

HUM May 2022

WISPS FROM THE CLOUD FOREST: GLASS FROGS OF RESERVA LAS GRALARIAS

By Jane A. Lyons

Growing up in hot dry central Texas I had little experience with amphibians of any kind. My first encounter with frogs was in elementary school at about age 9 when I took my mother's baking dish to the nearby creek and collected tadpoles to bring them home to raise them in my bedroom. Someone had told me that they would turn into frogs and I wanted to see that. My father thought it was a fun project but my mother was horrified at the thought of my rearing wild animals in my bedroom (I think she threw away the baking dish). My father did agree with her so I took the small blackish tadpoles back to the creek. I recall years later having to dissect frogs in high school biology class, which was pretty gross in my view. I remember vividly my first visit to Costa Rica in 1985 where, after 12 hours of heavy rain, we ventured out on a trail at Monteverde and found an amazing breeding frenzy of golden toads (*Incilius periglenes*) in a big puddle of rainwater collected around the roots of a large cloud forest tree. That species of golden toad is now extinct.

On a later trip to Costa Rica in 1987 we encountered a humongous toad of some sort on the ground next to our table at an outdoor restaurant in Manuel Antonio National Park. I worked on salamander conservation in the 80s and 90s in Austin as part of the Balcones Canyonlands Habitat Conservation Plan's efforts to conserve the underground aquifer and its many endemic denizens. When I fell in love with the Venezuelan cloud forest in 1991 it was because of its amazing avifauna, although the wonderfully cool montane tropical climate was a near-equal attraction. But I really had no idea of the amphibians found in the cloud forest. I do recall in 2002 while first exploring RLG seeing a brown frog lay tiny round golden eggs in a hole in the ground. That pretty well sums up my experience with amphibians until 2005. I did understand the importance of frog diversity and also as an indicator group of certain types of habitat quality.

After years of travelling, living, and working throughout South America I also had come to realize that indigenous peoples of this continent almost 'worshipped' frogs, or at least thought of them as very important. Why? Because frog toxins allowed for poison-tipped arrows used to hunt and kill enemies, and the songs of frogs indicated pure water. I have a quiver of poison-tipped arrows made from the poison dart frogs of Venezuela and a hand-made clay water jug decorated with clay frogs from Paraguay as well as various other indigenous craft items decorated with frogs. Frogs are in fact important symbols of a healthy habitat to indigenous peoples of South America.

By 1997 I was asking myself, "Where better to live and enjoy clean air and pure water and amazing biodiversity than in the cloud forest?"



My real official frog education began when I met Kathy Duffey on her first visit to RLG. Friends were visiting and we all toasted the inauguration of our new facilities at RLG.



Photo by Jane A. Lyons, 2005

Kathy had worked with glass frogs in Panama and Ohio on a project to prevent amphibian extinction due to a deadly and apparently world-wide fungus. But until Kathy and her then-boyfriend/now-husband Tim Krynak began to look for frogs at RLG, no one knew what frogs and other amphibians were found on our various creek-crossed properties. They have been back to RLG many times, with grant money for frog transects, with biology students, with a Fulbright fellowship and just on their own, to do all sorts of frog work at RLG. They even discovered a new species that has changed the manner in which frogs are now studied.

When they found some interesting frogs and ones they could not identify, Tim and Kathy contacted herpetologists at various universities including the Catholic University in Quito. These were serious world-class frog experts who soon began to bring their students and other researchers to RLG. Since then we have had regular frog field projects by Kathy, Tim, and numerous other researchers. With their help I began to learn about frogs and participated in some way in the many field projects at RLG. I even discovered some new frog vocalizations and natural history data. I am still a novice but have managed to absorb more and more frog information. I have certainly become another frog fanatic and seriously concerned about conservation of these amazing and ancient creatures in our modern and fast-changing world.

At RLG we do ‘worship’ our frogs. We have rain frogs, pond frogs, tree frogs, glass frogs and possibly something else still to be discovered. Our various species of rain frogs are active most of the year except in summertime. Tree frogs seem to be busy by the end of the year; our pond frogs are busy by February when our pond is full of water plus small puddles of water are found elsewhere. May is our “Frog Month” because by May we have had enough

rain over many months to make our creeks flow steadily with the pure water and high air humidity that our rarest frogs, the glass frogs, need for successful reproduction. Although some of our glass frogs begin their breeding season as early as January, by May all of our creeks are flowing with enough water to allow our rarest frogs to lay their eggs on leaves overhanging the creeks. The humidity is high enough to allow those eggs to expand and develop and eventually hatch as the tadpoles leap into the pure water creek below. In May the last male glass frogs finally begin to call and come down from high in the trees to proclaim and defend their breeding territory. Females hear their calls and know the creeks are flowing, so the scene is set for pairing, egg laying and ‘rearing’ the young. It is a perfect example of nature’s intertwined systems and the critical role of a well-functioning healthy water cycle.

Hidden Pools at RLG. The clean clear free-flowing water of our creeks is the perfect home to glass frog tadpoles.



Photos by Jane A. Lyons

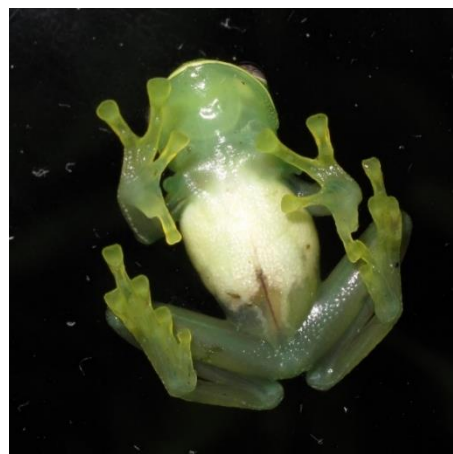
All of these frogs have their corresponding scientific data although very little is actually known about their behavior and life histories. Over the years at RLG we have added to the understanding of the life cycles of some of these species while others remain basically understudied.



Searching for glass frogs in Lucy's Creek. Why take a ladder to study the glass frogs of a creek? Because the males stay high in the trees until breeding season begins.

Photo by Henry Imba

So, what exactly is a glass frog? A glass frog is a member of the frog family Centrolenidae. As with many neotropical taxa, changes, new research, confusion, disagreement, splits and lumps, etc. are common within the evolving versions of frog taxonomy. For now, Centrolenidae is the family of glass frogs and which are found only in the neotropical region. They are defined by certain morphological features which include rounded snout, transparent skin usually on the belly but also on toes and legs, often allowing internal organs and even bones to be visible through the skin. The dorsal area is not transparent but is normally a solid green background sometimes with various spots. No glass is involved!



Roundish snout, transparent skin on belly, toes and even on legs but not on the frog's back which is normally some shade of green.

Photos by Segundo Imba

We have glass frogs all across the 1500 acres of RLG, including at all elevations. This is likely because we have creeks, waterways, drainages throughout the reserve that run in all directions due to the complexity of our flat plus hilly topography. This complexity also makes it hard for humans and domestic animals to access many areas which in turn helps protect out frogs from human-caused disturbance.

Here is a list of the eight species in three genera of glass frog that we currently know we have at RLG and their conservation status.

Centrolene ballux: Golden-flecked Glass Frog, considered Critically Endangered

Centrolene heloderma: Bumpy Glass Frog, considered Critically Endangered

Centrolene lynchi: Lynch's Glass Frog, considered Endangered

Centrolene peristictum: Dappled Glass Frog, considered Near Threatened

Espadarana prosoblepon: Emerald Glass Frog, considered Least Concern, abundant and widespread

Nymphargus grandisonae: Red-spotted Glass Frog, considered Least Concern, locally abundant and widespread

Nymphargus griffithsi: Pepper Glass Frog, considered Vulnerable, small range and specific habitat

Nymphargus lasgalarias: Las Galarias Glass Frog, considered Data Deficient, little studied and known only from two locations.



Distribution and data known from RLG

Thanks to field work and research projects conducted by many people at RLG since 2005 we have some extensive knowledge of a few of our glass frog species and at least basic documentation about others.

♦ *Centrolene ballux* : Golden-flecked Glass Frog, considered Critically Endangered

Even though considered critically endangered, at RLG *C. ballux* is one of our most abundant species of glass frog. It becomes active in the earlier part of the rainy season mostly at our mid-elevation sites and continues reproductive activity through much of the late spring rainy season. It seems to be somewhat aggressive to other species found in its preferred habitat (dense, wet cloud forest) and appears to be confined primarily to this narrow elevational zone.

(Below) Metamorph of *C. ballux* along Kathy's Creek. It will slowly grow to absorb the tail and then be a full adult frog. This 'metamorphosis' from tadpole to frog occurs within the creek water and often at least partially under the (normally sandy) substrate.

Photos by Ray So



(Right) Egg mass of *C. ballux* found hanging on a Melastomataceae leaf along 5 Frog Creek at RLG, March 2017.

(Below) Adult *C. ballux*, flecking visible



◆ *Centrolene heloderma*: Bumpy Glass Frog, considered Critically Endangered

C. heloderma has always been our rarest of the glass frogs at RLG, with the only known reproductively active population. In 2017 Dr. Katherine Krynak discovered a previously unknown population of this rare species outside of RLG and which makes us hopeful that additional populations may be found.



Adult Bumpy Glass Frog

Photo by Ray So

At RLG it is found only at our higher elevation sites along flowing creeks that have substantial overhanging native vegetation. We have documented problems with fruit fly (*Drosophilidae*) parasitism with this species where the flies lay their eggs inside the frog eggs, and the fly larvae develop as they feed on (and kill) the frog eggs. This parasitism of glass frog eggs by flies has been documented since the 1970s in Central America. We noted at RLG that the incidence of fly parasitism of *C. heloderma* eggs seemed to be much increased in areas where forest had been cut to make way for cow pasture. In more forested zones the parasitism was significantly less.



Healthy eggs of *C. heloderma* (left); parasitized eggs of *C. heloderma* (right)

Photos by Segundo Imba

◆ *Centrolene lynchi*: Lynch's Glass Frog, considered Endangered

At RLG *C. lynchi* is not abundant or at least is very difficult to find. It has a very narrow elevational range, found between 1800 - 2050 m elevation at RLG along a permanent creek. In January-April 2010 a team led by Nicole Dautel studied the calls and behavior of this little-known species. They studied 21 males along an 800 m transect at RLG. Their research resulted in altitudinal and range extension records as well as the first records of four different calls and physical combat behavior of this species. This study also resulted in documentation of courtship, egg deposition, male egg attendance of up to 3 egg masses at a time, egg development and hatching. No egg parasitism nor fungal disease was reported in this study.



Adult male *C. lynchi*

Photo by Ray So

Healthy egg mass

Photo by Segundo Imba

◆ *Centrolene peristictum*: Dappled Glass Frog, considered Near Threatened

C. peristictum is a very small frog and a glass frog that begins its breeding season somewhat earlier than our other glass frogs. It is the most studied glass frog at RLG and is found primarily in a dispersed colony along lower Lucy's Creek. Individuals (usually calling males) have also been found in all of our mid-elevation permanent waterways. Thesis work by Ana Salgado and her team was the first to document the behavior, and especially the parental care of *C. peristictum*. Later projects at RLG led by Jesse Delia, Ray So and Anyalet Valencia added much data about *C. peristictum* primarily focused on parental care, combat behavior and egg parasitism. The adult males of this species will "protect" multiple clutches of eggs on the same leaf, even bring water to them via a fold in their skin, if the egg masses need rehydrating. The males will fight physically with each other to control the best site for females, and then the male will try to control that site until he has 3-4 egg masses to care for. Parental care by these adult males includes the rehydrating to keep eggs from desiccating as well as keeping insects, parasites and predators away from the eggs and defending them from other adult males.

(Next page photos) The bright green eggs of *C. peristictum* hanging in a gelatinous mass from a fern stem (left). Even still within the egg these are already showing their 'tadpole tail' indicating they are close to hatching. When each egg hatches the tadpole inside leaps from the enclosing egg into the air and then drops into the flowing creek below. One of our volunteer researchers managed to film a leaping *C. peristictum* tadpole!



Normally, but not always, the female *C. peristictum* lays her eggs on the underside of a leaf overhanging a creek. These eggs (right) are clearly in the soon-to-emerge tadpole stage.



A *C. peristictum* tadpole still showing its bright green color even under water (left). A calling adult male *C. peristictum* (right)

C. peristictum eggs in various sites at RLG have also been parasitized by fly larvae. We reared one of the parasitic fly larvae in our lab to see which fly it was, and it appears to be a large-red-eyed species of Drosophilidae (fruit flies). Photo below.



Photos by Ray So

- ◆ *Espadarana prosoblepon*: Emerald Glass Frog, considered Least Concern, abundant and widespread

This easily identifiable glass frog has been found only at our lower site and with only a few individual males. It is normally more abundant at elevations some 1000 m lower than our lowest site. Seemingly it is not as dependent on flowing water but more dependent on leaf litter habitat. The eggs and tadpoles of this species are blackish, likely for better camouflage in leaf litter.



Adult male *E. prosoblepon*

Photo by Dana Wessels



Photo by Segundo Imba

- ♦ *Nymphargus grandisonae*: Red-spotted Glass Frog, considered Least Concern, locally abundant and widespread

At RLG this species is found at our lower, mid-and upper-elevation sites. From 2 April – 3 July 2011 Carl Hutter studied this species at RLG’s “5 Frog Creek”. Based on the activity of 25 males and 14 females, he and his team were able to document high site fidelity of males, details of male combat behavior and territoriality as well as six different kinds of calls made by males. The unique pattern of the red spots on each male made it possible to identify individuals.



Photo by Segundo Imba



Photo by Ray So

- ◆ *Nymphargus griffithsi*: Pepper Glass Frog, considered Vulnerable, small range and specific habitat

This species may be the most mysterious of all our glass frogs. At RLG it is known from only one small area. The initial study of this species identified it by its call as well as its black-speckled dorsum. Then a nearby different call of a similar species eventually resulted as a new species altogether, *N. lasgalarias*. Both the call and appearance of *N. griffithsi* seem obviously different from any of the other glass frog species in our area. It has been reported both above and below RLG and in a wide elevational band but is scarce within our properties. It appears that perhaps *N. lasgalarias*, which has a much more restricted elevational range and habitat preference, may in fact dominate the creeks where it is found, pushing *N. griffithsi* to different areas. More field research is necessary to understand the interactions, if any, between these two species.



N. griffithsi is characterized by its peppered-looking back and legs

Photo by Henry Imba

- ◆ ***Nymphargus lasgalarias*:** Las Galarias Glass Frog, considered Data Deficient, little studied and known from only two locations.

The only known regular site of this species is at RLG along the Kathy's Creek complex of waterways at about 2100 m elevation. Based initially on its distinct vocalization, this species was found to be a cryptic species, very similar to *N. griffithsi*, and previously unknown until it was discovered and described from RLG in 2011. Beside its different vocalizations, it is distinguishable by its unspotted body and larger size, and light golden-colored reticulated iris.



Adult male *N. lasgalarias*

Photos by Ray So



Adult male calling next to its egg mass



Tear-drop-shaped egg masses of *N. lasgralarias*

Photo by Ray So

These four egg masses have expanded in the rain and are hanging heavy on the leaves, all protected by one male which is perched at the top of the palm leaf on the upper right. Each egg mass is laid by a different female. As they absorb rainwater and humidity the egg masses expand and become heavier so that the leaf eventually ends up fairly close to the creek below. This should make it easier for the tadpoles to find the creek when they leap out of the low-hanging egg masses.



The white crystalline-looking egg masses of *N. lasgralarias*.



This metamorph of *N. lasgralarias* shows the totally unspotted body of the species.

Photos by Ray So.



One of the main problems of most of our glass frog species seems to be the presence of parasitic fruit flies that are found throughout the reserve. As adults they likely feed on fruit, but the fly eggs are laid inside the frog eggs, thus causing the demise of most, if not all, of the frog eggs in an egg mass. On this leaf a *N. lasgralarias* sits with a parasitic fruit fly on the same leaf. No eggs are seen nearby but ideally the male frog will defend his egg masses from parasitic flies.

Photo by Eric Snyder

We will continue to study, document and conserve the fascinating glass frogs found at RLG.

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