

A person in a dark, short-sleeved shirt is working with a metal frame structure in a forest. The person is looking towards the camera. In the foreground, a small, black and green frog is perched on a dark, mossy rock. The background is filled with green foliage and trees.

# 2017 ANNUAL REPORT

*PANAMA AMPHIBIAN RESCUE AND CONSERVATION PROJECT*

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



### **MISSION**

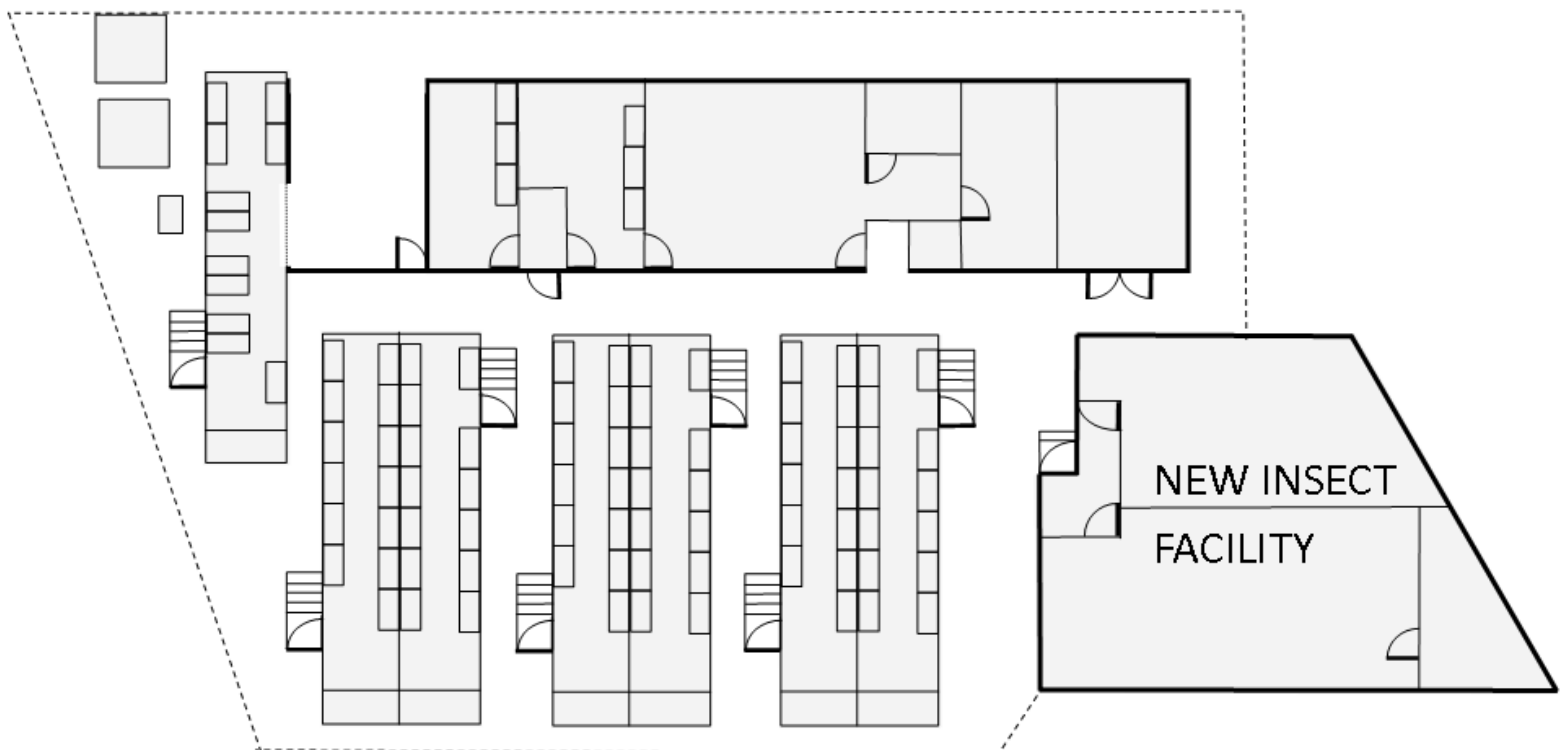
*Our mission is to rescue and establish sustainable assurance colonies of amphibian species that are in extreme danger of extinction throughout Panama. We will also focus our efforts and expertise on developing methodologies to reduce the impact of the amphibian chytrid fungus and proceed to reintroduction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

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

In 2017, we began planning to consolidate our collection currently held at the Nispero Zoo to the Panama Amphibian Rescue and Conservation Project Center in Gamboa. This requires a larger facility in Gamboa to accommodate the increased number of frogs. In 2017 we commissioned architectural designs for a new 1,600 square foot insect production facility. This building will expand our insect production capabilities and free up two pods that will house frogs. Our goal is to have the new facility constructed in 2018, and to move frogs out of the Nispero facility before the end of the year. An agreement is being prepared to sign with the newly formed, independent EVACC Foundation to ensure that some of the species continue in El Valle. This agreement will include surplus equipment and animals.

We continued to make progress on increasing the total available frog space in Gamboa. With help from the National Zoo’s Reptile Discovery Center husbandry team and numerous volunteers, we outfitted a pod dedicated entirely to dart frogs. This brings us to five completely outfitted frog pods and a total of 2,000 square feet of frog space.

In 2017, we also began colonies of soldierflies and earthworms. We successfully maintained and expanded the earthworm colonies, which are a new high-calcium food option for several of our larger frog species, but were not successful with the soldier flies. The addition of earthworms to our food supply helps to diversify our existing food supply colonies of fruit flies, springtails, crickets, pantry moths, super worms and cockroaches with a high calcium food item.



*Plan layout of the Panama Amphibian Rescue and Conservation Project in Gamboa showing the new insect expansion in relation to the existing facility*



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

We made good progress on reproducing priority species in 2017 (Table 1) and now care for more than 4,000 frogs in our captive collection. Highlights include the first-ever captive reproduction of rusty robber frogs *Stabomantis bufoniformis* using assisted reproduction methods, and the first-ever captive reproduction of the vanishing robber frogs *Craugastor evanesco*. Our growing captive frog population exceeds our captive population management goals for several species, allowing us to begin release trials. This important milestone establishes an outlet for surplus frogs, builds capacity in our team to produce larger numbers of frogs, provides a source of frogs for future research, and advances our strategic plan objectives to begin release trials that will allow us to learn and improve upon our methods.

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

2017 REPORT	% min required founder population*	Pairs bred to F 1 (goal 10 pairs bred)	Pairs Bred to F2	Number of frogs (min goal 300)
<i>Atelopus certus</i>	1	13		352
<i>Atelopus glyphus</i>	1	10		351
<i>Gastrotheca cornuta</i>	1	13	6	158
<i>Atelopus limosus (chevron)</i>	1	13		159
<i>Oophaga vicentei</i>	1	10		45
<i>Andinobates geminisae</i>	1	11		150
<i>Atelopus varius (lowland)</i>	1	8		1334
<i>Craugastor evanesco</i>	1	3		85
<i>Anotheca spinosa</i>	0.5	7	8	160
<i>Atelopus varius (highland)</i>	0.5	4		502
<i>Strabomantis bufoniformis</i>	0.3	3		34
<i>Agalychnis lemur</i>	0.25	2		89
<i>Atelopus zeteki</i>	0.2	3	6	685
				<b>4104</b>

\* = 10 males and 10 female represented or 20 males and 20 females collected, whichever achieves highest ratio

Key
Goal Attained or Exceeded
75% of Goal Attained
50% of Goal Attained
25% of Goal Attained
Goal not met







Dr. Brad Wilson and Chelsea Thomas in the frog pod at the Atlanta Botanical Gardens. Re-importation plans are underway to help expand the genetic representation of *Agalychnis lemur* and *Anotheca spinosa* in Panama

We are working to carefully manage our captive collection and have entered all animals from both facilities into the Zoological Information Management System database. Keeping these records up to date has been a significant challenge for staff, and we are now working on additional training for a dedicated registrar. In collaboration with Dr. Diorenne Smith we tested pit tag insertions in adult *Atelopus*, and an alternate elastomer tagging and toe-clipping approach in order to develop a more time-efficient way than photographic cage-cards to identify individuals held in group tanks.

We coordinated two field trips to continue collecting additional founding populations of the *Gastrotheca cornuta*, *Craugastor evanescens* and *Andinobates geminisae* located in the Minera Panama S.A. mining concession area. We are also working with Atlanta Botanical Gardens to repatriate *Agalychnis lemur* and *Anotheca spinosa*. These species were part of amphibian rescue efforts in Panama in 2004, which exported animals to the USA before any local ex-situ facilities were established. This reimportation will help to bolster the number of genetically represented founders in Panama, helping us to achieve our population management goals for those species.



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

We conducted research into spindly leg syndrome which is a major rate-limiting factor affecting our production of frogs. We investigated water quality, diet composition and ration as potential factors associated with the syndrome.



Spindly leg froglet (left) vs healthy froglet (right)

We found lower rates of spindly leg syndrome and improved survival of tadpoles reared in reconstituted reverse-osmosis water and in groups that were fed less food. We suspect that lack of calcium in tap water may be responsible for these observations and will conduct additional experiments to test this hypothesis in 2018. We have conducted additional husbandry experiments that have helped us to improve our production and post-mesomorph survival of *Andinobates geminisae* with help of Kathleen Higgins from Simon Fraser University.

Dr. Gina DellaTogna from the InterAmerican University in Panama leads our assisted reproduction research program. We have begun cryo-banking sperm from *Atelopus varius*, *A. glyphus*, *A. certus*, *A. limosus*, *Strabomantis bufoniformis* and *Craugastor evanesco*. Establishing a bank of living frozen sperm cells will insure our captive population against unforeseen genetic bottlenecks, and help long-term stability of the living collection. While we have successfully thawed viable sperm, we still need to demonstrate proof of concept by producing viable embryos.

We continue to process and analyze samples collected from two major disease trials in Panamanian Golden Frogs and Hellbenders in collaboration with Alyssa Wetterau and researchers from Cornell University and Liberty University. We are investigating microbiome changes during the course of infection and conducting a comparative host transcriptome response, during the course of an infection in highly susceptible and resistant species.

### 2017 Research Publications

- Becker, M.H. & Gratwicke B. (2017) Minimum lethal concentration of sodium hypochlorite on *Batrachochytrium dendrobatidis*. Plos One 12(4): e0176439. <https://doi.org/10.1371/journal.pone.0176439>
- Della Togna, G., Gratwicke B., Evans M., Augustine L., Chia H., Bronikowski E., Murphy J.B. Comizzoli P. (2017) Influence of extracellular environment on the motility and structural properties of spermatozoa collected from hormonally stimulated Panamanian Golden Frog (*Atelopus zeteki*). Theriogenology 108: 153-160.
- Della Togna, G., Trudeau V.L., Gratwicke B., Evans M., Augustine L., Chia H., Bronikowski E.J., Murphy J.B. Comizzoli P. (2017) Effects of hormonal stimulation on the concentration and quality of excreted spermatozoa in the critically endangered Panamanian golden frog (*Atelopus zeteki*). Theriogenology 91:27-35.
- Gratwicke, B. & Murphy, J.B. (2017) History of Captive Management and Conservation Amphibian Programs Mostly in Zoos and Aquariums. Part I—Anurans. Herpetological Review 48:1 138-157
- Ibáñez D., R., Griffith E.J., Lips K.R. & Crawford A.J. (2017) Altitudinal distribution and advertisement call of *Colostethus latinasus* (Amphibia: Dendrobatidae), endemic species from eastern Panama and type species of *Colostethus*, with a molecular assessment of similar sympatric species. Zootaxa 4254: 91–101.
- Rodríguez, C., Rollins-Smith L., Ibáñez R., Durant-Archibold A.A., Gutiérrez M. (2017) Toxins and pharmacologically active compounds from species of the family Bufonidae (Amphibia, Anura). Journal of Ethnopharmacology 198: 235–254.
- Woodhams, D.C., LaBumbard B.C., Barnhart K.L., Becker M.H., Bletz M.C., Escobar L.A., Iannetta A.A., Joyce M.D., Rabemananjara F., Gratwicke B., Vences M., Minbiole K.P.C. (2017) Prodigiosin, Violacein, and volatile organic compounds produced by widespread cutaneous bacteria of amphibians can inhibit two *Batrachochytrium* fungal pathogens. Microbial Ecology. doi:10.1007/s00248-017-1095-7



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

We do not have any tools likely to mitigate the primary chytridiomycosis threat. Nonetheless, our in-country facilities place us in a unique position to conduct small-scale releases of surplus offspring at sites where they have declined, rather than euthanize them. We conduct intensive post-release monitoring to understand the challenges faced by amphibians transitioning from captive to wild conditions.

We released 90 captive-bred *Atelopus limosus* in the Mamoni Valley Preserve in central Panama as a pilot study conducted by Blake Klocke from George Mason University and Angie Estrada and Daniel Medina from Virginia Tech University. This allowed us to test radiotracking and mark-recapture methods and evaluate the effects of soft release mesocosms on improving post-release survival. The lessons learned from this study will help us to improve future study design. Once we have refined our release trial methodology, systematic replicated releases in multiple localities will allow us to begin to incorporate real world experimental data into population models and to test landscape-level hypotheses in a field situation.



**Top:** Kevin Kratt (Cheyenne Mountain Zoo) Caroline Gabel (Shared Earth Foundation), and Dr. Roberto Ibáñez (STRI) release frogs along a transect in the Mamoni Valley.

**Middle:** Frogs have been individually marked with numbered visual implant tags and yellow elastomer dye, we found that the implant tags were challenging to read in field situations.

**Bottom:** A female *Atelopus limosus* sports a 0.3g radio transmitter allowing us to track frog movements post-release.



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

We continued our online and exhibit-based offerings at the Punta Culebra Nature Center and the Nispero Zoo. We helped to coordinate and organize events around this year’s golden frog festival. We had pro-bono assistance from the public relations company Stratego and the event covered by 49 different news, TV, radio and online outlets resulting in publicity valued at \$33,000 with a public relations value of \$181,000. Festivities began with a beautiful golden frog mural on Avenida Nacional painted by four local artists: Lauri Preschi, Rolo De Sedas, Amir Luchky and EvaDe. The Golden Frog Festival this year marked by several major events throughout the month of August. In El Valle de Antón, the representante del corregimiento Mr. Evilio Morales and the Junta Comunal led and organized the 2017 Golden Frog Day Parade, a free entrance day at the Nispero zoo, and family day at Paseo El Valle. Caminando Panama hosted the annual La Dorada 5k/15k Trail Run that sold out to 400 participants for the second year in a row. In Panama City, we hosted an open house at the Gamboa Amphibian Rescue and Conservation Center and the Punta Culebra Nature Center attended by 1,500 people, and hosted a family frog day at the Altaplaza mall. SENACYT generously printed and helped to distribute a third edition of our very popular ‘Anfibios de Panamá’ posters.”

Eleven independent English and Spanish news stories covering our project appeared in 2015 with more Spanish news articles than English. Our online constituency continued to grow steadily. We now have 4,800 twitter followers, 11,000 Facebook fans, 6,000 instagram Instagram followers. Website visitors dropped 6% compared to last year with 38,000 unique visitors (about 9,000 of these were Spanish-speaking visitors).



Our golden frog mascot makes a splash at Caminando Panama’s annual golden frog trail run, sold out 2 years in a row.



49 local news outlets covered golden frog day festivities



New Avenida Nacional golden frog mural painted Lauri Preschi, Rolo De Sedas, Amir Luchky and EvaDe.

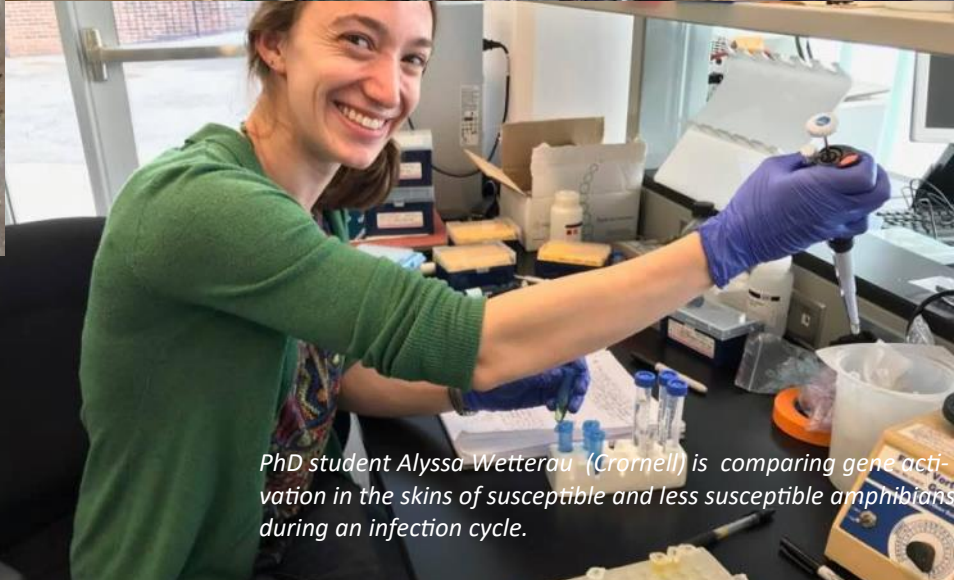




Jorge Guerrel prepares frogs for release trial



First ever captive-bred *Craugastor evanesco* at EVACC



PhD student Alyssa Wetterau (Cromell) is comparing gene activation in the skins of susceptible and less susceptible amphibians during an infection cycle.

## SALVEMOS NUESTRO TESORO NACIONAL

**Festival de la RANA DORADA**  
Agosto 2017

**CALENDARIO DE EVENTOS**

EL VALLE DE ANTÓN	PUNTA CULEBRA	GAMBOA
<b>DOMINGO 13 DE AGOSTO</b> <b>CARRERA LA DORADA</b> 4:00 - 11:00AM Camina o corre 5 a 15km para salvar un tesoro nacional.	<b>DEL 16 AL 18 DE AGOSTO</b> <b>ENCUENTRO DIDACTICO</b> 1:00 - 5:00PM Actividades: Charlas educativas, exhibición: Las Fabulosas Ranas de Panamá, y arte para niños.	<b>SÁBADO 19 DE AGOSTO</b> <b>CASA ABIERTA</b> 9:00AM - 4:00PM Proyecto de Rescate y Conservación de Anfibios
<b>LUNES 14 DE AGOSTO</b> <b>EL NISPERO</b> 1:00 - 5:00PM Tour guiado a la exhibición de ranas. Entrada gratuita.	<b>AVENIDA NACIONAL</b> 10 y 11 DE AGOSTO <b>MURAL PARA TODOS</b> 10:00AM - 5:00PM Un grupo de artistas estarán pintando un mural sobre la Aventura Nacional en honor a la rana dorada.	<b>DOMINGO 27 DE AGOSTO</b> <b>ACTIVIDADES EN FAMILIA</b> 3:00 - 7:00PM Actividades para toda la familia celebrando el Tesoro Nacional.
<b>SÁBADO 19 DE AGOSTO</b> <b>DÍA FAMILIAR PASEO EL VALLE</b> 11:00AM - 5:00PM Ven a conocer a las ranas doradas en persona! Exhibición de ranas doradas.		



Collaborators Angie Estrada and Daniel Medina (Virginia Tech) monitored released frogs for 30 days in these mesocosms as part of a 'soft release' experiment



**GOAL 6: Ensure the financial sustainability of the project.**

We continued to raise funds to support the operational expenditures of this project (below). About 35% of funds was from project partners, 45% from corporate contributions and 20% from foundations and restricted grants. We also secured \$200,000 in additional pledges from private donors to support construction of the insectarium. Our financial goals in the coming year are to increase the number of project partners and to build an operational support fund that can help us to carry staff salaries in between grant periods. In addition, we hope that academic partners who wish to make use of the living collection can help us to support the husbandry expenses associated with producing the frogs.

**Financial Report for Calendar Year 2017\***

<b>FUNDING SOURCE</b>	<b>Purpose</b>	<b>Amount (US \$)</b>
Houston Zoo	Salaries and operating costs	94,872
Minera Panama	Salaries, operating costs, expedition, swab analysis	301,150
BBVA	Supplies, travel, meetings, education, internships	42,968
Zoo New England	Salaries	14,869
Cheyenne Mountain Zoo	Salaries	23,336
Smithsonian and donors	Coordinator salary, supplies, internships, fellows, travel	139,512
Mohammed Bin Zayed Species Conservation Fund	Reintroduction research	820
Wood Tiger Fund	Research and program support	45,000
Friends of the National Zoo	Reintroduction research	3,000
EVIM foundation	Program support	3,000
SENACYT	Research costs	8,500
<b>Total</b>		<b>677,027</b>

\*These funds include direct project costs, but do not reflect the in-kind institutional administrative support, utilities, fundraising, public affairs and programmatic support costs generously provided by the





## 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, Stratego, the Woodtiger Fund, The Shared Earth Foundation, The Anele Kolohe Foundation, National Geographic Society, Mohammed Bin Zayed Species Conservation Fund, The Smithsonian Women's Committee and the EVIM foundation.

## 2016 Online Contributions

Our sincere thanks to the following individuals who contributed \$25 or more online:

Whitney Barnebey, Alison Beckman, Gayle Boss, Sandeep Cheema, Michael Gaglia, Josh Gibbs, Rich Goldsworth, Mary Gunderson, Lisa Gutheil, Hanna Haberkern, Matthew Hecht, Bear Kirkpatrick, Stefanie Klodt, Kayla Lawson, Maggie M, Kristin Majeska, Christine McKinnon, Carole Onasch, Tomasz Pachulski, Matthew Simon, Eric Stubbs, Elizabeth Wade, Kurt Walton, Gregory Wilson.

## Staff

*Lead Scientist & International Coordinator* - Dr. Brian Gratwicke

*Project Director, Panama* - Dr. Roberto Ibáñez

*Gamboa Amphibian Research and Conservation Center Manager*– Jorge Guerrel.

*Technical Staff* - Nair Cabezón, Lanki Cheucarama, Rigoberto Díaz, Nancy Fairchild, Estefany Illueca.

*Interns* – Orlando Garcés, Jennifer Warren, Kathleen Higgins, Valeria Franco, Maryuri Estrada, Julio Campeiro

*El Valle Amphibian Conservation Center Manager* – Heidi Ross.

*Technical Staff* – Milagro González, Yeisson Muñoz. *Interns* – José Alonso, Erika Herrera.

## Steering Committee

Bob Chastain, President and CEO *Cheyenne Mountain Zoo*;

Lee Ehmke, CEO, *Houston Zoo*;

Dr. Steve Monfort, Director *Smithsonian Conservation Biology Institute*;

Dr. Matthew Larsen, Director *Smithsonian Tropical Research Institute*;

John Linehan, President and CEO *Zoo New England*.

## Implementation Committee

Dr. Eric Klaphake & Dr. Liza Dadone *Cheyenne Mountain Zoo*; Peter Riger *Houston Zoo*; Dr. Brian Gratwicke *Smithsonian Conservation Biology Institute*; Matthew Evans *Smithsonian's National Zoological Park*; Dr. Roberto Ibáñez, Jorge Guerrel & Heidi Ross *Smithsonian Tropical Research Institute*; Dr. Eric Baitchman & Bryan Windmiller *Zoo New England*; Dr. Brad Wilson *Atlanta Botanical Gardens*.



## 2017 Volunteers

We are grateful to the following volunteers for their generous assistance: Vincent Premel, Lauren Jones, Madisson Steege, Tommaso Toldo, Juan David Sepulveda, Kevin Krahn, Nicolaas Beschier Van Der Giesen, Blake Klocke, Mark Anthony Vassallo, Aurora Guizado.

## 2017 Golden Frog Festival

**Organizing Committee:** Linette Dutari, Roberto Ibáñez, María L. Prechi, Jorge Alemán, Nelly Florez, Crystal DiMiceli, Sonia Tejada, Jimena Pitty, Álvaro González, Rigoberto Díaz, Adrián Benedetti, Ana Matilde Ruíz, Ana Endara, Sean Mattson, Heidi Ross, Lanki Cheucarama.

**Participating organizations:** Ministerio de Ambiente de Panamá, Smithsonian Tropical Research Institute, Fundación Smithsonian, SENACYT, YMCA, Rana Dorada Pub, Stratego, Zoológico El Nispero, Caminando Panamá.

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