Identifying novel smacoviruses in fecal samples of the Salt River Horses

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CISA
Student
Showcase


Photo: Hannah McGraw

Introduction and Objectives:

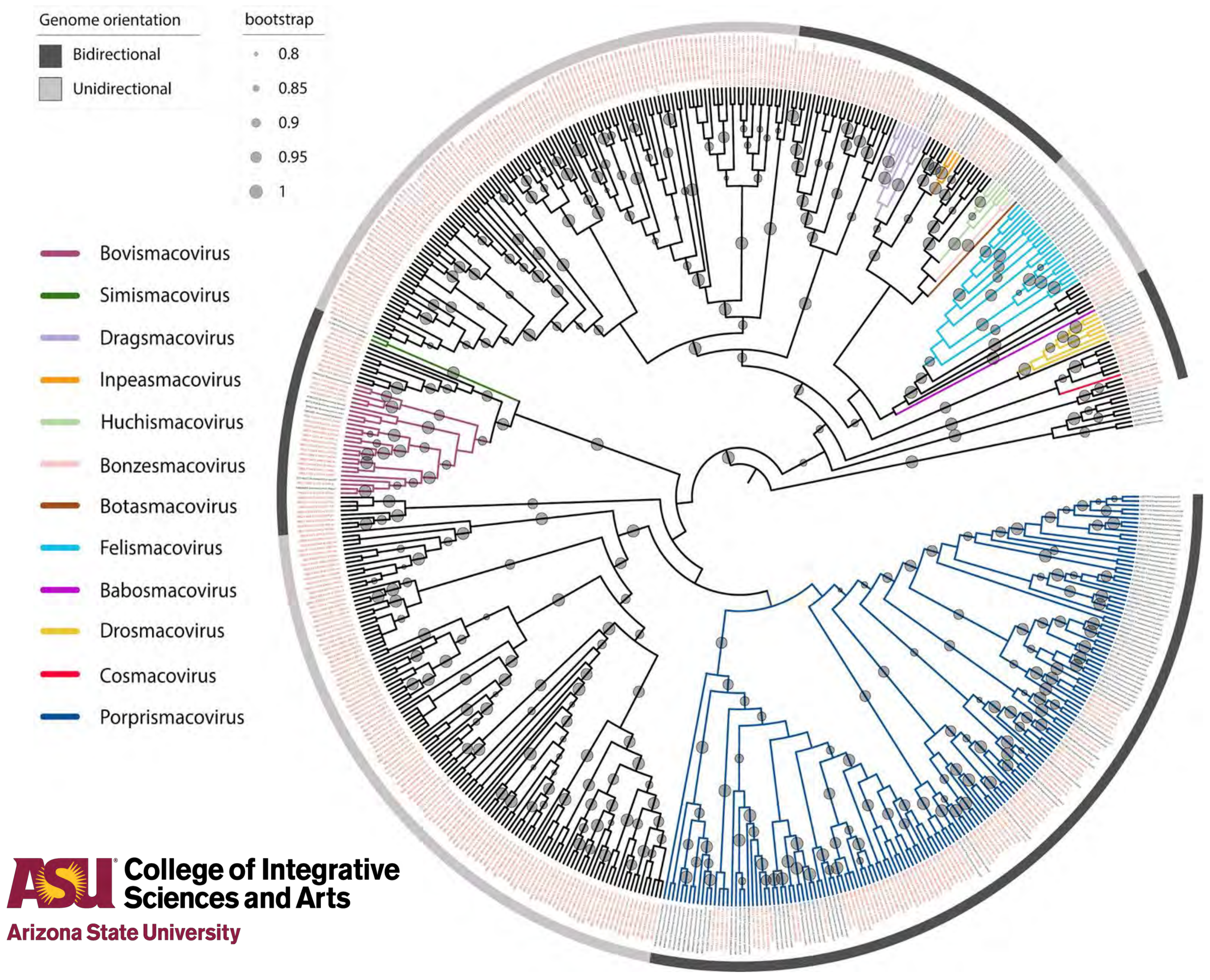
- The goal of this study was to identify DNA viruses within the fecal samples of the Salt River horses.
- The Salt River wild horses have generated much public interest in Arizona. These wild horses have been around since the early 1800s but have been considered an invasive species, with concerns that they are damaging the salt river ecosystems. While the Wild Horse and Burro act of 1971 states that wild horses are protected on public land, there was controversy as to whether these horses were actually wild or just stray livestock. The Salt River Wild Horse Management Group fought for legal protection of these horses to be recognized by Arizona, and in 2016 a law was passed putting them under protection. While the Management Group monitors the health of these horses, there are minimal investigations on the viruses associated.
- Twenty-five DNA and RNA virus families have been found in horses previously including one genome from *Smacoviridae*, which is the focus of this study. Smacoviruses encode a replication-associated protein (Rep) and a unique capsid protein (Cp) that does not share similarities with other families in Cressdnaviricota and are small in nature with only a 2.3kb - 2.9kb genome. They have been identified in the feces of a large number of vertebrate species, including humans and horses. No definitive host for smacoviruses has been determined, however one study using CRISPR analyses showed gut-associated archaea as a putative host.
- Smacoviridae is divided into twelve genera including Babosmacovirus, Bonzesmacovirus, Bostasmacovirus, Bovismacovirus, Cosmacovirus, Dragsmacovirus, Drosmacovirus, Felismacovirus, Huchismacovirus, Inpeasmacovirus, Porprismacovirus, and Simismacovirus. These genera required a ≥ 40% Rep protein sequence identity to be classified in the same genera. Genomes require a 77% pairwise identity to be grouped into the Smacovirus species as a whole.

Methods

- Six samples were collected on the 11th of September 2022 next to Coon Bluff road and seven samples were collected on the 19th of May 2023 next to Goldfield road, Mesa, AZ. Samples were confirmed to be from different horses by collecting only fresh samples. The samples were all collected into 50 ml conical centrifuge tubes and transported on ice packs to Arizona State University.
- For viral identification, fecal processing within the ASU lab included using the Roche high pure viral nucleic acid protocol and the templiphi processing protocol. The result was sent to Macrogen Inc. to finalize the genome sequences. Computer analysis included using SDTv1.2 to find pairwise identities of full genomes and creating a maximum likelihood phylogenetic tree from the Rep amino acid sequence from known species and the novel species using PhyML 4.0.

Results

- We identified 422 full genomes of smacoviruses across the 13 fecal samples using a viral metagenomic approach. The 422 smacovirus genomes recovered in this study all encode a Rep and Cp. Of these, 171 have bidirectionally transcribed genomes and 251 have unidirectionally transcribed genomes.
- The bidirectional genomes shared 53-74.5% pairwise identity with other known full genomes of smacoviruses. The unidirectional genomes share 53-76.8% pairwise identity with other known full genomes of smacoviruses. The novel genomes that share greater than 77% pairwise identity were grouped into species. The 171 bidirectional genomes are represented by 85 species and the 251 unidirectional genomes are represented by 59 species.
- The figure below shows the relationship between the novel smacovirus genomes and identified smacovirus genomes. The red taxa are the novel genomes and the black taxa are the identified genomes. The colored branches correspond to the genera the smacoviruses fall within.



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Discussion and Conclusion

- These smacovirus genomes were the first identified in Arizona and the first identified in the Salt River wild horses. There was previously only one genome of smacovirus identified in horses.
- The genomes classified in the previous genera of *Smacoviridae* include 102 genomes across Propirsmacovirus, Bovismacovirus, and Dragsmacovirus.
- There were 73 novel bidirectional genomes that created 8 new major clades and 247 novel unidirectional genomes created 3 new major clades, which adds to the only one previous genera of smacoviruses that had unidirectional genomes.
- Further research is needed to determine the significance of the variety of unidirectional genomes of smacoviruses in horses and whether this diversity is unique to these horses and their environment. Further research is also needed to determine the true host of Smacoviridae, but it seems likely the host is a gut-associated archaea as horses have a diverse population of archaea in the microbiota of their intestinal tract.
- **These results expand the smacovirus viral diversity and viruses associated with wild horses.**


Photo: Hannah McGraw

Literature Cited & Acknowledgements

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