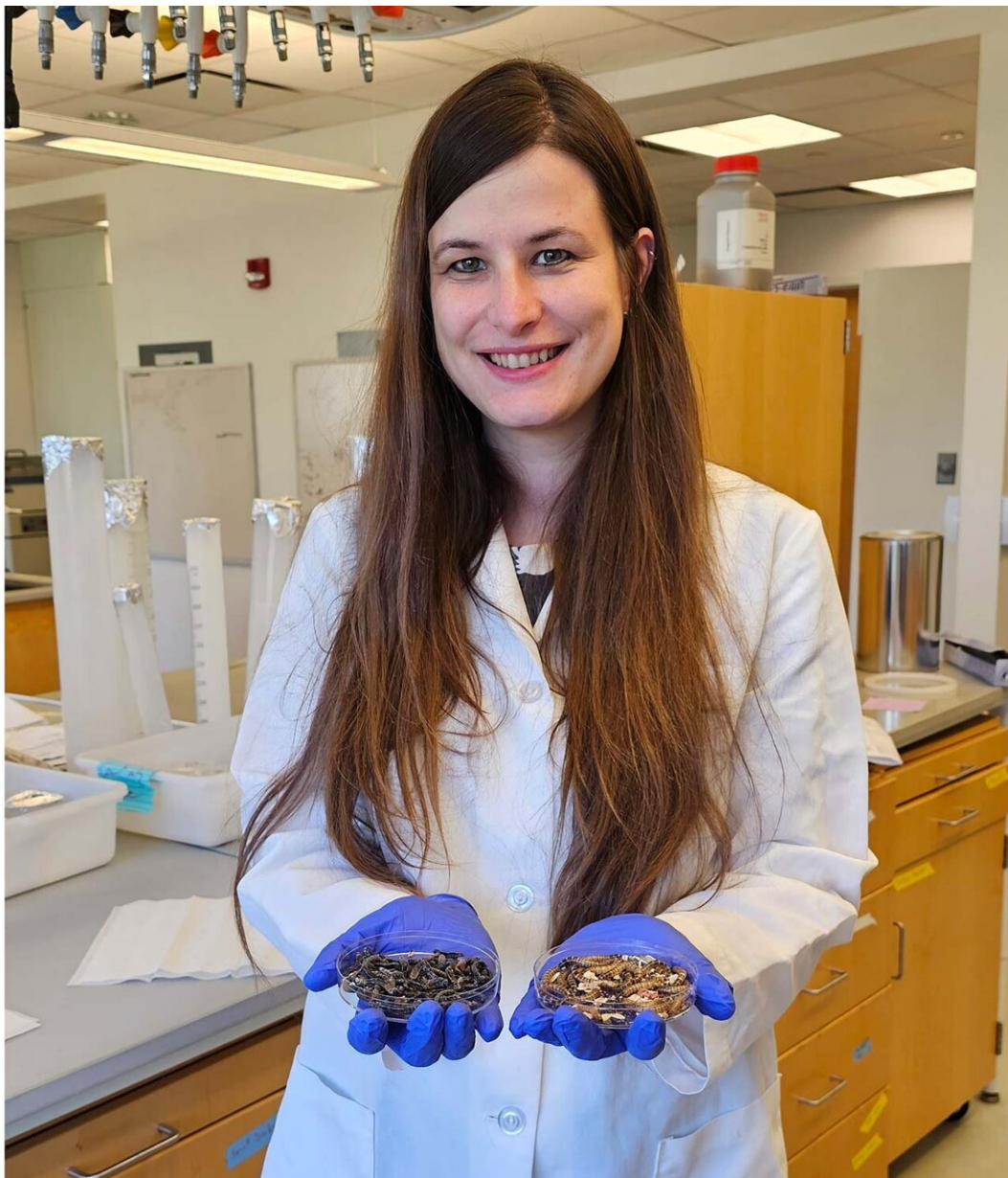


How beetle juice led to the discovery of a virus and solved the mystery of a superworm die-off

August 28 2024, by Kitta MacPherson



Insect virologist and virus co-discoverer Judit Penzes displays infected, dead superworms (at left) and healthy worms. Credit: J. Penzes

Rutgers University-New Brunswick scientists have discovered a virus that caused a nationwide die-off of superworms, a common food for birds, reptiles, other pets and, more and more so, even for humans as an alternative protein source. In doing so, they pioneered a different way to search for and identify emerging viruses and pathogens in humans, plants and animals.

Using chopped up beetle carcasses forming a slurry and an electron microscope cooled by [liquid nitrogen](#), the scientists [report](#) in *Cell* that they have discovered what they have titled Zophobas morio black wasting [virus](#).

The name is derived from the virus's deadly effect on a species of darkling beetle, Zophobas morio, native to the subtropics, particularly in the insect's immature larval stage when it emerges from its eggs as large, brown superworms. This species was named "superworm" because its larvae are bigger, at about 2 inches in length, than any others grown as feed.

The protein-rich larvae of Z. morio, which are dietary staples for captive, often exotic reptiles, birds, fish and amphibians worldwide, mysteriously began dying off in 2019, puzzling pet food suppliers and pet owners.

Jason Kaelber, an author of the study and an associate research professor at the Institute for Quantitative Biomedicine (IQB) at Rutgers-New

Brunswick, worked with Judit Penzes, the first author of the study and a postdoctoral associate at IQB.

"Judit was looking to identify the reason beetle farmers were losing all their superworm colonies to a deadly disease and I was looking to develop ways of discovering new viruses that don't depend on DNA or RNA sequencing," Kaelber said. "We ended up discovering the virus that has been sweeping the country and killing superworms."

The [scientific investigation](#) began more than a year ago, when Penzes, a molecular virologist, was contacted by beetle farm owners whose superworms were mysteriously dying off at alarming rates. Penzes was already well known in the industry because of earlier work in which she isolated a virus that was killing crickets, another popular food for pets.

She started by collecting superworms at pet stores in New Jersey.

"Whenever I went to a pet store, I immediately went to the feeder insect section, opened the containers and looked at the worms," she said. "They were all infected. I told the owners of the stores what I was seeing that I was researching this virus, and asked if I could have the container. They were immediately on board. They told me to take as many as I needed."



Superworms killed by the virus now discovered and identified by Rutgers scientists. Credit: Judit Penzes

She returned to her lab, took a Magic Bullet blender, dropped the worm carcasses in and blended them at a high speed. The process created a slurry of beetle juice which she took and processed using a virus purification method that separated the virus out due to its density. In the final step, she shined a fluorescent light on the centrifuge tube and the virus glowed blue.

"I said, 'I got you,' when I saw it," Penzes said. "I knew then it was, indeed, a virus."

Next, Penzes worked with Kaelber, a fellow electron microscopist, to

examine the virus using a cryo-[electron microscope](#), which allows a three-dimensional view of the virus, including its interior.

"You're taking a virus, a protein, a cell, etc., and you're freezing it so quickly that the water solidifies without turning into ice crystals," Kaelber said. "We actually can figure out what the amino acid sequence of the protein is without analyzing the DNA, and just by looking at that 3D structure, because we have such sharp resolution."

They compared the structure of the protein with all known proteins using the database of the Protein Data Bank hosted at Rutgers and found that it is similar to a virus affecting cockroaches, but not identical, and part of a family of animal viruses known as parvoviruses.

"It's a new one, different from anything that's been sequenced or imaged before," Penzes said.

The scientists are also grateful to superworm farmers nationwide who sent samples voluntarily, once word of the study got out. "The eagerness of the farmers to help us out researching the virus had an enormous role in helping this published study to be born," Penzes said.



Healthy superworms have been in rare supply for several years because of a mysterious die-off of the popular pet food source. Rutgers scientists have pinpointed the cause of the illness and are developing a vaccine. Credit: Judit Penzes

The effort, Kaelber said, provided a "proof of concept" that cryo-electron microscopy can be employed to directly discover and characterize new pathogens.

"In the future, if there's ever a really important outbreak, we're going to want to throw every tool we can at it to see what we can find," Kaelber said. "We'd like to make diagnostic cryo-electron microscopy routinized, so that when there's some unknown infectious disease, we have a lot of options for same-day identification of the causative agent."

Cryo-electron microscopy has gained popularity in recent years, becoming a more prevalent method for 3D analysis of known specimens. However, the Rutgers work represents the first time the method was used on an unknown pathogen.

After discovering the virus, the researchers tested a way to protect the *Z. morio* beetles from disease, by injecting a closely related virus from another species that doesn't cause symptoms. They are developing a vaccine based on that work.

"The discovery is important for two reasons," Kaelber said. "First, beetle farmers can use this information to protect their colonies and understand which actions will be effective or ineffective at managing the epidemic. Second, the beetle epidemic was a real-world test of the technology that we hope can be useful to rapidly investigate future outbreaks in humans, plants or animals."

Scientists Martin Holm of the Rutgers Institute for Quantitative Biomedicine and Samantha Yost of REGENXBIO Inc., in Rockville, Md., also authored the study.

More information: Judit J. Penzes et al, Cryo-EM-based discovery of a pathogenic parvovirus causing epidemic mortality by black wasting disease in farmed beetles, *Cell* (2024). [DOI: 10.1016/j.cell.2024.07.053](https://doi.org/10.1016/j.cell.2024.07.053)

Provided by Rutgers University

Citation: How beetle juice led to the discovery of a virus and solved the mystery of a superworm die-off (2024, August 28) retrieved 28 August 2024 from <https://phys.org/news/2024-08-beetle-juice-discovery-virus-mystery.html>

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