Science for Saving Species

Research findings factsheet

Project 2.3.5



The discovery of two novel papillomaviruses in native and invasive Christmas Island geckos

In brief

Papillomaviruses are widespread in the animal kingdom. In this study, we discovered two new papillomaviruses in the highly invasive Asian house gecko (*Hemidactylus frenatus*) and mute gecko (*Gehyra mutilata*) on Christmas Island and Cocos (Keeling) Islands, and in the Critically Endangered Lister's geckos (*Lepidodactylus listeri*) in their captive breeding colony on Christmas Island.

These two viruses are very similar genetically, show unique genomic features, and are more closely related to a bird papillomavirus than other reptile papillomaviruses.

We examined seven lizard species for the presence or absence of the two viruses, and found it in three, including one Critically Endangered species, Lister's gecko, which now exists only in captive breeding populations on Christmas Island and in Taronga Zoo in Sydney. As the viruses may pose a threat to this species and potentially to other threatened species, research into their ecology and ability to cause disease in affected reptiles is a high priority.



Context

Papillomaviruses commonly infect their host's skin and mucosal membranes (mucous membranes include the surface of the mouth, the conjunctiva, the entire digestive tract and the reproductive tract). Papillomaviruses infect all animals, including humans, with amphibians the sole exception. The majority of papillomavirus infections either present no symptoms or present as benign wart-like lesions; however, a minority of them can cause cancer. A well-known example of a papillomavirus that causes cancer is the virus that causes cervical cancer in women. Unlike some other types of viruses, papillomaviruses are considered highly "host specific", which means they are rarely transmitted between different species, even very closely related ones.

The rate of discovery of new papillomaviruses is increasing, and more than 400 of these viruses have now been identified across all host species. The majority of these viruses have humans and other mammals as hosts. We have discovered two papillomavirus species in geckos inhabiting Christmas Island and the nearby Cocos (Keeling) Islands, and although such viruses are not unknown in reptiles, these two represent the first discovery of papillomaviruses in lizards.











What the research looked at

Our discovery that two new species of papillomaviruses are infecting lizards on Christmas Island and Cocos (Keeling) Islands has prompted investigation into the genetics and biology of the viruses. To add to the urgency and importance of the research, one of the affected lizards, although once common on Christmas Island, is now a threatened species.

To uncover as much as possible about these newly discovered viruses, we developed a number of diverse research aims. These included:

- Identifying the presence and significance of the viral genes, which is important for understanding their diseasecausing mechanisms
- 2. Describing the evolutionary relationship of these two new papillomaviruses to other viruses
- 3. Determining the distribution of these viruses in the tissues of affected lizards
- 4. Determining the proportion of lizards affected with papillomavirus DNA, which is vital for understanding how common the viruses are within the island populations.



What we did

We undertook a number of procedures to uncover answers to the aims of this research project. First, we set out to look at the genetic make-up of the two viruses. To do this for a virus or any other organism, it is necessary to determine the order of the bases (part of DNA that stores information and gives DNA its genetic code) that make up the DNA. This we achieved by sequencing the DNA using advanced genetic technology. Once we had sequenced the DNA, we confirmed the number, type and ordering of each gene for each virus.



The genome layout of the papillomavirus. Image: Jess Agius

After we resolved the genetic structure of each of the two new viruses, we were interested in understanding how they are related to papillomaviruses that had already been described, and their evolutionary similarities and differences. This kind of study is known as phylogenetics. We aligned the gene sequences of a diverse range of papillomaviruses that are distantly and closely related to those that we had discovered. When we had the final alignment, we entered it into a statistical model to create a tree showing common ancestors, viral relatedness, and how the viruses evolved through time.



Simplified phylogenetic tree showing relatedness of gecko papillomaviruses to other reptile and bird papillomaviruses. Image: Jess Agius

To understand the types of tissues the papillomaviruses were able to infect we collected a variety of organs from lizards on Christmas Island and Cocos (Keeling) Islands and extracted the DNA from the organ cells. We tested DNA from six species of gecko and one species of skink for the presence or absence of papillomavirus DNA:

- Lister's gecko (*Lepidodactylus listeri*)
- Asian house gecko (*Hemidactylus frenatus*)
- Mute gecko (Gehyra mutilata)
- Sri Lankan house gecko (*Hemidactylus parvimaculatus*)
- Flat-tailed gecko (*Hemidactylus platyurus*)
- Mourning gecko (*Lepidodactylus lugubris*)
- Blue-tailed skink (Cryptoblepharus egeriae)

Once we knew about the presence or absence of disease in various tissues from multiple animals, we could determine the proportion, or prevalence of affected lizards to non-affected lizards. We then compared these proportions between locations and species.

Key findings

Our genetic analysis of both the new papillomavirus genomes revealed that they are relatively similar to one another, sharing 89% of their genetic identity. The two viruses contain the same number and types of genes, and in identical positions in the genome; and they each contain the four core genes that are found across all papillomaviruses. In addition, they each have one accessory gene that is not found in all papillomaviruses and which has been associated with interference with immunity, successful establishment of infection to allow the virus to replicate, and the transformation of cells to a cancerous form. However, unlike other papillomaviruses, which all contain at least six genes, both of the two new papillomaviruses we identified contained only five. They therefore represent the smallest, simplest structure of any currently known papillomaviruses.

Our phylogenetic analysis of the viruses revealed that, as we expected, both are more closely related to bird and reptile papillomaviruses than to those that are hosted by mammals. Interestingly, though, we found that both these lizard viruses are more closely related to a papillomavirus that is hosted by a sea bird, the northern fulmar (*Fulmarus glacialis*), than to other reptile papillomaviruses. The evolutionary tree we generated also showed that both these lizard viruses are primitive, meaning that they evolved before any other known bird or reptile papillomavirus.

We found papillomavirus DNA across all the organ types tested (liver, skin, mouth and colon), and in three of the seven lizard species sampled (Asian house gecko, mute gecko and Lister's gecko). The viruses were more frequently detected in samples collected from the head and skin of the lizards, with positive samples from the colon and liver less common.

The new papillomavirus species were detected on both Christmas Island and Cocos (Keeling) Islands, with one species more common than the other for both locations. The Cocos (Keeling) Islands had a higher number of lizards affected with papillomaviruses (32%) than did Christmas Island (27%). Of the lizards we tested for papillomaviruses, the widely distributed Asian house gecko showed most frequent infection.



RIGHT: Asian house gecko where papillomavirus DNA was first discovered Image: Jess Agius

Implications and recommendations

The discovery of two novel papillomaviruses in three gecko species on two distinct islands presents multiple concerns for species conservation and management. In addition to infecting two of the most invasive and widespread geckos, the Asian house and mute geckos, these viruses have also been identified in the Critically Endangered Christmas Island Lister's gecko. The Lister's gecko is conservation-dependent, surviving only in captive breeding programs on Christmas Island and at Taronga Zoo, Sydney. Although the ultimate effects of the papillomaviruses on the health of affected animals is

uncertain as yet, these viruses have the potential to cause declines in Lister's geckos and to devastate the remaining populations.

Further work towards uncovering the ecology of these viruses and their ability to cause disease in affected animals is urgently required. The knowledge acquired from this work can then feed into conservation management actions for the Lister's gecko, and also the Christmas Island blue-tailed skink, which is also Critically Endangered and extinct in the wild, and a species that is in close contact with Lister's geckos. Additional surveillance of Lister's, mute and Asian house geckos can help to assess the patterns of papillomavirus disease, that is, whether it is declining, stable or increasing in the lizard populations. Although we have not detected papillomavirus infection in the Christmas Island blue-tailed skink, continued surveillance of these skinks, and the last free-ranging Christmas Island endemic giant gecko (Cyrtodactylus sadleiri), will be important to confirming the continuing freedom from disease, and assuring the continuing health of the population.

Jess in the lab microscopically examining lizard samples for papillomaviruses. Image: Laura Pulscher



Cited material

Agius, J. E., Phalen, D. N., Rose, K. & Eden, J.-S. 2019. New insights into Sauropsid *Papillomaviridae* evolution and epizootiology: Discovery of two novel papillomaviruses in native and invasive Island geckos. *Virus Evolution*, 5(2), doi: 10.1093/ve/vez051

Further Information

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