

2015 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 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) so that one day captive amphibians may be re-introduced to the wild.

VISION

The Panama Amphibian Rescue and Conservation Project will be a sustainably financed, Panamanian-led organization that has stemmed the tide of extinctions caused by amphibian chytrid fungus and other threats to amphibians. We will lead successful recovery programs for Panama's endangered amphibians and serve as an exemplary model that can be replicated to address the threat of chytridiomycosis to the survival of amphibians worldwide.

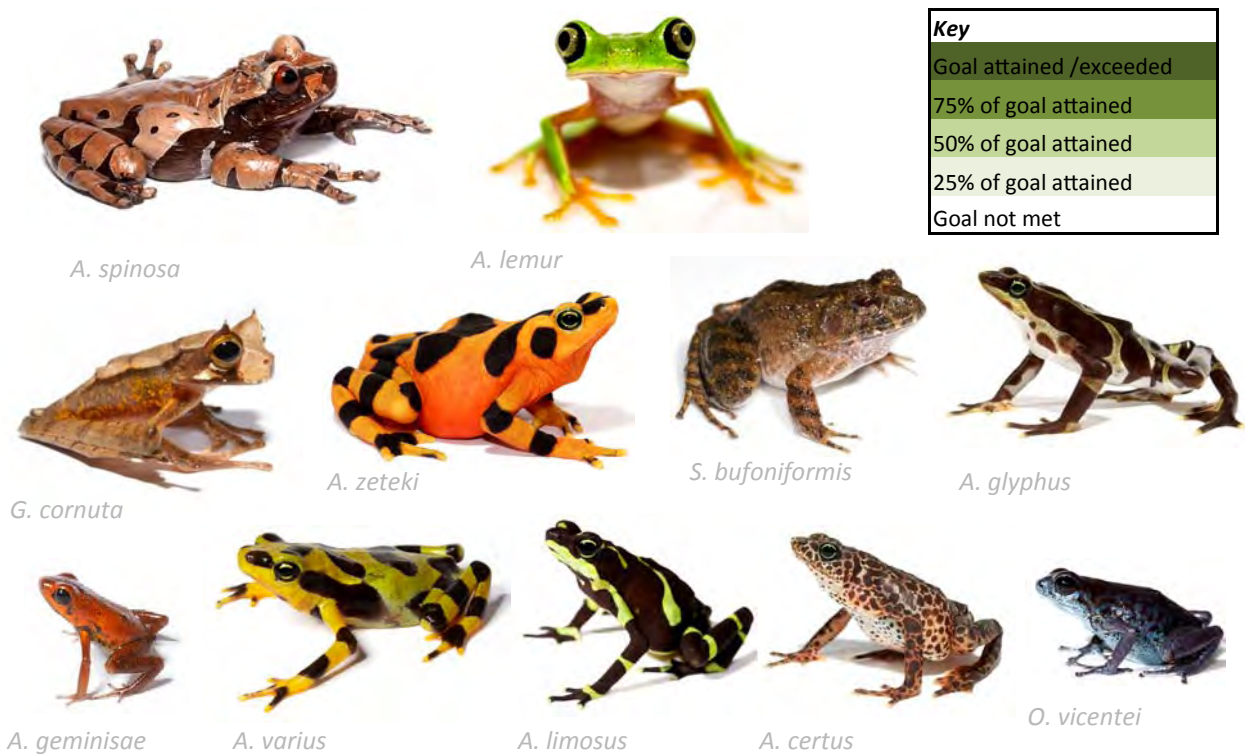
*Cover image: A horned marsupial frog *Gastrotheca cornuta* female with a pouch full of eggs, this is a high priority conservation species being held and bred at the El Valle Amphibian Conservation Center. Photo © Joel Sartore, Photo Ark, reproduced with permission.*

GOAL 1: Create assurance colonies of Panama’s most vulnerable amphibian species

To date we acquired 20 pairs or bred 10 pairs, whichever ratio is greater, for 8 species and bred at least 10 founding pairs of two species (Table 1). Collectively, we are working with 12 species of conservation concern.

Table 1: Progress toward captive population management goals at EVACC (El Valle Amphibian Conservation center) and the Gamboa ARCC (Amphibian Research and Conservation Center).

Species	% min required founder population	Founder pairs bred (goal 10 pairs)	Number of frogs (goal 500)
<i>Atelopus certus</i>	1	12	250
<i>Gastrotheca cornuta</i>	0.95	13	149
<i>Atelopus limosus (chevron)</i>	1	8	82
<i>Atelopus glyphus</i>	1	5	66
<i>Oophaga vicentei</i>	1	5	49
<i>Andinobates geminisae</i>	0.925	4	38
<i>Atelopus varius (lowland)</i>	1	1	52
<i>Craugastor evanesco</i>	1	0	44
<i>Anotheca spinosa</i>	0.5	7	150
<i>Atelopus varius (highland)</i>	0.4	4	50
<i>Strabomantis bufoniformis</i>	0.575	2	41
<i>Agalychnis lemur</i>	0.25	1	72
<i>Atelopus zeteki</i>	0.2	2	23
<i>Atelopus limosus (brown)</i>	0.2	2	9





Chiriqui harlequin frog Atelopus chiriquiensis was reevaluated during the IUCN redlisting workshop and was listed as extinct, likely due to the effects of the chytrid fungus. Photo (c) Marcos Guerra,

Prioritization

We have learned a lot about how chytridiomycosis has affected Panamanian amphibians in the last 10 years and we conducted an expert survey to take stock of its effects. We found that of Panama's 214 described amphibian species, about 100 species can still be reliably found even in places where the chytrid fungus is present, and experts consider these species less susceptible to the fungus. Approximately 80 species are very rare, and we simply do not have any idea about their susceptibility to chytridiomycosis, or their current population numbers. Thirty-six species were considered highly susceptible to the chytrid fungus and were once reliably encountered but have experienced, or are predicted to experience, severe chytridiomycosis-related declines. We evaluated the probability of avoiding extinctions of these susceptible species through captive breeding efforts and published our findings in *Animal Conservation*.

We partnered with the IUCN Red List to help reevaluate all Panamanian amphibian species and have conducted literature reviews of all publications since 2004. In 2015, we conducted a Red List workshop with about 20 Panamanian experts to 1) evaluate all new species; 2) revise all species our expert survey identified as needing to be reconsidered; and 3) revise all species in our captive conservation programs. Combined with our previous 'Least Concern' species update, this leaves about 70 species for Panama that still require updating.

Husbandry

This year we continued to improve and update our record keeping using the ZIMS software program. This was facilitated at EVACC through a generous donation of satellite internet from Odebrecht. We updated all of our husbandry manuals and now have facility-specific husbandry manuals for EVACC, Gamboa and the Punta Culebra Nature Center. We began a tadpole nutrition research project to investigate the role of protein content in the development of tadpoles in conjunction with the University of Derby and the Waltham Center for Pet Nutrition, funded by the Waltham Foundation. We published our findings on the stress effects of group housing on *Atelopus* (Cikanek et al 2014) and on the effects of captivity on the skin microbiome of Panamanian golden frogs (Becker et al 2014).



Assisted reproduction and sperm cryopreservation

This year Gina DellaTogna graduated from the University of Maryland after successfully defending her thesis “Structural and Functional Characterization of the Panamanian Golden Frog Spermatazoa – Impact of Medium Osmolality and Cryopreservation on Motility and Cell Viability.” Gina is the first Panamanian to earn her PhD studying Panama’s national amphibian. Gina successfully tested the methods she developed for her

Ph.D. on three other *Atelopus* species and cryopreserved the samples. She will continue her assisted reproduction work on a post-doctoral fellowship supported by the Wood Tiger Foundation and will assist the project to help meet our reproduction and genetic management goals.

Veterinary care

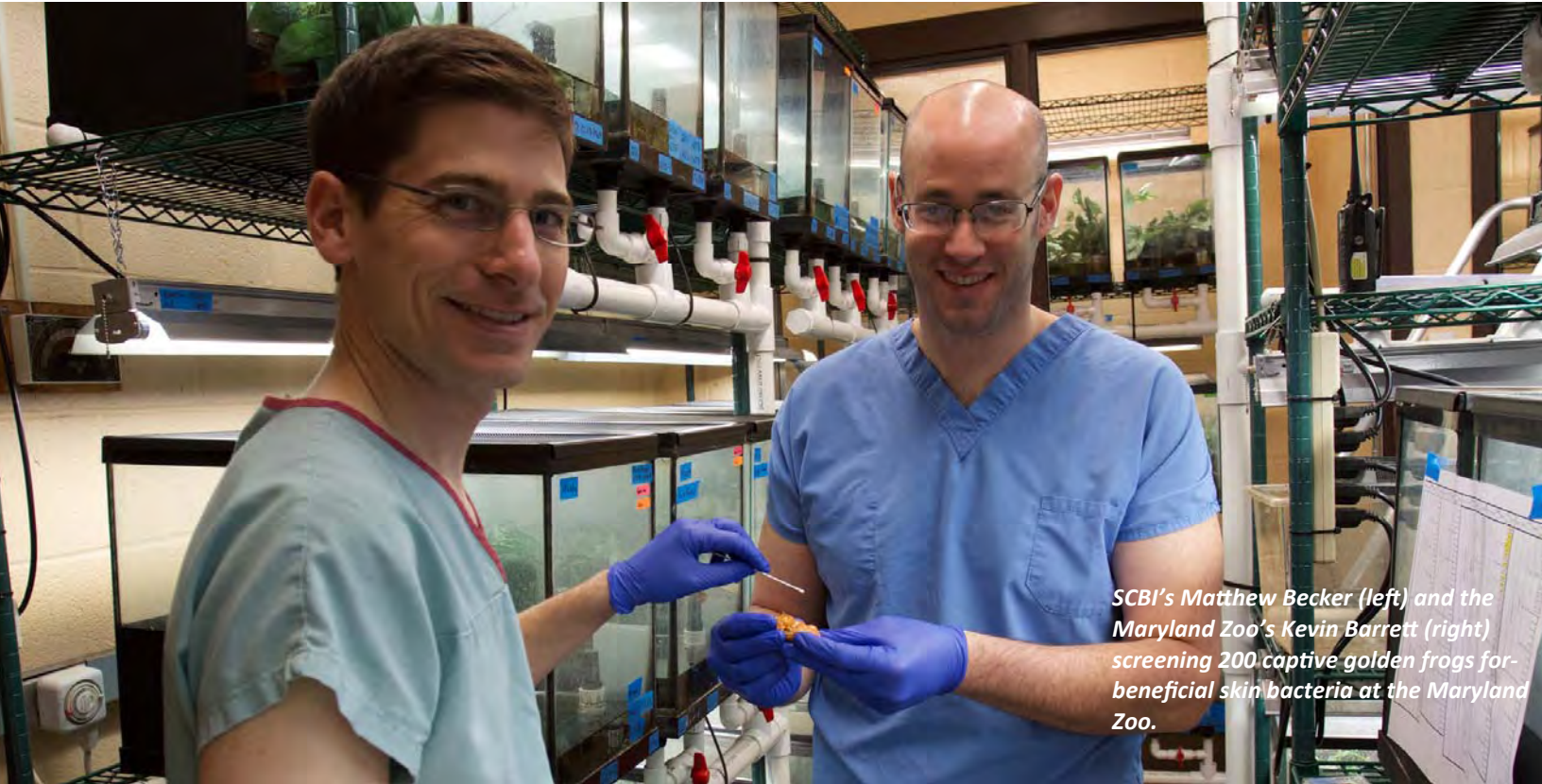
We continue to coordinate with the veterinary team from remote locations. The team is collaborating on developing quarantine and treatment protocols for the lowland *Atelopus varius* population that have been entering the collection with coinfections with chytridiomycosis and *Fusarium*. The *Fusarium* species was identified by our colleagues at the USDA as *F. keratoplasticum*. We conducted some inhibition assays to inform treatment plans for infected animals. We also conducted experiments to fulfill Koch’s postulates with the *Fusarium* isolates using non-priority frog species, but the results were not conclusive. The other major veterinary issue we have been focused on is an edematous condition linked to kidney lesions that has been observed in our *Atelopus* species. We are planning to conduct some diagnostic pathology tests this year in collaboration with Allan Pessier from San Diego Zoo to identify causes and husbandry experiments to resolve the issue. Diet and nutrition continue to be an important topic in the prevention of certain diseases and conditions that can be commonly seen in captive amphibians.

Expeditions

The facilities have coordinated eight field trips together to collect founding populations of the priority species located in the Minera Panama S.A. mining concession area. Between the two facilities there are now representative founders from six separate species. We will continue to produce and execute these work plans into the future.



Atelopus stream in the Donoso Region



SCBI's Matthew Becker (left) and the Maryland Zoo's Kevin Barrett (right) screening 200 captive golden frogs for beneficial skin bacteria at the Maryland Zoo.

GOAL 2: Re-establish healthy wild populations of Panamanian amphibians

Developing tools to inform reintroductions in Bd-positive areas

Smithsonian post-doctoral Fellow Matthew Becker worked with support from the Golden Frog Species Survival Plan and the Maryland Zoo in Baltimore to screen the skin microbiomes of 200 captive golden frogs in order to identify individuals that might have the same microbiome signatures that were associated with survival in previous chytrid-exposure trials. We analyzed these swabs and 16 frogs had microbes associated with survival from previous experiments and created cultures of bacteria isolated from golden frog skin. Dr. Becker has begun the final stage of the experiment, which will involve testing different types of probiotic applications to the frogs and looking for probiotic effects, or simply differential disease outcomes. We have established a biorepository of samples that will allow us to examine the metatranscriptomes of frogs, microbes and the fungus during the course of an infection and hopefully to explain the relative influence of the host vs. the microbiome in determining a disease outcome.

We also began analyzing results of a separate study conducted by Smithsonian post-doctoral fellow Anna Savage examining differential disease outcomes in lowland leopard frogs that are moderately susceptible to the chytrid fungus. The aim is to understand why some individuals in a species are more susceptible to the disease than others by analyzing various immune indicators and transcriptomes.

Reintroduction planning

We translated the IUCN Conservation Breeding Specialist Group report “The Golden Frogs of Panama (*Atelopus zeteki*, *A. varius*): A Conservation Planning Workshop Final Report” into Spanish. The plan lays out a detailed framework of actions to lay the groundwork for experimental reintroductions of Panamanian golden frogs in the wild. A formal implementation group meets periodically to coordinate implementation of the plan among different stakeholders.

GOAL 3: Build capacity in Panama to safeguard the nation's amphibian fauna over the long-term

This year completed phase two of our Gamboa Amphibian Research and Conservation Center and officially opened it in April. The new building has office space, storage, a lab and quarantine room as well as an exhibition with interpretation about our project.

Table 2: Ex situ amphibian conservation space built, or under construction, in Panama.

Ex situ conservation space	Complete	Incomplete	Completion date
EVACC exhibit	900		2008
EVACC frog holding area	1,500		2008
EVACC Minera annex	1,000		2013
EVACC pod		400	2016
Gamboa ARCC Phase 1	2,800		2013
Gamboa ARCC Phase 2	2,200		2015
Total sq ft	8,380	400	

The new Minera annex at EVACC is open and housing frogs, and has 500 square feet of living space on the upper floor. The main building at EVACC is eight years old and has several infrastructural issues that have consumed staff time this year. We are conducting a general infrastructure appraisal on the main building, to understand and prioritize needed upgrades and maintenance. A termite issue, coupled with years of humidity in the building has rendered all wooden shelves inside the main facility obsolete and need to be replaced with metal shelving. We have replaced twenty shelving units this year and the remainder will be replaced in 2016.

Twelve full time staff run the rescue project. As we continue to reach project goals and see increased numbers in the amphibian collection, the demand of labor also increases. We have identified the need for additional full-time staff at each facility. With our growing frog collection we would like to hire an additional keeper at each facility, but this will require commitments from new long-term partners that we are actively soliciting. From a funding perspective, we are actively working to build a support base of potential contributors and supporters in Panama that have toured our facilities extensively. We renewed our grant from Minera Panama that together with core partner-provided funding continues to provide funding for staff salaries, training, and animal care over the longer term. This is supplemented by project-specific grants and external collaborations.



Cutting the ribbon at the opening ceremony for the new Gamboa Amphibian Rescue and Conservation Center



Exhibition niche where visitors can peek inside a working amphibian rescue pod at the Gamboa Amphibian Research and Conservation Center.

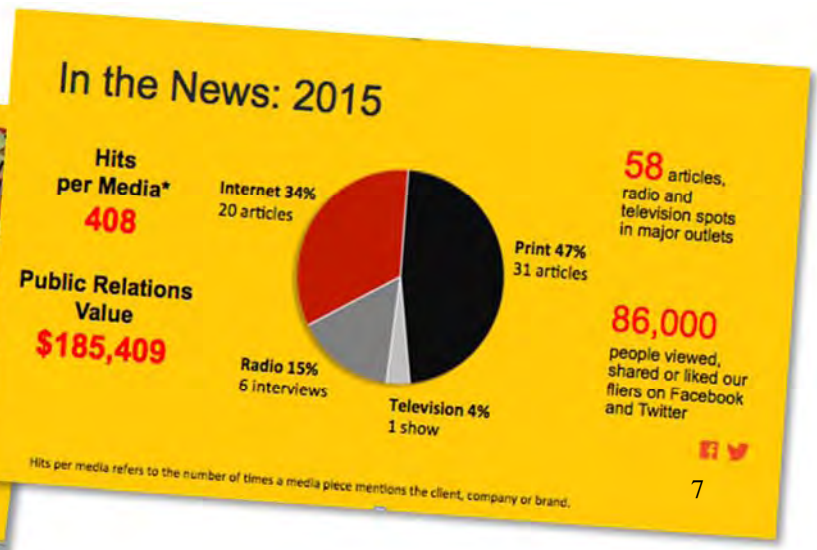
GOAL 4: Cultivate and foster an appreciation for amphibians in the public mindset

Exhibits

Our education masterplan identified the Punta Culebra Nature Center facility at the Smithsonian in Panama City and the El Valle Amphibian Conservation Center as our two venues with the highest visitor rates. The “Fabulous Frogs of Panama” at the Punta Culebra Nature Center in Panama is very popular and continues to draw approximately 58,000 people. About 15,000 of these visitors were schoolchildren and teachers who took part in the school program that includes an informal learning curriculum with frog-based activities. The exhibition area at EVACC is visited at least 100,000 visitors annually and is in need of new interpretive signage, maintenance, and repair. We are seeking funding for these upgrades to be able to reach a larger audience to share the important conservation messages and aims of the project. The exhibition area at EVACC is located inside the privately owned Nispero Zoo, and continues to be the only place in the country where the Golden frogs are exhibited. Gamboa does not receive a lot of visitors, this year we developed a small interpretive exhibit explaining the project and allowing passersby in the community an opportunity to look inside a working amphibian pod. We are seeking funding to complete the security fence and gate to this area that would allow passers-by access to the exhibit area.

Media

Our project was covered in 15 independent English and Spanish news stories in 2015, with more Spanish news articles than English ones this year! Our online constituency continued to grow steadily: we now have 4,000 Twitter followers, 9,700 Facebook fans, 3,000 Instagram followers and 42,000 unique visitors to our bilingual website (about 7,000 Spanish visitors). We have not been very successful, however, at leveraging this constituency for online contributions which raised about \$2,000.





Golden Frog Festival 2015

Several events throughout the month of August marked the Golden Frog Festival that has a growing list of co-sponsors and event partners. In El Valle de Anton, ANAM led and organized the 2014 Golden Frog Day Parade and in Panama City we hosted a frog-themed education night at the newly opened BioMuseo, which was attended by more than

900 people. Over the weekend we organized fun family days at Rey Supermarkets in El Valle (700 people attended), Punta Culebra Nature Center (600 attendees) and MultiPlaza Mall in Panama City, where thousands of mall goers took part in the festival exhibits and activities in the La Luna Plaza. All of the events included live frog exhibits, frog restaurants (insects!), as well as a variety of frog-themed art, games, and talks, and even a juggler and acrobatic show with the acrobats dressed up as golden frogs. Caminando Panama hosted the second annual La Dorada 5k/15k Trail Run that was sold out (275 runners or walkers participated), doubling the number of participants from the previous year. The Gamboa lab also hosted an open house for the local community, and scientists announced the Festival at the National Lottery, where the ticket featured a golden frog.

With the help of an in-kind donation from Stratego, a local communications firm, we hosted media tours of the new Gamboa facility and exhibit, and carried out an extensive traditional and social media campaign during the Festival, creating a variety of promotional materials (see below) capitalizing on the slogan 'Saving a National Treasure.' The Festival received extensive publicity: 58 articles in print, online and TV outlets; 86,000 hits on our fliers or event announcements; and a visit by Smithsonian scientists to the National Lottery. This all had the potential to reach an estimated audience of 3 million people. The estimated public relations value of the media effort was more than \$185,000.





New Gamboa Amphibian Research and Conservation Center



Heidi Ross with captive-bred *Atelopus* in the Minera annex at EVACC.



New Gamboa Amphibian Facility



La Dorada Trail Run hosted by Caminando Panama



Gamboa frog team



Captive-bred *Geminis'* dart frog tadpole *Andinobates geminisae*

FINANCIAL REPORT FOR CALENDAR YEAR 2015

FUNDING SOURCE	Purpose	2015 expenses
<i>Houston Zoo and EVACC donors</i>	<i>Salaries and operating costs EVACC</i>	<i>\$ 63,150</i>
<i>Minera Panama</i>	<i>Salaries and operating costs EVACC & Gamboa, Donoso expeditions</i>	<i>\$ 415,125</i>
<i>BBVA</i>	<i>Panama project operating costs</i>	<i>\$ 80,000</i>
<i>USAID</i>	<i>Final construction expenses Gamboa facility</i>	<i>\$ 166,829</i>
<i>Zoo New England</i>	<i>Salaries</i>	<i>\$ 20,000</i>
<i>Cheyenne Mountain Zoo</i>	<i>Salaries</i>	<i>\$ 20,000</i>
<i>USFWS</i>	<i>Research for cure</i>	<i>\$ 36,900</i>
<i>SCBI and donors</i>	<i>Coordinator salary & travel, postdoctoral fellow</i>	<i>\$150,000</i>
		\$ 952,004



Donors

In addition to the contributions from project partners and sponsors listed above, we are grateful to the following donors who have made additional contributions to the project directly or via the Houston Zoo: Buffalo Zoo, David Castro, Dickerson Park Zoo, Susan and Frank Mars, George and Mary Rabb Foundation, the Wood Tiger Foundation, the Waltham Foundation, The Shared Earth Foundation, The Anele Kolohe Foundation.

Online Contributions

Our sincere thanks to the following individuals who contributed \$25 or more online: Heather Comfort, Kenneth Foster, Brian Gratwicke, Cindy Hoffman, Fred Kromm, Gladwyn Leiman, Peter MacPherson, Sophie Redfern, Iain Robertson, Julio Rodriguez, Virginia Salzman, Edward Samuel, Howard Schloss, Eric Stubbs.

Golden frog day sponsors

National Science Foundation, Rachel Page, Mike Ryan, Ryan Taylor, Fundacion Smithsonian, Jamie Voyles, Multiplaza, Oferta Simple, EVACC, SENACYT.

2015 Scientific Publications Related to the PARC Project or involving PARC staff collaboration.

Gratwicke B., Ross H., Batista A., Chaves G., Crawford A.J., Elizondo L., Estrada A., Evans M., Garelle, D., Guerrel J., Hertz A., Hughey M., Jaramillo C. A., Klocke B., Mandica M., Medina D., Richards-Zawacki C. L., Ryan M. J, Sosa-Bartuano A., Voyles J., Walker B., Woodhams D. C., and R. Ibáñez (2015). Evaluating the probability of avoiding disease related extinctions of Panamanian amphibians through captive breeding programs. *Animal Conservation*. DOI: 10.1111/acv.12249

Belden LK, Hughey MC, Rebollar EA, Umile TP, Loftus SC, Burzynski EA, Minbiole KPC, House LL, Jensen RV, Becker MH, Walke JB, Medina D, Ibáñez R and Harris RN (2015) Panamanian frog species host unique skin bacterial communities. *Front. Microbiol.* 6:1171. doi: 10.3389/fmicb.2015.01171

Rebollar EA, Hughey MZ, Medina D, Harris RN, Ibáñez R and Belden LK (2015) Skin bacterial diversity of Panamanian frogs is associated with host susceptibility and presence of *Batrachochytrium dendrobatidis*. *ISME Journal*. doi:10.1038/ismej.2015.234

Becker, Matthew H., Walke, Jenifer B., Cikanek, Shawna, Savage, Anna E., Mattheus, Nichole, Santiago, Celina N., Minbiole, Kevin P. C., Harris, Reid N., Belden, Lisa K. and Gratwicke, Brian. 2015. Composition of symbiotic bacteria predicts survival in Panamanian golden frogs infected with a lethal fungus, *Proceedings of the Royal Society of London B: Biological Sciences* 282: 20142881.



*Jorge Guerrel with an experimental tadpole rearing setup to investigate the effect of protein on *Atelopus* tadpole growth, funded by the Waltham Foundation.*

Steering Committee

Bob Chastain, President and CEO, *Cheyenne Mountain Zoo*;
Peter Riger, Director of Conservation, *Houston Zoo*;
Steve Monfort, Director, *Smithsonian Conservation Biology Institute*;
Matthew Larsen, Director, *Smithsonian Tropical Research Institute*;
John Linehan, President and CEO, *Zoo New England*.

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Dr. Roberto Ibáñez, *Project Director, Panama*;
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Nair Cabezon, Lanki Cheucarama, Rigoberto Díaz, Nancy Fairchild, Estefany Illueca, *Technical Staff*.

Heidi Ross, *El Valle Amphibian Conservation Center Director*;
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Brad Nissen, *Intern*;
Diana Troetsch, Abileth González, Edgardo Griffith, *Minera Panama support staff*.

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2015 Volunteers

We are grateful to the following volunteers for their generous assistance: Erick Barria, Antonio Delgado Velayos, Wong Chuk Kwan, Juan Antonio Licea, Jordi Maggi, and Joe Porter.

2015 Golden Frog Festival

Organizing Committee: Sharon Ryan, Crystal Dimiceli, Nelly Florez, Sonia Tejada, Alvaro Gonzalez, Jimena Pitty, Rigoberto Diaz, Roberto Ibanez, Adrian Benedetti, Ana Endara, Sean Mattson, Carlos Celis, Ana Lucrecia Arosemena, Heidi Ross, Rebecca Rissanen. **Volunteers:** Karold Agrioyanis, Asquena Algilar, Kary Almengor, Carla Baldan, Haydee Basto, Kiria Berdiales, Karen Blackall, Angelica Casaretto, Manuel Chanis, Crystal Di Miceli, Rigoberto Díaz, Angie Estrada, Nancy Fairchild, Nelly Flórez, Anna Frogge, Maria Garcia, Katherine Gomez, Katherine Gonzalez, Lina Gonzalez, Jorge Guerrel, Wendy Guerrero, Maria Gutierrez, Jeymmi Hinds, Beth King, Marina Leccesse, Ana Lesne, Adrian Lezcana, Gisselle Liao, Katherine Lievano, Alicia Mata, Juan Murillo, Florencia Nunez, Emmy Quintero, Emigdio Romero, Ana Matilde Ruiz, Sharon Ryan, Ramiro Solis, Sonia Tejada, Nicole Thompson, Francis Torres, Katie Uckele, Pino Yesuri.

Participating organizations: Ministerio de Ambiente de Panamá, Smithsonian Tropical Research Institute, Fundacion Smithsonian, El Valle Amphibian Conservation Center, Panama Amphibian Rescue and Conservation Project, La Rana Dorada Pub, SENACYT, USAID, National Science Foundation, APRADAP, Uber, Biomuseo, Under Cover Rain Boots, Oferta



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