Screening for Frog Virus 3 in Frog Genera from Gabon, Africa

BIOTECHNOLOGY
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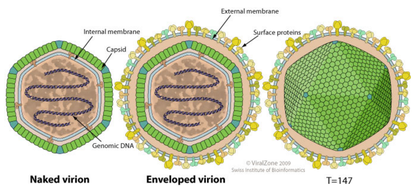
Abstract

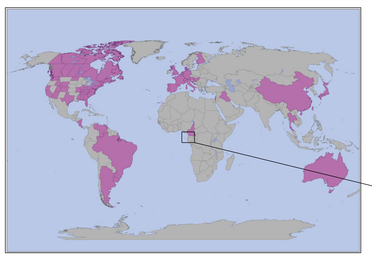
Ranaviruses are a genus within the family Iridoviridae. These are large double-stranded DNA viruses that infect fish, reptiles, and amphibians. Pathogenicity of infection poses a significant threat to amphibian and fish aquaculture industries, as well as the preservation of endangered species. There is a need to better understand these viruses and their distribution world-wide. We have collaborated with Dr. Bryan Stuart (North Carolina Museum of Natural Sciences) to acquire a large number of DNA samples representing a variety of frog genera native to Gabon, Africa. The samples were isolated from skin swabs and have previously been used to detect the fungal pathogen, *Batrachochytrium dendrobatidis*. Using a quantitative Polymerase Chain Reaction assay with primers specific to Frog Virus 3, we have tested for the presence of this virus. Frog Virus 3 is the prototypical member of the Ranavirus genus. It shares genetic similarity with other members in the genus and has been detected in all classes of species known to be infected by these viruses (fish, reptiles, and amphibians). We will compare our findings on the presence of Frog Virus 3 with previous data on occurrence of *Batrachochytrium dendrobatidis*. While Ranavirus has been previously detected in a frog species in Africa, this is the first study specifically screening for Ranavirus in frog genera in Gabon. Due to presence of Ranavirus in neighboring Cameroon, we hypothesize that Frog Virus 3 will be detected in at least one of the frog genera that we are screening.

Introduction



Ranaviruses are a member of the family, Iridoviridae, which are large double-stranded DNA viruses. Their genome is packaged in an internal membrane which is then surrounded by a capsid structure. The virions can be either enveloped or non-enveloped, both are infectious however.

The host range of Iridoviridae and subsequently Ranaviruses include fish, amphibians and reptiles. Therefore, they pose a threat to not only endangered species but also aquaculture industries.



Countries highlighted in purple indicate where Ranaviruses have been detected in the world. As seen on the map they have a worldwide distribution with presence on 6 out of the 7 continents. In many of the countries not highlighted there have not been studies to detect these viruses.

As seen on the map above there is only one country in Africa where Ranaviruses have been detected, this is Cameroon. By collaborating with Dr. Bryan Stuart (North Carolina Museum of Natural Sciences) we obtained a large number of DNA samples isolated from skin swabs from several frog genera in neighboring Gabon.

Chiromantis rufescens

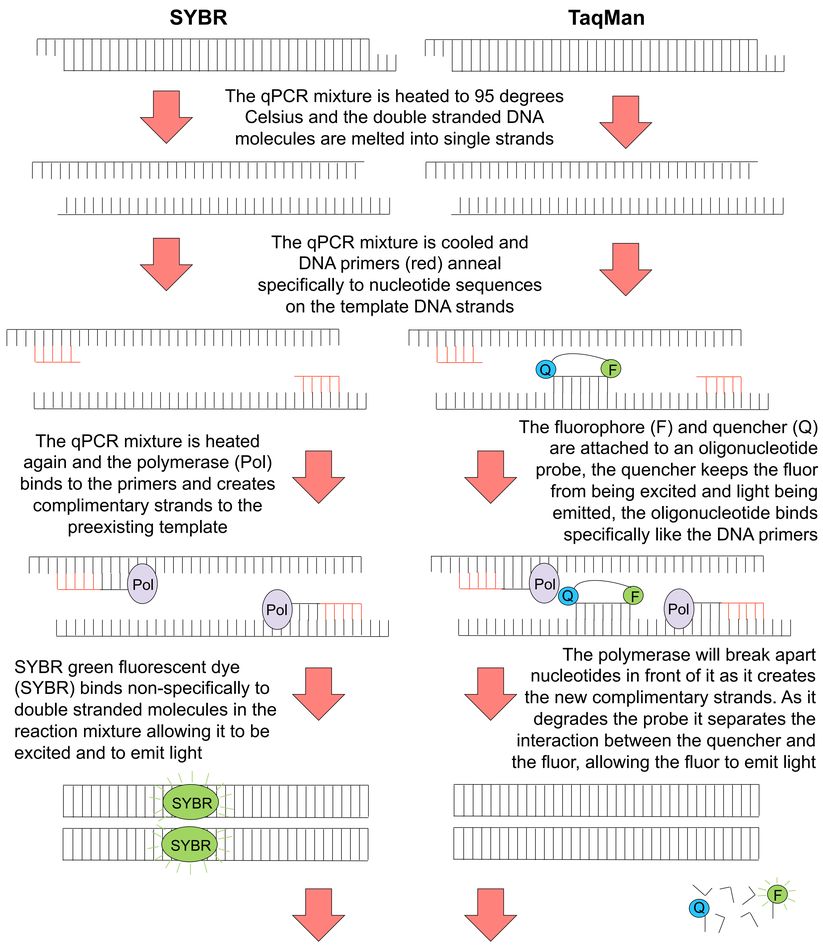
Frog Virus 3 is the prototypical member of the Ranavirus genus. It shares genetic similarity with other members in the genus and has been detected in all classes of species known to be infected by these viruses (fish, reptiles, and amphibians).

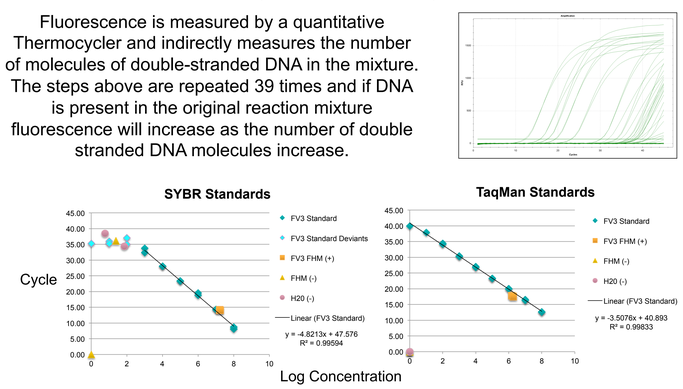
Due to presence of Ranavirus in neighboring Cameroon, we hypothesize that Frog Virus 3 will be detected in at least one of the frog genera that we are screening.

Methods

Hyperolius cinnameoventris

Skin swabs were taken from several frog genera. We received DNA that was purified from these samples. A reaction mixture containing the template DNA sample, DNA primers specific for the Frog Virus 3 Major Capsid Protein, and a polymerase master mix was prepared. The polymerase master mix includes a temperature dependent polymerase, deoxynucleotide triphosphates, and a fluorescent dye or probe. We used two different master mixes, SYBR green and TaqMan.





As seen in the standard curves above, the SYBR green assay gave a high non-specific fluorescent background as compared to the TaqMan assay. The specificity of the fluorescent probe in the TaqMan assay produces lower background fluorescence with greater sensitivity.

Results

Afrixalus fulvovittatus

Hyperolius tuberculatus

Leptopelis aubryi

Species	IUCN Listing	Bd+	FV3+	Bd+ / FV3+	Samples
Afrixalus fulvovittatus	Not Threatened	1	1	0	6
Afrixalus paradorsalis	Not Threatened	1	0	0	3
Amnirana species A	Not Threatened	4	0	0	12
Artholeptis species A	Not Threatened	1	0	0	6
Chiromantis rufescens	Not Threatened	3	0	0	8
Conraua crassipes	Not Threatened	1	0	0	7
Hyperolius bolifambae	Not Threatened	0	0	0	3
Hyperolius cinnamomeoventris	Not Threatened	0	0	0	7
Hyperolius ocellatus	Not Threatened	4	0	0	10
Hyperolius tuberculatus	Not Threatened	1	1	0	4
Leptopelis aubryi	Not Threatened	2	1	0	8
Leptopelis cristallinoron	Not Threatened	1	2	1	3
Leptopelis species C	Not Threatened	2	2	1	5
Leptopelis zebra	Near Threatened	0	1	0	4
Opisthothylax immaculatus	Not Threatened	0	1	0	2
Petropedetes palmipes	Endangered	2	1	0	5
Phlyctimantis leonardi	Not Threatened	0	0	0	4
Ptychadena species A	Not Threatened	1	2	0	6
Scotobleps gabonicus	Not Threatened	2	0	0	4
Total		**26**	**12**	**2**	**107**

Table 1: qPCR Result Summary: Bd = *Batrachochytrium dendrobatidis,* FV3 = Frog Virus 3, IUCN = International Union for Conservation of Nature

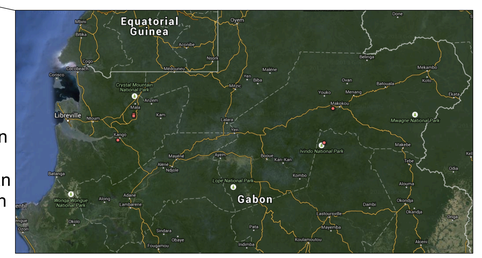
Opisthothylax immaculatus

Petropedetes palmipes

Scotobleps gabonicus

Conclusions

Frog Virus 3 was detected in 12 of the 107 samples that we screened. This is the first instance of detection of Ranavirus in Gabon. The map below shows the locations where the Frog Virus 3 positive samples were collected. One point is equal to one or more positive samples.



While we cannot confirm infection from the samples, presence of Frog Virus 3 in these samples further extends the known global distribution of Ranaviruses. Given the high sensitivity and specificity, the TaqMan assay outperformed the SYBR green assay as a tool for environmental sampling for Ranaviruses.

Acknowledgements

Dr. Matthew C. Allender, University of Illinois – qPCR Primers and Standard
Dr. Bryan Stuart, North Carolina State University – Skin Swab DNA
Dr. Jacques Robert, University of Rochester – FV3-GFP Virus

References

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