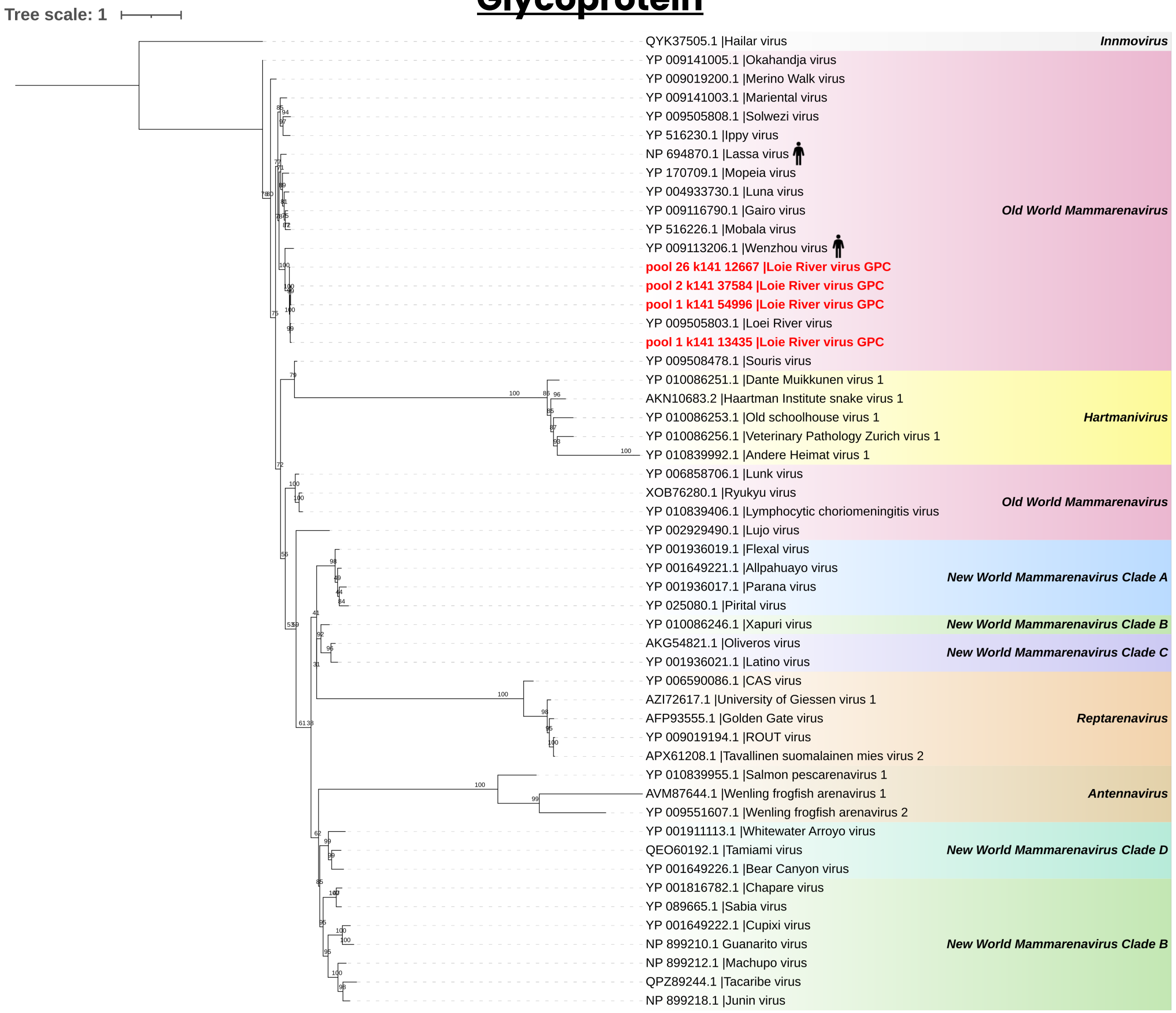
Arenaviridae

To determine the cross-species transmission potential of a candidate virus in rodents

L protein



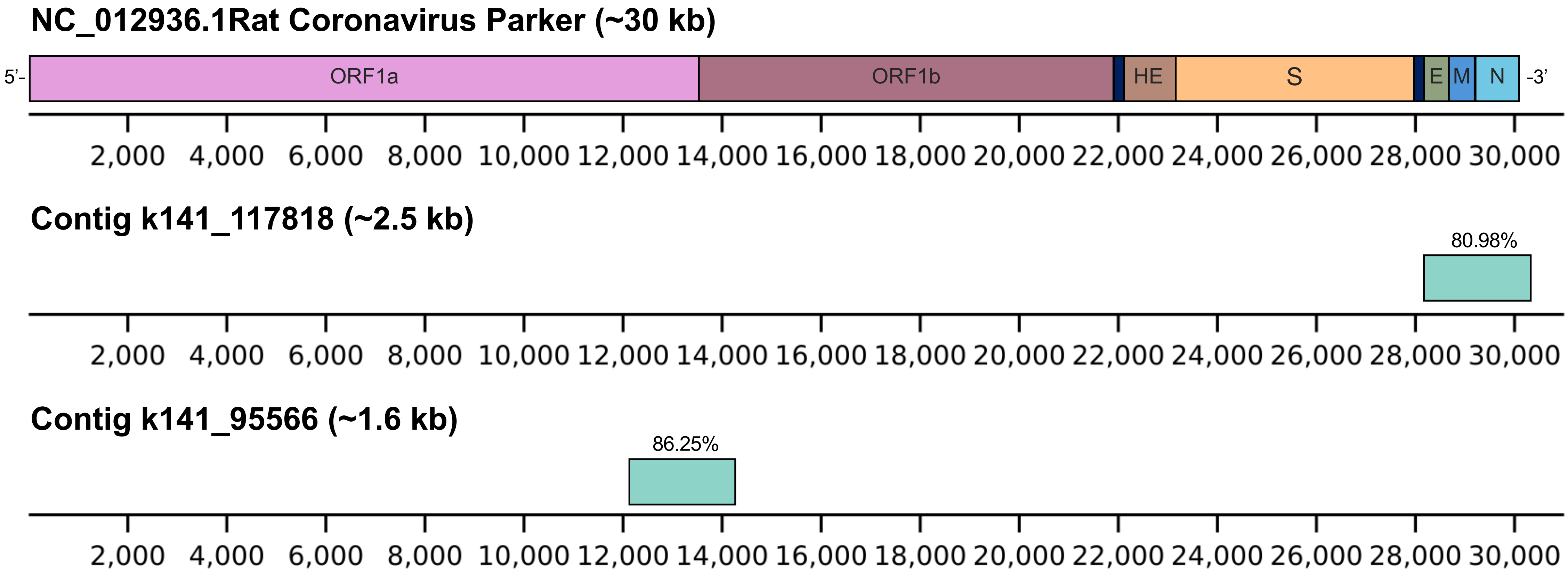
Glycoprotein



Sequences clustered tightly with Loie River virus and Wenzhou virus within the Old World Mammarenavirus lineage.

Part 3– Phylogenetic tree analysis

Coronaviridae To determine the cross-species transmission potential of a candidate virus in rodents

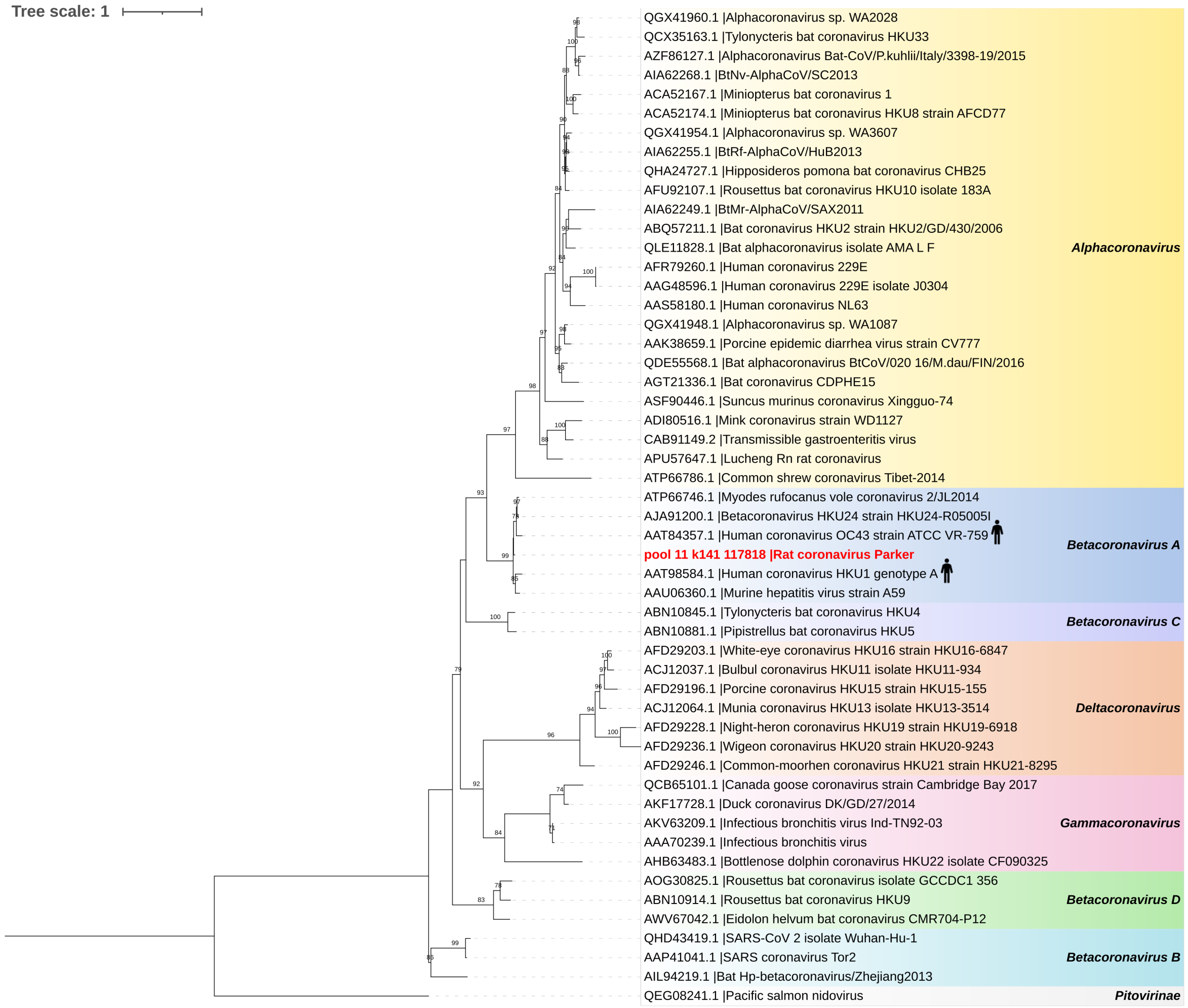


Part 3- Phylogenetic tree analysis

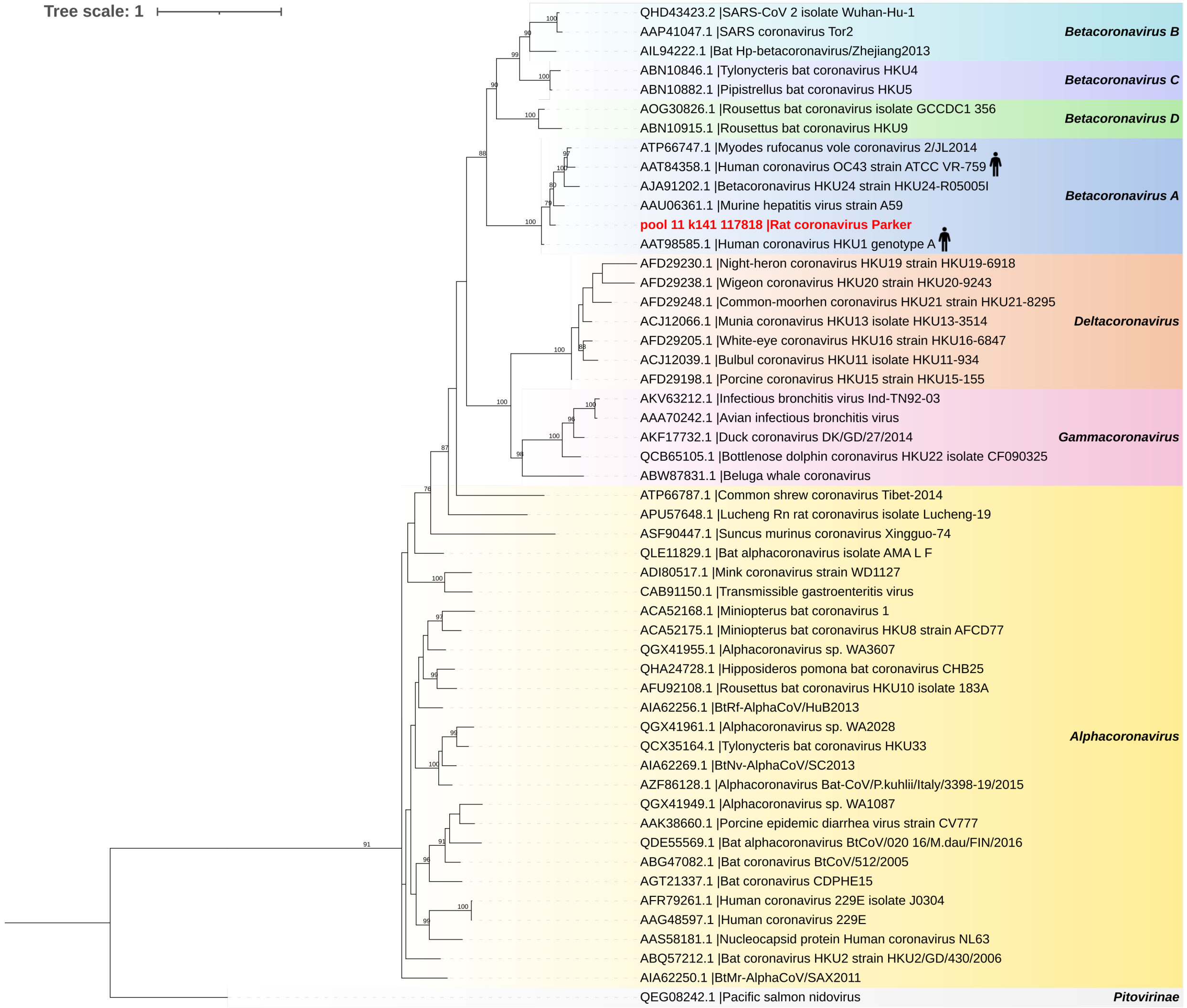
Coronaviridae

To determine the cross-species transmission potential of a candidate virus in rodents

Membrane protein



Nucleoprotein

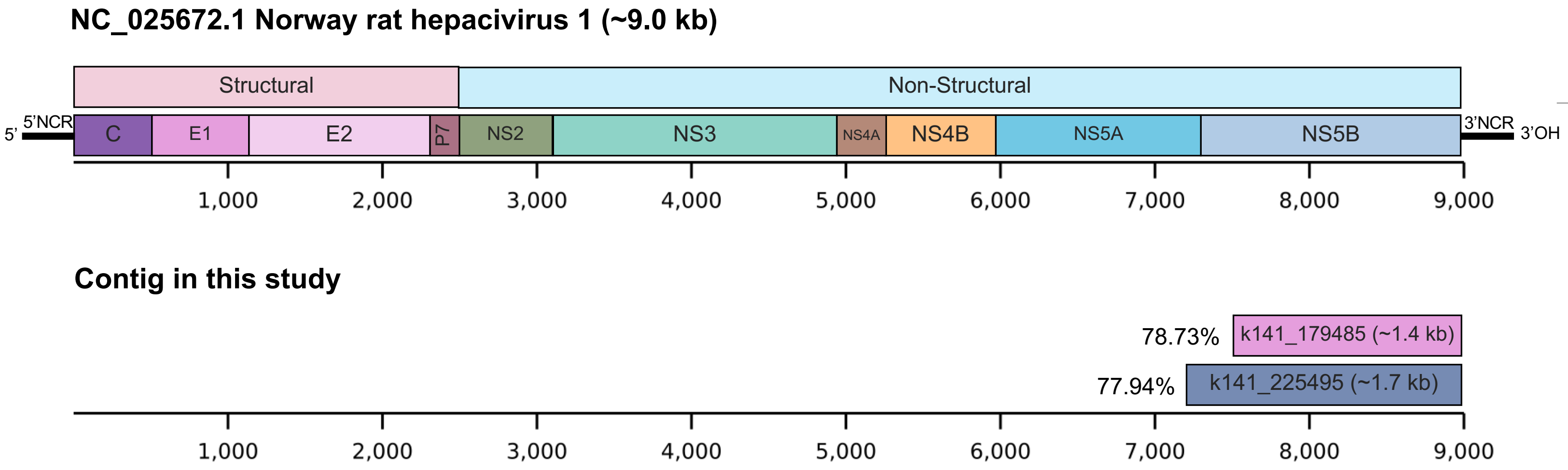


Rat coronavirus Parker in our study placed them within the *Betacoronavirus A* clade, that have a human coronavirus.

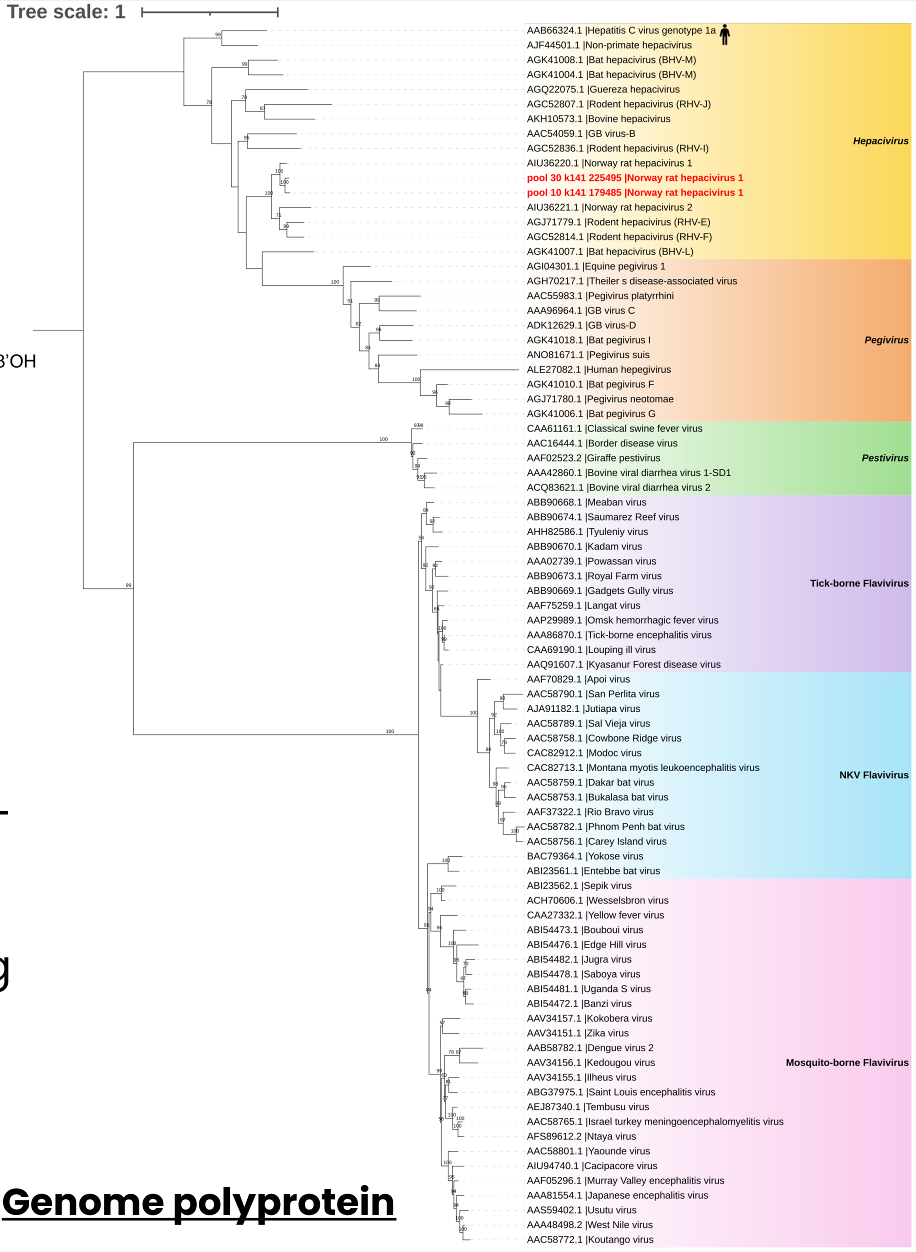
Part 3- Phylogenetic tree analysis

Flaviviridae

To determine the cross-species transmission potential of a candidate virus in rodents



- ➢ *Hepacivirus* in our study clustered within a clade of rodent-associated hepaciviruses,
- ➢ Evolutionarily related to Hepatitis C virus (HCV), which causing chronic liver disease in humans.



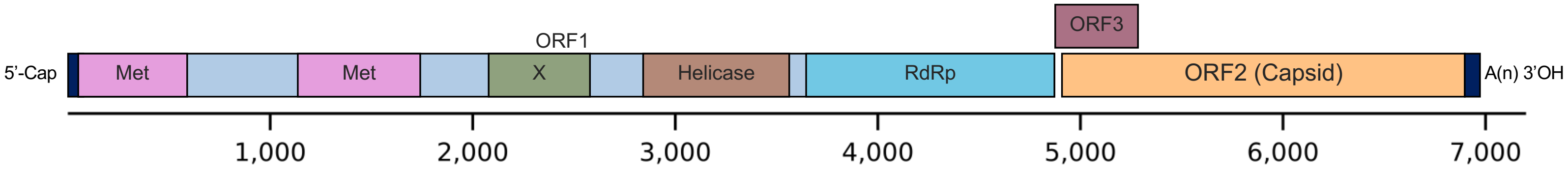
Genome polyprotein

Part 3- Phylogenetic tree analysis

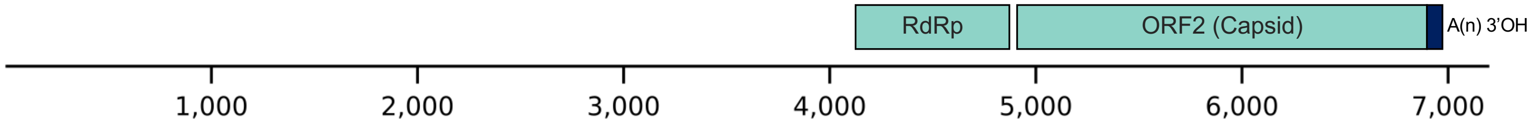
Hepeviridae

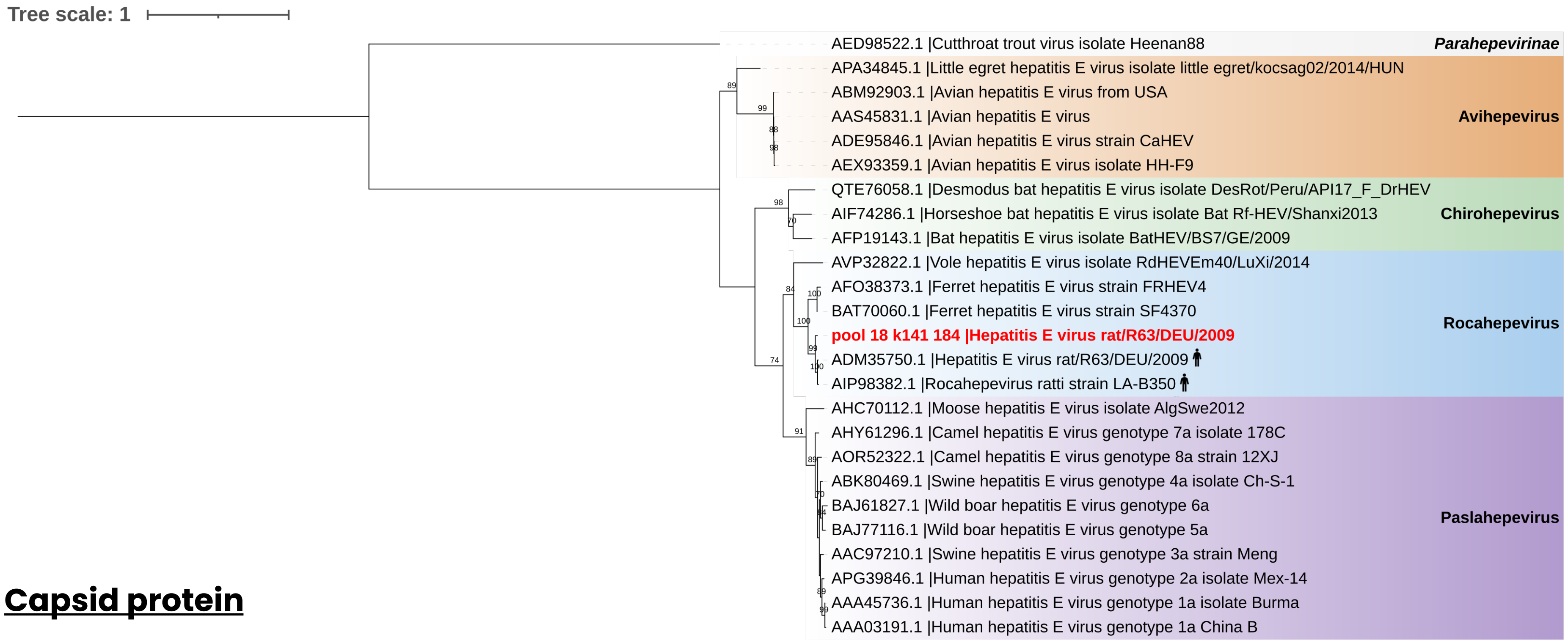
To determine the cross-species transmission potential of a candidate virus in rodents

NC_038504.1 Rat hepatitis E viruses (~6.9 kb)



Contig k141_184 (~1.7 kb) : 79.84%



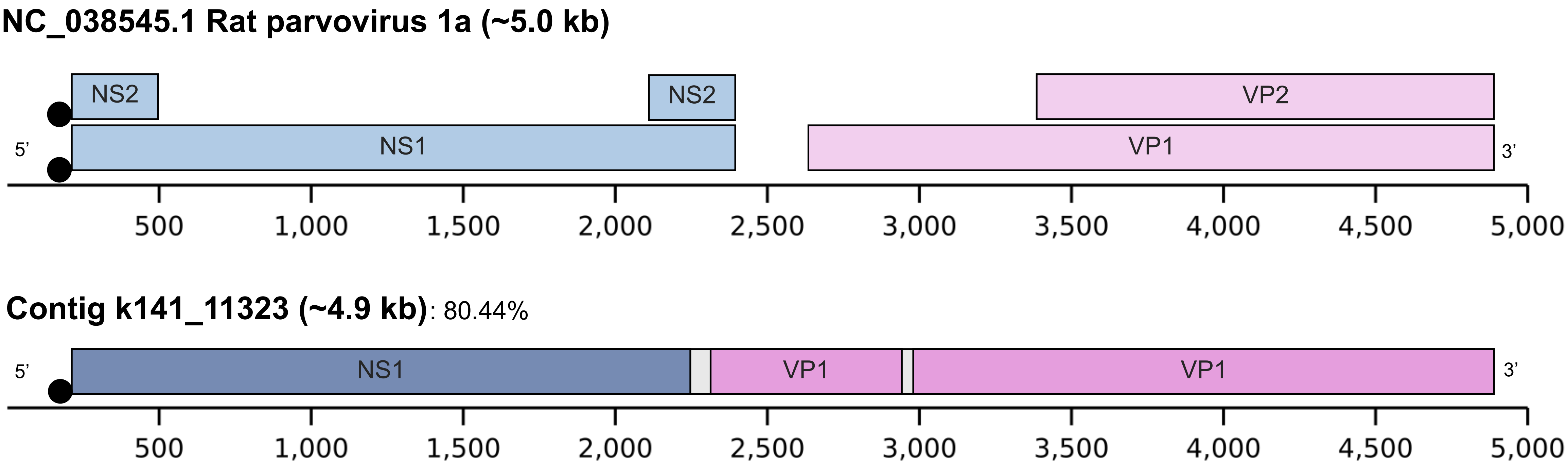


Capsid protein

Part 3– Phylogenetic tree analysis

Parvoviridae

To determine the cross-species transmission potential of a candidate virus in rodents



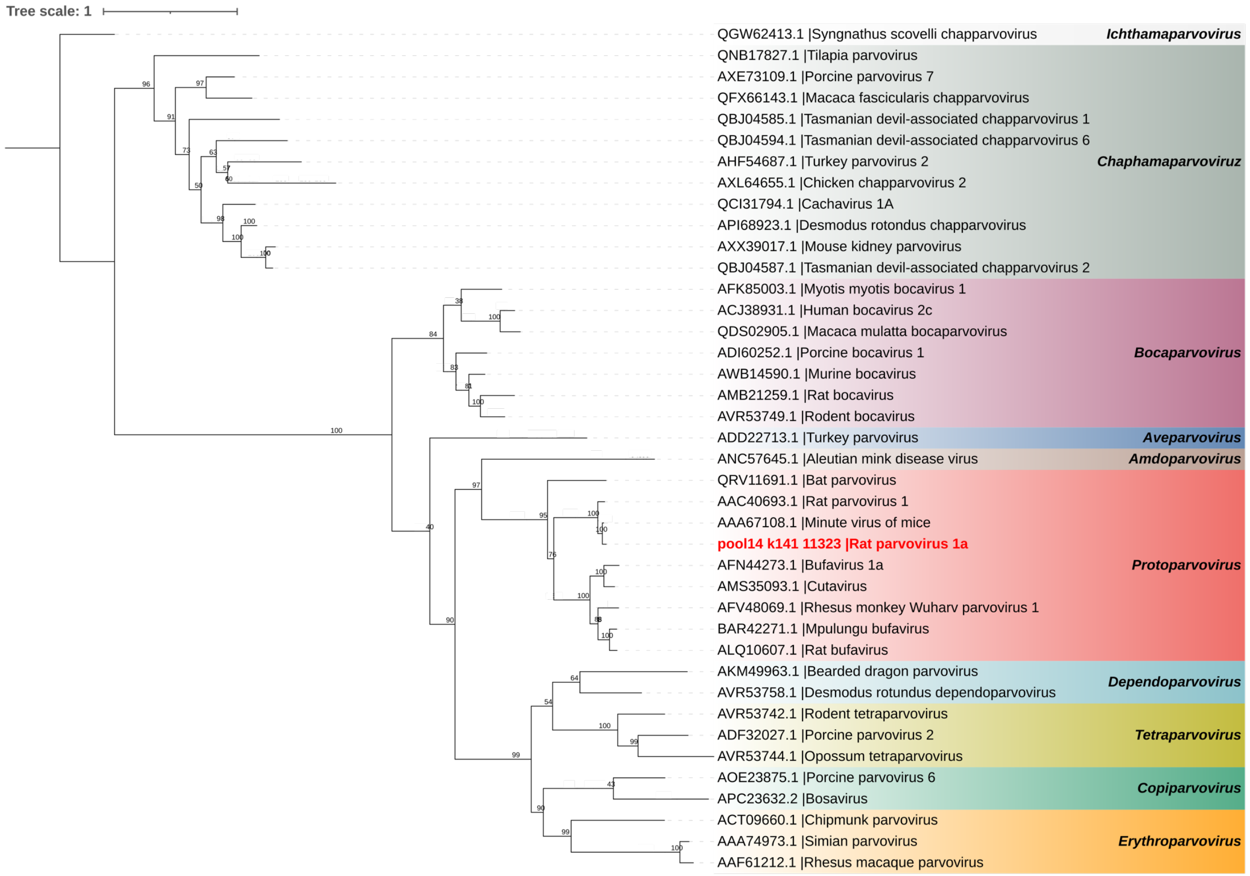
NC_038545.1 Rat parvovirus 1a (~5.0 kb)

Contig k141_11323 (~4.9 kb): 80.44%

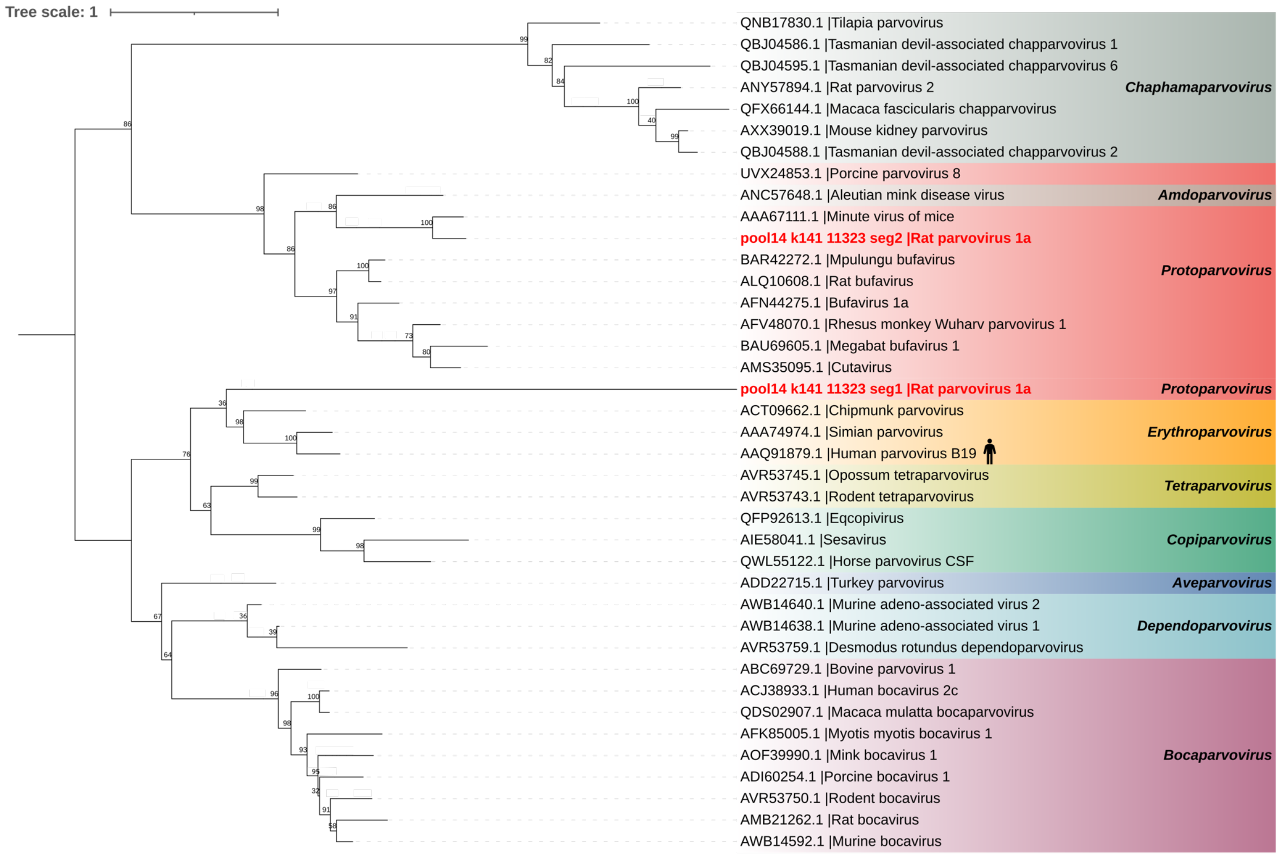
Part 3- Phylogenetic tree analysis

Parvoviridae To determine the cross-species transmission potential of a candidate virus in rodents

NS1 protein



VP1 protein



Genetic recombination events may occur within the Rat parvovirus 1a.

Conclusion

Virome identification

- A total of viral contigs found that mammalian viruses comprising the largest category, followed by bacteriophages.
- We identified mammalian-associated viruses across 15 families in rodents.

Diversity of viral community

- Viral community shows no significant differences in alpha diversity across host species and habitats.
- Beta diversity analysis revealed a significant difference in viral composition between rodent species.

Evolutionary of viruses

- Loei River virus, Rat coronavirus Parker, Rat Hepatitis E virus share evolutionary roots with Wenzhou virus, Human coronavirus HKU1, *Rocahepevirus ratti* respectively, highlighting the presence of viral lineages capable of cross-species transmission.
- Phylogenetic analysis of Rat parvovirus 1a using different protein found that they are in different clade, which may suggest recombination events.

Thesis plan

Activities	Timelines																	
	2023		2024				2025				2026				2027			
	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
1. Coursework	🟩	🟩	🟩	🟩														
2. Literature review and planning		🟩	🟩	🟩	🟩	🟩												
3. Qualify examination				🟩														
4. Proposal examination							🟩											
Part 1 Sample collection and preparation for sequencing																		
5. Sample collection			🟩		🟩													
6. Nucleic acid extraction			🟩	🟩	🟩	🟩												
Part 2 Diversity of bacterial pathogen in rodents of each habitat																		
7. 16S rRNA sequencing						🟩	🟩	🟩	🟩	🟩								
8. Bacterial identification							🟩	🟩	🟩	🟩								
9. Diversity of bacterial pathogens								🟩	🟩	🟩								
10. qPCR of interesting pathogens									🟧	🟧								
11. Prevalence of interesting pathogen										🟧	🟧							
Part 3 Virome profile in rodents of each habitat, and phylogenetic reconstruction																		
12. Pan PCR amplification				🟩	🟩	🟩	🟩	🟩										
13. Hybrid-capture based viral enrichment						🟩	🟩	🟩										
14. Shotgun NGS							🟩	🟩	🟩									
15. Virome identification								🟩	🟩	🟩								
16. Comparison of virome profile in rodents of 2 provinces										🟩	🟩							
17. Phylogenetic reconstruction										🟩	🟩							
18. Host and virus correlation network analysis										🟧	🟧							
19. Prevalence of interesting viruses using qPCR											🟧	🟧						
20. Determination of spillover risk of candidate viruses											🟧	🟧						
21. Selection of candidate viruses											▨	▨						
Part 4 Candidate virus with cross-species transmission potential																		
22. Retrieval of receptor binding domain sequence of candidate virus												▨	▨	▨				
23. In sillico receptor binding prediction model in each animal and human															▨	▨		
24. Selection of potential cross-species transmitted virus based on the best docking score															▨	▨		
25. Thesis preparation and defense																▨	▨	▨

✓ Finished

- Viral species identification
- Comparison of viromes profiles in rodents of 2 provinces
- Phylogenetic tree analysis

Further works

- qPCR of interesting bacterial pathogens
- Prevalence of interesting pathogen
- Prevalence of interesting virus

🟩 Finished tasks
🟧 Ongoing tasks
▨ Further tasks

10 credits

Acknowledgements

Advisor

Asst.Prof.Dr.Sirinart Aromseree

Co-advisor

Prof. Dr. Chamsai Pientong

Co-advisor

Dr. Suwalak Chitcharoen

HEC Research group

Medical Microbiology

MDKKU
คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
FACULTY OF MEDICINE KHON KAEN UNIVERSITY

panda
Pandemic risk prevention - Asia

PANDASIA
PREVENTING FUTURE PANDEMICS

HEC
Research Group

CESM
Chulalongkorn University

THANK YOU

FOR YOUR KIND ATTENTION

Q&A I SUGGESTION