# 2020 Annual Report

Panama Amphibian Rescue and Conservation Project


A project partnership between: Cheyenne Mountain Zoo, Houston Zoo, Smithsonian's National Zoo, Smithsonian Tropical Research Institute, and Zoo New England


# Mission

Our mission is to rescue and establish sustainable assurance colonies of amphibian species that are in extreme danger of extinction throughout Panama. We will also focus our efforts and expertise on developing methodologies to reduce the impact of the amphibian chytrid fungus (Bd) and proceed to reintroduction trials.

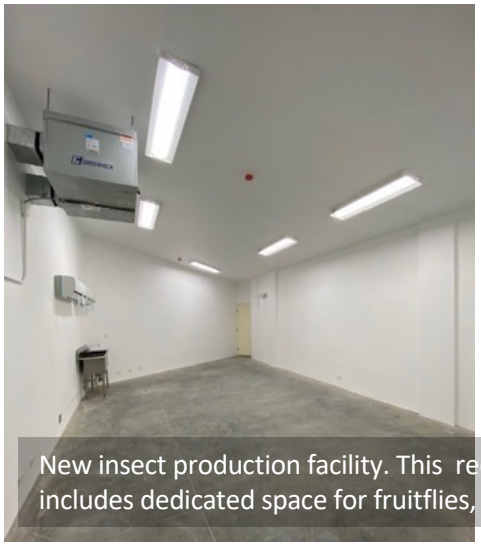
Cover: We feature the exceptional efforts of our conservation staff working at the Panama Amphibian Rescue and Conservation Center in Gamboa in a time of COVID. Left to Right: Kenia Cabezón, Amaranto Cabezón, Nancy Fairchild, Tina Mejía, Jennifer Warren, Orlando Garcés, Lanki Cheucarama, and Jorge Guerrel.

## Goal 1: Ensure adequate physical infrastructure and staffing capacity to effectively manage and breed the living collection.

2020 was a challenging for the Panama Amphibian Rescue and Conservation (PARC) project, as it was for most people and institutions in the world, as we grappled with the COVID-19 human pandemic. COVID-19 severely restricted our operations in 2020 presenting us with a significant challenge in maintain the safety of our employees and the living collection. Our essential staff are now working on staggered schedules and in partitioned areas to reduce overlapping physical work spaces, and we were unable to accommodate volunteers or interns representing a significant reduction in our staffing capacity this year. COVID-19 lockdowns in Panama occurred for much of 2020, and also disrupted our regular research and conservation activities. Research activities have been halted pending guidance from the Panamanian Government and the Smithsonian phased reopening plans. It is in times of difficulty that we truly appreciate being part of a village of committed people. We are especially grateful to our staff who managed to figure out how to keep the wheels turning with considerable personal sacrifice and effort to abide by significant new safety measures. We would also like to thank to our regular donors and foundations who recognized the extent of the impact on our operations and kindly extended performance periods on existing grants and to the individuals who kindly maintained or even increased their regular financial contributions.

Lanki Cheucarama feeds *Atelopus* tadpoles produced in one of our rescue pods.

Construction of our new insect facility was halted mid-construction due to COVID-19 safety concerns, but the building was completed in early 2021, we are awaiting installation of fire suppression systems and building inspections before we can occupy the new facility. This new 1,600 square feet insectarium has two climate-controlled rooms that can be maintained at different temperatures, allowing a diversity of food items with varying sizes and nutritional properties to meet the needs of our diverse amphibian collection. Each room had redundant AC capacity and is connected to a backup generator. A larger room will be used for rearing crickets, pantry moths, super worms and cockroaches, and a smaller room for rearing springtails and fruit flies in cooler conditions. Entry to the facility is through an entry vestibule to limit escape of insects and the entry of organisms that may disrupt the insect cultures. This space will increase our amphibian rescue and conservation work by expanding our insect production capabilities and diversify our existing food supply for our growing collection of amphibians, which consist of 12 species and more than 1600 individual adult animals. A huge thanks to the STRI Office of Facilities and Engineering who oversaw this project and to the many individual donors, the Cheyenne Mountain Zoo, Zoo New England, the Houston Zoo, the Holtzman Foundation, the Shared Earth Foundation for enabling us to complete it.



New insect production facility. This recently completed 1,660 sq ft facility improvement includes dedicated space for fruitflies, crickets and storage.

## Goal 2: Manage genetically viable assurance colonies of 12 species in captivity that are at risk of extinction from chytridiomycosis.

In April 2020 we experienced a failure of both the primary, and backup air conditioner in one of our 7 amphibian pods. The resulting increased temperatures overnight lead to a significant loss amounting to 20% of the genetic lines of Limosa harlequin frogs *Atelopus limosus*, Toad mountain harlequin frogs, *A. certus* and Pirre harlequin frogs *A. glyphus*. We believe the failure was connected to a voltage fluctuation, and have developed a recovery plan focused on preventing recurrence and increasing breeding of the existing collection.

In 2020 we managed to successfully breed 12 pairs of harlequin frogs of the genus *Atelopus,* 1 pair of Crowned treefrogs *Triprion spinosus*, 4 pairs of Lemur leaf frogs *Agalychnis lemur*, develop a new method to rear Vicente's dart frog *Oophaga vicentei* using artificial bromeliads (7 pairs bred) and to produce numerous offspring from Geminis' dart frog *Andinobates geminisae* (9 pairs bred). We have more than 1,600 adult frogs in our captive collection and an updated summary of our existing holding in relation to our population management goals for each species is presented in Table 1.

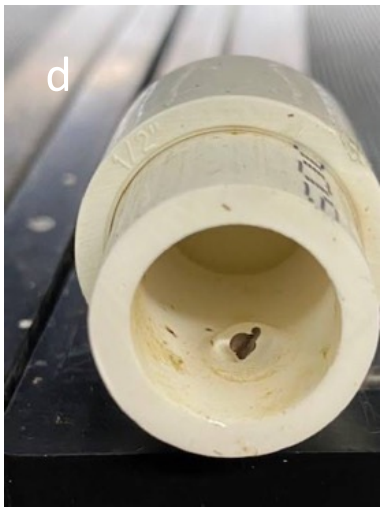
### Table 1: 2020 Population management report card for the 12 priority conservation species maintained by the Panama Amphibian Rescue and Conservation Project.

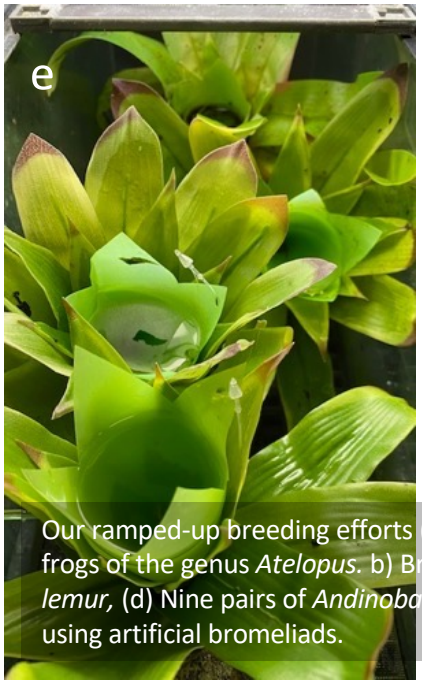
| | Founders alive or represented (Goal = 20) | Pairs Bred to F1 (Goal = 10) | Pairs bred to F2 (Goal = 10) | Total number of frogs in collection (Goal = 300) |
|---|---|---|---|---|
| *Andinobates geminisae* | 40 | 18 | 3 | 128 |
| *Gastrotheca cornuta* | 22 | 12 | 1 | 29 |
| *Oophaga vicentei* | 36 | 10 | 0 | 50 |
| *Atelopus varius (lowland)* | 51 | 11 | 0 | 552 |
| *Craugastor evanesco* | 45 | 3 | 0 | 61 |
| *Atelopus limosus* | 18 | 8 | 0 | 78 |
| *Atelopus certus* | 17 | 8 | 3 | 85 |
| *Atelopus glyphus* | 17 | 8 | 0 | 242 |
| *Triprion spinosa* | 11 | 6 | 6 | 65 |
| *Agalychnis lemur* | 8 | 6 | 0 | 55 |
| *Strabomantis bufoniformis* | 8 | 2 | 0 | 8 |
| *Atelopus varius (highland)* | 6 | 4 | 0 | 30 |
| *Atelopus zeteki* | 4 | 5 | 2 | 241 |

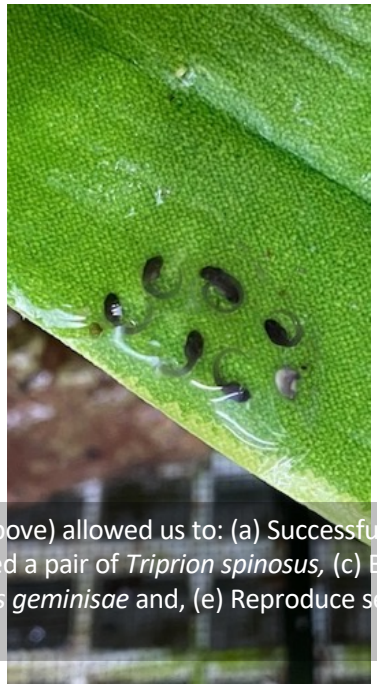
| *Progress towards goal* |
|---|
| 0-24% |
| 25-49% |
| 50-74% |
| Goal met |

Male Geminis' dart frog *Andinobates geminisae* transporting a tadpole.

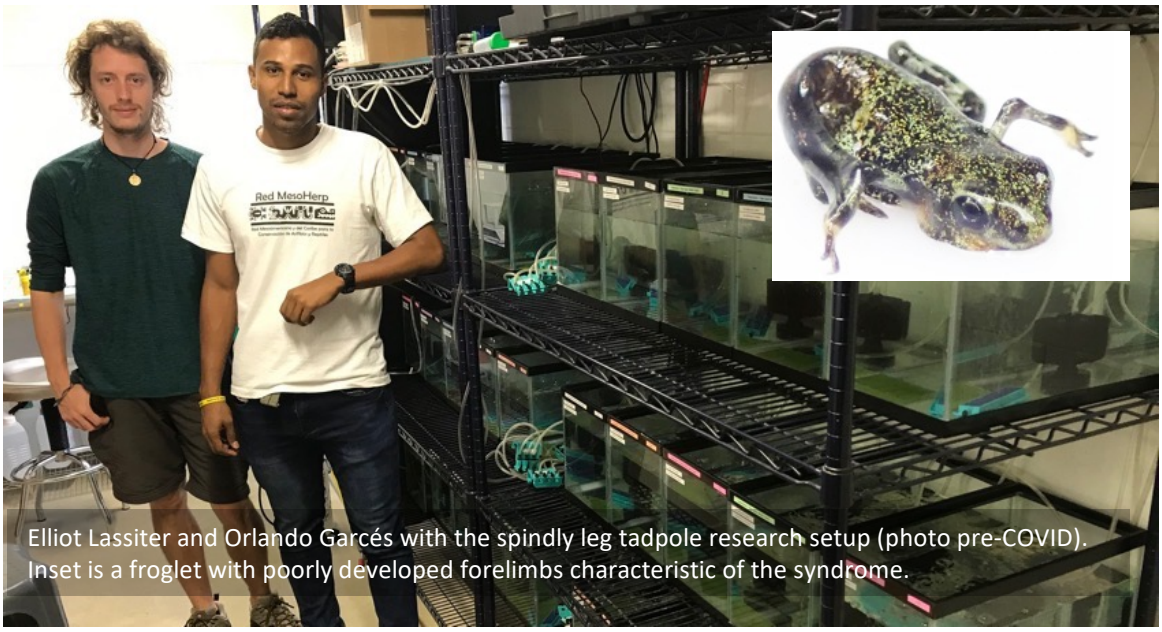


Our ramped-up breeding efforts (above) allowed us to: (a) Successfully breed 12 pairs of harlequin frogs of the genus *Atelopus.* b) Breed a pair of *Triprion spinosus,* (c) Breed four pairs of *Agalychnis lemur,* (d) Nine pairs of *Andinobates geminisae* and, (e) Reproduce seven pairs of *Oophaga vicentei* using artificial bromeliads.




Elliot Lassiter and Orlando Garcés with the spindly leg tadpole research setup (photo pre-COVID). Inset is a froglet with poorly developed forelimbs characteristic of the syndrome.

## Goal 3: Research factors to improve long-term sustainability of the captive collections and increase success of release trials.

Spindly leg syndrome is a relatively common musculoskeletal abnormality that leads to poorly developed forelimbs of newly metamorphosed froglets. This condition is usually associated with captive-rearing of frogs and has been rarely observed in the wild, making it an animal welfare concern for zoos and aquariums. We completed a project with support from the Morris Animal Foundation and The Woodtiger Fund investigating spindly leg syndrome and the role of calcium and phosphate in tap water. The experiment, led by Elliott Lassiter and Orlando Garces found clear evidence linking spindly leg syndrome calcium / phosphate deficiencies in the water used to rear tadpoles. We found that rates of spindly leg syndrome were not connected to the tadpole's diet, but to low levels of dissolved calcium and phosphate in the water used to rear the tadpoles. We recommend that other people encountering spindly leg syndrome test their water for calcium hardness and supplement it with calcium chloride if it is too soft and to reduce the daily quantity of food offered to tadpoles so that they grow more slowly.

We continued to investigate the comparative susceptibility of species in our captive collection using a non-lethal mucusome approach. The project led by Dr. Luke Linhoff is funded by the National Geographic Society, The Woodtiger Fund and the Smithsonian Scholarly Studies Grant. It involves taking a skin mucus sample from the frog and exposing that to the chytrid fungus in a test tube. A dye that selectively stains live zoospores helps us to quantify the inhibitory activity of the frog skin mucus. In addition to ranking the Bd susceptibility of species in our existing amphibian collection, we hope to identify species that have a wide range of susceptibility, that could allow us selectively breed resistant frogs. Luke has found promising results documenting differences in mucusome activity and susceptibility within and between species, but the research is on hold due to COVID-19 restrictions.

We are collaborating with Dr. Gina Della Togna from the InterAmerican University in Panama and is a Smithsonian Research Associate. Gina chairs the IUCN amphibian reproduction working group on developing assisted reproduction methods. To date, Gina's efforts have resulted in 37 pairs from 5 species resulting in 17 egg clutches and viable offspring from all 5 species. We have collected 110 samples from 7 species, 3 different hormone treatments. We have cryopreserved sperm from 6 species and installed a -80 freezer from SI for tissue and genome resource banking. (This ultra-cold freezer, however, has been loaned to the government of Panama to help with Covid vaccine storage). Gina has several Panamanian trainees, and their research has been supported by the government of Panama SENACYT and The Woodtiger Fund.

# 2020 Research Publications involving collaborations with PARC

**Lassiter, E., Garcés, O., Higgins, K., Baitchman, E., Evans, M., Guerrel, J., Klaphake, E.,** Snellgrove, D., **Ibáñez, R. and Gratwicke, B.,** 2020. Spindly leg syndrome in *Atelopus varius* is linked to environmental calcium and phosphate availability. PloS one, 15(6), p.e0235285.

Rodriguez, C., **Ibáñez, R.**, Rollins-Smith, L.A., Gutiérrez, M. and Durant-Archibold, A.A., 2020. Antimicrobial secretions of toads (Anura, Bufonidae): bioactive extracts and isolated compounds against human pathogens. Antibiotics, 9(12), e843.

Kelehear, C., **Ibáñez, R.**, Rodríguez, C., Buitrago, S. and Durant-Archibold, A.A., 2020. Sarcophagid myiasis in the bufonid *Rhinella alata* in Panama. Journal of wildlife diseases, 56(3), pp.667-672.

Martin H, C., **Ibáñez, R.**, Nothias, L.F., Caraballo-Rodríguez, A.M., Dorrestein, P.C. and Gutiérrez, M., 2020. Metabolites from microbes isolated from the skin of the Panamanian rocket frog *Colostethus panamansis* (Anura: Dendrobatidae). Metabolites, 10(10), e406.

Byrne, A.Q., Richards-Zawacki, C.L., Voyles, J., Bi, K., **Ibáñez, R**. and Rosenblum, E.B., 2021. Whole exome sequencing identifies the potential for genetic rescue in iconic and critically endangered Panamanian harlequin frogs. Global Change Biology, 27(1), pp.50-70.

Yovanovich, C.A., Pierotti, M.E., Kelber, A., Jorgewich-Cohen, G., **Ibáñez, R.** and Grant, T., 2020. Lens transmittance shapes ultraviolet sensitivity in the eyes of frogs from diverse ecological and phylogenetic backgrounds. Proceedings of the Royal Society B, 287(1918), e20192253.

Ramírez, J.P., Jaramillo, C.A., Lindquist, E.D., Crawford, A.J. and **Ibáñez, R.,** 2020. Recent and rapid radiation of the highly endangered harlequin frogs (*Atelopus*) into Central America inferred from mitochondrial DNA sequences. Diversity, 12(9), e360.

Rodríguez, A., Mundy, N.I., **Ibáñez, R.** and Pröhl, H., 2020. Being red, blue and green: the genetic basis of coloration differences in the strawberry poison frog (*Oophaga pumilio*). BMC Genomics, 21, e301.

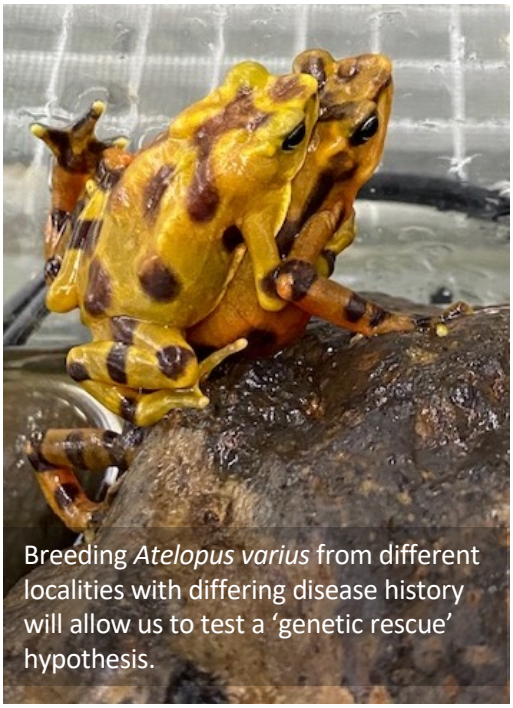
Savage, A.E., **Gratwicke, B.**, Hope, K., Bronikowski, R.E., Fleischer, R.C. 2020. Sustained immune activation is associated with susceptibility to the amphibian chytrid fungus. Molecular Ecology, 29(15), pp.2889-29903. DOI: 10.1111/mec.15533

Rodriguez, C., **Ibáñez, R.,** Ng, M., Spadafora, C., Durant-Archibold, A.A. and Gutiérrez, M., 2020. 19-Hydroxy-bufalin, a major bufadienolide isolated from the parotoid gland secretions of the Panamanian endemic toad *Rhinella centralis* (Bufonidae), inhibits the growth of *Trypanosoma cruzi.* Toxicon, 177, pp.89-92




## Goal 4: Begin experimental frog reintroduction trials with surplus offspring.

This year Dr. Allie Byrne joined SCBI as a post-doctoral fellow working to examine the genes of *Atelopus* in specimens at the Smithsonian's National Museum of Natural History that were collected before Bd related declines and comparing them with genes from surviving animals. A second piece of the project, once Covid travel restrictions are lifted, will be a release trial of *Atelopus varius.* Allie will compare the survival probability of frogs collected pre-Bd, and post-Bd with crosses of the two groups to test the idea of genetic rescue as a reintroduction tool. Allie is working with Dr. Carly Muletz in SCBI's Center for Conservation Genomics and is supported by grants from the Smithsonian Fellowship and Revive & Restore.



Breeding *Atelopus varius* from different localities with differing disease history will allow us to test a 'genetic rescue' hypothesis.


## Goal 5: Cultivate and foster an appreciation for amphibians in the public mindset and work on community engagement at the field level.

COVID-19 has forced us to close our primary exhibition area at the Punta Culebra Nature center, and to scale back golden frog day celebrations to a series of online talks. We developed a Spanish-language public service announcement from a golden frog, who draws parallels between the COVID 19 pandemic, and the pandemic affecting amphibians. Overall our public communications abilities were negatively impacted by the pandemic and we generated less new content to share, web traffic was down 40% compared to previous years, while social media followings remained similar to 2019.

## Goal 6: Ensure the financial sustainability of the project.

We gratefully acknowledge the financial support from the following primary partners, corporations, foundations and societies who have supported this work. Note these figures reflect expenditures in calendar year 2020 by funding source, not contributions.

| Funding Source | Purpose | 2020 Expenses (US$) |
|---|---|---|
| **SCBI and donors** | Coordinator salary, supplies, fellows, travel, insectarium construction. | 375,798 |
| **First Quantum Minerals (Cobre Panama)** | Salaries, operating costs, swab analysis | 340,650 |
| **The Woodtiger Fund** | Research and program support | 45,000 |
| **Houston Zoo** | Insectarium construction | 35,198 |
| **Zoo New England** | Salaries | 22,429 |
| **Holtzman Wildlife Foundation** | Insectarium construction | 23,367 |
| **Cheyenne Mountain Zoo** | Salaries | 17,864 |
| **National Geographic Society** | Reintroduction Research | 15,023 |
| **SENACYT** | Research | 2,911 |
| **Morris Animal Foundation** | Research | 1,960 |
| **STRI and donors*** | Supplies | 273 |
| **TOTAL** | | **880,200** |

* These funds include direct project costs incurred in the calendar year 2020, but do not reflect unexpensed funds or in-kind institutional administrative support, utilities, fundraising, public affairs and programmatic support costs generously provided by the Smithsonian Tropical Research Institute who host this project.

## Donors

In addition to the contributions from project partners, we are grateful to the following donors who have made additional contributions to the project directly: Susan and Frank Mars, Linda Mars, Sey and Pearl Moskowitz, Liliane Willens, The Woodtiger Fund, The Shared Earth Foundation, The Anela Kolohe Foundation, National Geographic Society, Cervecería La Rana Dorada, Baton Rouge Zoo.

## 2019 Online Contributions

Our sincere thanks to the following individuals who contributed $25 or more online: Ryan Amenta, Promise Bauers-Garcia, Lauren Burdette, Shannon Dade, Gregory Heilman, Sabrina Hickerson, Zoe Hilton, Aimee Jackson, Ian McKinney, Mallory Miles, Martijn Oei,Tenley Peterson, Hugh Rand, Eric Stubbs, Hung-Shiu Ting, Elizabeth Wade, Gregory Wilson, Bryan Yonka.

## Staff

*Lead Scientist & International Coordinator* - Dr. Brian Gratwicke
*Project Director, Panama* - Dr. Roberto Ibáñez
*Facility Manager Gamboa* – Jorge Guerrel.
*Technical Staff* - Amaranto Cabezón, Kenia Cabezón, Lanki Cheucarama, Nancy Fairchild, Orlando Garcés, Estefany Illueca, Tina Mejía, Jennifer Warren
*Graduate Students* Blake Klocke, Alyssa Wetterau. *Post Doctoral Fellows* – Luke Linhoff, Andreas Hertz.

## Steering Committee

Bob Chastain, President and CEO *Cheyenne Mountain Zoo*;
Dr. Steve Monfort, Director *SCBI;*
Dr. Oris Sanjur, Acting Director *Smithsonian Tropical Research Institute*;
John Linehan, President and CEO *Zoo New England;*
Jamie Kratt, *at-large member.*

## Implementation Committee

Nicole Chaney *Cheyenne Mountain Zoo*; Dr. Brian Gratwicke & Matthew Evans *Smithsonian's National Zoological Park and Conservation Biology Institute*; Dr. Roberto Ibáñez & Jorge Guerrel *Smithsonian Tropical Research Institute*; Dr. Eric Baitchman & Bryan Windmiller *Zoo New England.*

## 2020 Volunteers

John Berkholtz, Michelle Castellanos, Leslie Finster, Elliot Lassiter, Gerald McNeil, Amadeus Plewnia, Julie Weisman.

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