

## Scientists establish cell cultures of endangered frog

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San Diego Zoo Global Wildlife Conservancy scientists have taken a tremendous leap forward in banking viable amphibian cells. This was achieved through the first successful establishment of cell cultures from frozen biopsy specimens of the critically endangered Mississippi gopher frog. A method called "tissue piecing" and immediate freezing in liquid nitrogen allows field biologists to collect samples that can later be processed in a laboratory.

"With amphibians we have found that we can routinely obtain viable cells from a fresh <u>biopsy</u>, but they fail to thrive and divide, leaving us often unable to establish and freeze cell cultures," said Oliver Ryder, Ph.D., <u>San Diego Zoo</u> Institute for Conservation Research director of genetics. "The question then is, how are we to know if there are viable cells in a tissue-pieced amphibian biopsy when we cannot grow the cells from a fresh biopsy?"

Thanks to a breakthrough achieved at the Institute for Conservation Research, the Mississippi gopher frog case provides proof that endangered amphibian cells can be grown and cells frozen from fresh or tissue-pieced and frozen biopsies.

The tissue-piecing technique has been used for some time with numerous species. In <u>mammals</u>, for example, scientists can mince a <u>skin</u> <u>biopsy</u>, treat it with cryoprotectant and freeze it. Later the tissue pieces can be thawed in a lab to establish a cell culture. But this method had not been previously successful with endangered amphibians until now.



"We are very pleased to have demonstrated for a critically endangered species that we have the techniques necessary for establishing cell cultures under field conditions, when quick access to a lab is not feasible," said Ryder. "For species for which we have not been able to successfully establish cell cultures, but have banked tissue-pieced samples, we know now that we have saved viable cells. In the future we now have the opportunity to go back when we hope to have worked out methods for growing cells from species whose cells have been recalcitrant to our cell culture efforts and use tissue-pieced specimens to obtain, grow and preserve cell cultures in support of conservation science applications."

This breakthrough expands the total Mississippi gopher <u>frog</u> cell cultures to 19 individuals. The Frozen Zoo now contains a substantial sampling of the gene pool of this critically endangered species.

Cell cultures have been a primary tool in the study of disease in many species; however, this method has been underutilized for endangered species and has had very limited application to the study of amphibian diseases, largely because of the lack of cell cultures from endangered amphibians. Globally, amphibians are threatened with severe population declines due to infectious pathogens such as chytrid fungus. Availability of cell cultures may help combat these and other diseases. Additionally, cell cultures can be utilized to advance reproductive studies.

## Provided by Zoological Society of San Diego

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