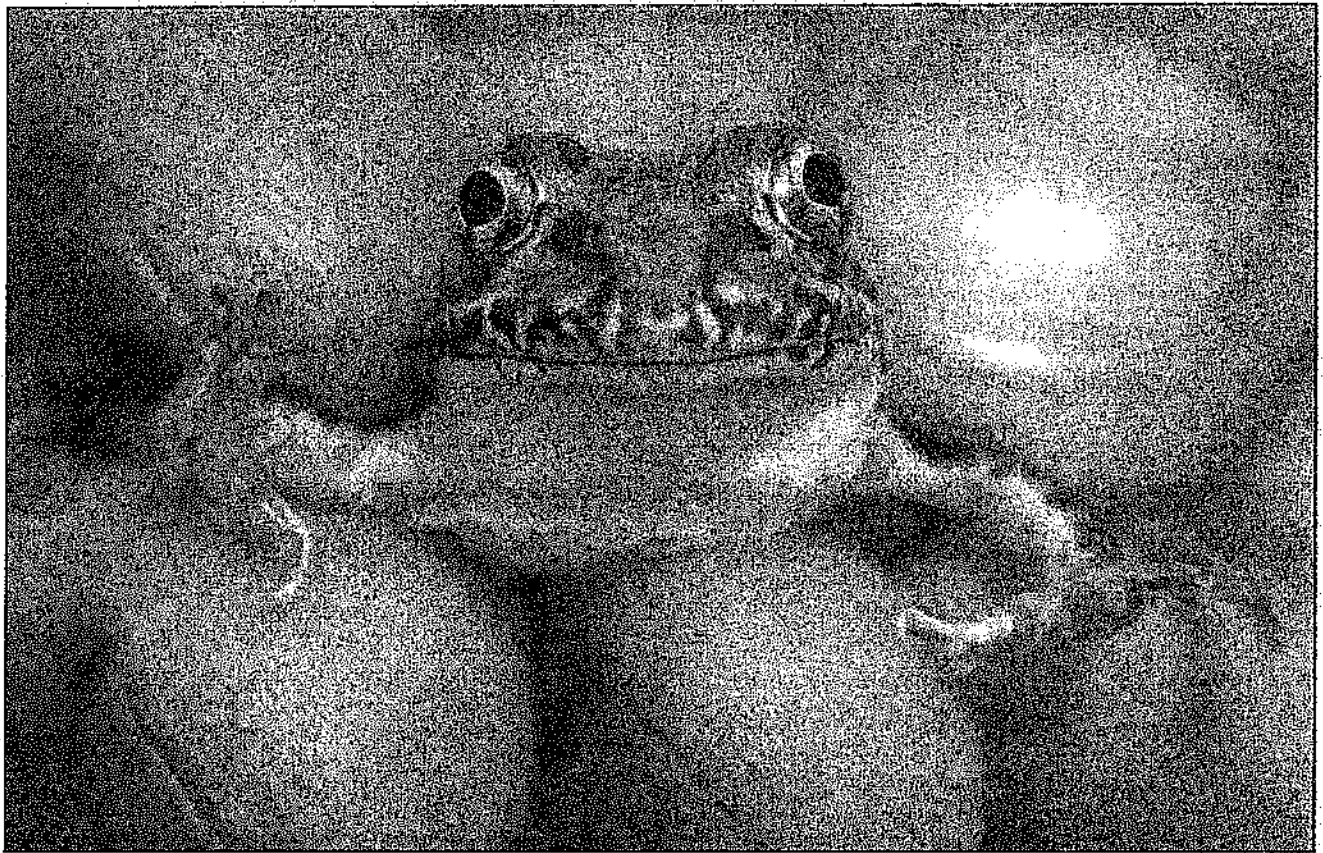


# Leopard frogs on the spot



Photos by Sarah Prall, The Arizona Daily Star

Researcher Don Swann gently holds a young leopard frog, which will be tested for the deadly chytrid skin fungus

## Fungus epidemic is blamed for the species' big decline

By Jim Erickson  
The Arizona Daily Star

With nets and buckets in hand, three biologists hiked into the Catalinas last week to see what was left of a leopard frog population decimated by deadly fungus last winter.

They were pleased to find a couple of dozen seemingly healthy juvenile lowland leopard frogs in the pools of Montrose Canyon, at Catalina State Park. But they saw just one adult, which concerned them.

"That tells me that a lot of the adult frogs died last winter in the epidemic," said Michael J. Sredl, a herpetologist with the Arizona Game and Fish Department.

In January, Sredl went to Montrose with researchers Don

Swann and Erik Enderson. They found 110 dead lowland leopard frogs and one dead canyon tree frog.

"Almost all the frogs we saw were dead, and if they weren't dead they were dying," said Swann, a wildlife biologist at Saguaro National Park. He returned to Montrose on Friday with Sredl and Enderson.

In January, four dead or dying adult leopard frogs, two tadpoles and a tree frog were sent to the Arizona Veterinary Diagnostic Laboratory in Tucson. All the samples tested positive for chytrid skin fungus, which has been blamed for frog die-offs in Australia, Central America and the United States.

The Montrose frog die-off was one of five that occurred in Arizona last winter, and all five



The frogs at Montrose Canyon aren't faring well

were caused by the chytrid fungus, Sredl said. The fungus kills adult frogs, not tadpoles.

Chytrids are microscopic organisms in soil and water that help decompose dead plant and animal matter. Until recently, they had never been known to cause disease in vertebrates.

But in June 1998, researchers reported that chytrid fungus killed frogs and toads in 19 species from Queensland, Australia, and western Panama. Weeks later, University of Arizona herpetologist Phil Rosen said chytrid

See FROGS, Page 3B

# Frogs

Continued from Page 1B

fungus killed leopard frogs at the Cienega Creek nature preserve, southeast of Tucson, and in the San Bernardino Valley east of Douglas.

Amphibian populations are declining worldwide — even in pristine, isolated locations — and scientists are trying to figure out why. In Arizona, native frog populations have been dropping since the late 1970s or early '80s, Sredl said.

Leopard frogs were once common around Tucson, including the Santa Cruz and Rillito rivers. Today they're "damn hard to find" in the Tucson basin and have retreated to about a dozen spots with year-round water, Sredl said.

Habitat loss and competition from introduced species — bass, sunfish, catfish, bullfrogs and crawfish — are the main causes of frog declines in Arizona. Air pollution, water pollution and even increased ultraviolet radiation because of a thinning ozone layer also have been blamed.

Now chytrid fungus has been added to the list of culprits. But scientists aren't sure how big a factor it is in the decline of Arizona frogs, how long the fungus has been here or exactly how it kills the frogs, Sredl said.

The researchers are watching leopard frogs at places like Montrose Canyon to see how infection rates and mortality vary over



Sarah Prall, The Arizona Daily

Enderson, left, and Swann hunt increasingly rare leopard frogs in a Montrose Canyon pool

time.

On Friday, the Montrose pools were alive with hopping young frogs and wriggling tadpoles. Runoff from recent rains splashed over boulders while deer grass, catclaw, seep willows, ash and cottonwoods crowded the banks.

The biologists caught five low-

land leopard frogs and one canyon tree frog. They checked for signs of chytrid fungus infection: blood-red streaks on the legs and between the webbing of the feet.

"They looked like healthy frogs, all of them," Sredl said.

The frogs were sent to the diagnostic lab, where veterinary pathologist Greg Bradley will check

for sporangia, microscopic filled with thousands of chytrid spores.

"It may just be a transient thing that passes through the populations. We don't know," Sredl said.

He urged anyone who finds dead frogs in the wild to call Game and Fish at 628-5376.

September 15, 1999

MR MICHAEL J SREDL  
AZ GAME & FISH DEPARTMENT  
2221 W GREENWAY ROAD  
PHOENIX AZ 85023-4312

Reference number: 99-2655

Dear Mr. Sredl:

Five live *Rana yavapaiensis* and one dead *Hyla arenicolor* were received on September 10, 1999. The frogs were collected from Montrose Canyon, Catalina Mountains, Pima County Arizona. This population had experienced a die off last winter due to chytrid fungal infection. These animals were not apparently ill.

**Gross necropsy findings:**

Grossly, the *Hyla arenicolor* is unremarkable. All of the *Rana yavapaiensis* contain tiny red nodules in the skin of the hind limbs and abdomen. All have full stomachs and are in good flesh. There are no other remarkable gross findings.

**Microbiology:**

Aerobic bacterial cultures of the skin, liver and small intestine from all of the frogs yield no growth or rare contaminant organisms. Virus isolation is in progress.

**Microscopic description:**

***Frog A (Hyla arenicolor)***

**Skin:** Unremarkable.

**Skeletal muscle, bone, stomach, intestine, kidney, liver, brain:** Unremarkable.

All of the following are *Rana yavapaiensis*:

***Frog B:***

**Skin:** Scattered subcutaneous granulomas contain cross sections of mites. The cornified epidermis of the digits contains a small number of chytrid fungi.

**Lung:** There are a few nematode larvae in the bronchus.

**Liver, gall bladder, heart, stomach, testes, spleen, intestine, kidney:** Unremarkable.

***Frog C:***

**Skin:** Cornified layers of the skin of the digits contains a small number of chytrid fungi. Skin of the thigh contains no chytrid fungi however there are multifocal subcutaneous granulomas containing cross-sections of mites.

Don -

Just got these. FYI

Mike

**Lung:** The lungs contain cross sections of nematode parasites.  
**Liver, stomach, intestine, heart, kidney:** Unremarkable.

***Frog D:***

**Skin:** Unremarkable.

**Lung, liver, testes, pancreas, intestine, stomach, kidney, heart:** Unremarkable.

***Frog E:***

**Skin:** The skin of the thigh contains scattered subcutaneous granulomas with mites. No chytrid fungi are visualized here or in skin from the digits.

**Kidney, spleen, stomach, intestine, heart:** Unremarkable.

***Frog F:***

**Skin:** Skin from the thigh contains subcutaneous granulomas with mites. No chytrid fungi are observed in this skin or in skin of the digits.

**Lung:** The lung contains a few cross sections of nematode parasites.

**Kidney, testes, liver, intestine, stomach, pancreas, heart:** Unremarkable.

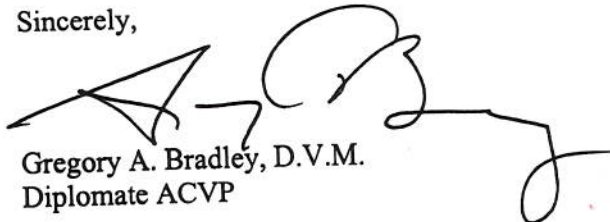
**Diagnosis:**

1. Chytrid fungal infection, multifocal, mild, skin of digits frogs B & C.
2. Granulomatous dermatitis, chronic, multifocal, moderate with mites, frogs B, C, D, E & F.
3. Intrapulmonary nematode parasites (probable *Rhabdias* sp.) multifocal, mild, lungs, frogs B, C & F.

**Comment:**

Two of the *Rana yavapaiensis* had very small number of chytrid fungi in the cornified layers covering the surface of the digits. This is interesting. These frogs may serve as carriers from which the population may be infected. When the environmental conditions change this winter it will be interesting to see if the severe infection and death loss reoccurs in the population. All of the *Rana yavapaiensis* also had dermal granulomas with mites as we've seen in the past. I think these are largely incidental. There were also a few frogs with parasitic infections of the lungs but these had not incited any inflammatory response and are also likely incidental.

Sincerely,



Gregory A. Bradley, D.V.M.  
Diplomate ACVP

Data Form for Winter Surveys of Ranid Frogs

Date 11/28/99 Observer(s) Swann, Caldwell  
Wolner Locality MC

Details of Locality Montrose Canyon (Catalina St Park)

TimeStart 1130 TimeStop 1445 Approx. Distance 0.5 mi

# Canyon treefrogs observed >25 # Sonoran mud turtles observed 0

Other species of interest observed \_\_\_\_\_

Lowland leopard frogs: Live adults 35 Dead adults 2 Larvae observed? Yes

Notes on abundance, health, and behavior of anurans:

Frogs were mostly sluggish - could not leap away from us. In cold water, cold. Not all showing red legs, but some signs apparent. Some frogs thin, ones we handled could not right themselves or did so slowly. A few lively ones, however.

Sick or dead anurans (if any) collected:

Collected 2 alive, very sluggish LLF's  
1 dead LLF  
1 alive, sluggish CTF

Date of specimens to VDL 11/29/99 Specimen ID# DS-MC-11-28

Photos taken (if any) Pool photos by DES

Additional comments or notes (if any)

Pools were in shade - even though it was hot outside canyon, cool inside at pools. Frogs were in water at edge, or on edge

Date: Wed, 1 Dec 1999 18:17:22 -0700 (MST)  
From: Donald E Swann <dswann@U.Arizona.EDU>  
To: DJCaldw111@aol.com  
Subject: Re: Montrose Canyon 11/28

Dennis,

Both of these look good, except "HYAC" should be "HYAR", which is short for *Hyla arenicolor*. And also, what does "distance or activity of sampling/observations" mean? Otherwise, go ahead and forward them or I will forward, whichever you prefer.

Don

On Wed, 1 Dec 1999 DJCaldw111@aol.com wrote:

> Don  
>  
> I set these up to be consistant with the rest of the reports. Look them over  
> and forward them to Sredl and Rosen and me.  
>  
> 11/28/99  
> Caldwell/Swann/Wolner  
> Montrose Canyon, Catalina St. Park  
> Started at lowest pools and worked 1/2 mile up canyon.  
> Time (start & stop): 11:30 - 14:45  
> Distance or Activity of Sampling/Observation: 30 meters  
> Total Anurans Seen (by species): 37 *R. yavapaiensis*, >25 *H. arenicolor*  
> Sick or dead Anurans collected: 2 sick, 1 dead RAYA, 1 sluggish HYAC  
> Sick or dead Anurans not collected: >20 sick RAYA, 1 dead RAYA  
> Date to VDL: 11/29/99  
> Specimen ID#: DS-MC-11-28 (batch)  
> Frogs were mostly sluggish - could not leap away from us. Cold water. Not all  
> showing red leg, but some signs apparent. Some frogs thin. Frogs handled had  
> trouble righting themselves. A few lively frogs observed. Larva observed.  
> Pool photos were taken by Swann  
> Pools were in shade - even though it was hot outside canyon, it was cool  
> inside at pools. Frogs were in water at edge or out of water at edge.  
>

December 8, 1999

MATT 909-1411

MR MICHAEL J SREDL  
AZ GAME & FISH DEPARTMENT  
2221 W GREENWAY ROAD  
PHOENIX AZ 85023-4312

Reference number: 99-3396  
Frog necropsies

Dear Mr. Sredl:

Three Lowland Leopard frogs (*Rana yavapiensis*) and one tree frog (*Hyla arenicolor*) were received on November 29, 1999. The specimens were obtained from the Montrose Canyon area of the Catalina Mountains. Your identification number is DS-NC-11-28.

**Gross necropsy findings:**

The three *Rana yavapiensis* all have reddened skin of the feet and legs. The tree frog has a few pinpoint, red nodules in the skin of the ventrum but does not have the same diffuse reddening of the skin.

**Microbiology:**

***Lowland Leopard Frog A***

2+ *Aeromonas hydrophila* and 2+ *Pseudomonas* sp. from the liver, lung and skin.

***Lowland Leopard Frog B***

**Liver:** No isolates.

**Lung:** A light growth of *Aeromonas hydrophila*.

**Skin:** A moderate growth of *Aeromonas hydrophila*.

***Lowland Leopard Frog C***

A light growth of *Aeromonas hydrophila* from the liver, lung and skin. A light growth of *Pseudomonas* sp. from the skin.

***Tree Frog (D)***

No bacterial growth from the liver and lung. A rare *E. coli* from the skin.

Reference number: 99-3396

Frog necropsies

December 8, 1999

Page 2

**Microscopic description:**

***Lowland Leopard Frog A***

**Skin:** The skin is artifactually absent from many of the sections. Where it is present, there is epidermal hyperplasia and multifocal hyperkeratosis. The superficial layers are colonized by zoosporangia of chytrid fungi. The underlying epithelium exhibits intercellular and intracellular edema with a rare infiltrate of heterophils. The dermis contains an occasional granuloma surrounding a mite.

**Liver, kidney:** Unremarkable.

**Lung:** A large bronchus contains a cross section of a metazoan parasite. The parasite contains a reproductive tract containing larvae.

**Small intestine:** Unremarkable.

**Heart:** Unremarkable.

***Lowland Leopard Frog B***

**Skin:** The skin of this specimen is well preserved. There is marked, diffuse epidermal hyperplasia and hyperkeratosis with superficial colonization by zoosporangia of chytrid fungi. The epidermis exhibits intercellular edema and occasional infiltrates of mononuclear cells.

**Ovary:** Unremarkable.

**Liver, heart, stomach, kidney:** Unremarkable.

**Spleen, small intestine, large intestine:** Unremarkable.

***Lowland Leopard Frog C***

**Liver, pancreas, heart, small intestine, kidney, ovary:** Unremarkable.

**Stomach:** Numerous cross sections of metazoan parasites are present in the lumen.

**Lung:** Multiple cross sections of metazoan parasites are present in the main stem bronchus. Several of these contain reproductive tracts containing larvae.

**Skin:** There is diffuse epidermal hyperplasia and hyperkeratosis. Superficial layers are colonized by sporangia of chytrid fungi. The basal layers of the epidermis exhibit intracellular edema and mild infiltrates of macrophages and a few heterophils. The dermis contains scattered granulomas centered around cross sections of mites.

***Tree Frog (D)***

**Liver, kidney, heart, intestine, pancreas, testes, stomach:** Unremarkable.

**Skin:** The dermis contains a few granulomas centered around cross sections of mites. There is no evidence of chytrid infection.

Reference number: 99-3396

Frog necropsies

December 8, 1999

Page 2

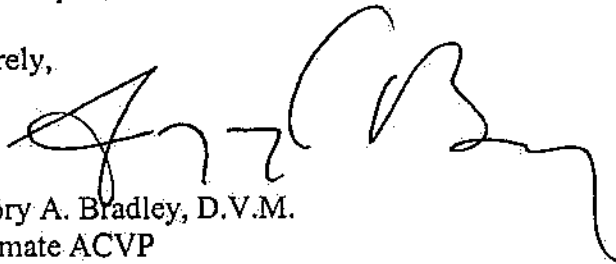
**Diagnoses:**

1. Dermatitis, chronic, with epidermal hyperplasia, hyperkeratosis and sporangia of chytrid fungi, skin of Lowland Leopard Frogs A, B and C.
2. Granulomas, dermal, multifocal, Lowland Leopard Frogs A, B, C and Tree Frog (D).
3. Endoparasitism, mild, lungs and stomach, Lowland Leopard Frogs.

**Comment:**

The three Lowland Leopard Frogs had well developed skin lesions of chytridiomycosis. The tree frog did not. All of the frogs had mite infestations of skin as we have seen in previous submissions. Some of the Lowland Leopard Frogs also had stomach and lung nematode parasites.

Sincerely,

A handwritten signature in black ink, appearing to read 'Gregory A. Bradley', with a stylized, cursive script.

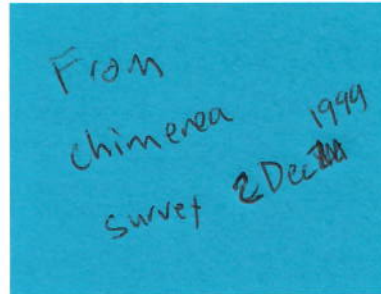
Gregory A. Bradley, D.V.M.  
Diplomate ACVP

sls

December 10, 1999

MR MICHAEL J SREDL  
AZ GAME & FISH DEPARTMENT  
2221 W GREENWAY ROAD  
PHOENIX AZ 85023-4312

Reference number: 99-3475  
Frog necropsies



Dear Mr. Sredl:

The body of a dead *Rana yavapaensis* was received on December 3, 1999. The specimen was collected by Don Swann and was given identification number SNP-2. There were 47 live frogs and one dead frog at the site.

**Gross necropsy findings:**

The frog is in a state of moderately advanced postmortem decomposition. Therefore no cultures were attempted. There were no gross lesions observed.

**Microscopic description:**

**Skin:** Due to the postmortem decomposition, the epidermis has sloughed from all of the sections and is not available for examination.

**Heart, lung, testes, kidney, liver:** Unremarkable.

**Stomach, intestine, spleen:** Unremarkable.

**Diagnosis and Comment:**

This frog was badly autolyzed. There was no epidermis present on the skin sections with which to evaluate the presence or absence of chytrid fungi.

Sincerely,

Gregory A. Bradley, D.V.M.  
Diplomate ACVP

sls

Date: Wed, 27 Jan 1999 09:15:53 -0700  
From: Michael Sredl <MSredl@gf.state.az.us>  
To: "'dswann@u.arizona.edu'" <dswann@u.arizona.edu>,  
"'eend@azstarnet.com'" <eend@azstarnet.com>  
Subject: Montrose

Don/Eric

I got the results back from AVDL. All specimens, including tadpoles, had some level of chytrid fungus infection. In general, the "deader" specimens had higher level of chytrid infection. Besides chytrids, Aeromonas (a bacteria implicated in red-leg) and Saprolegnia (see Andy Blaustein's papers) were found in the leopard frogs, but not the tree frogs.

On a related subject. I visited another die-off at Rock Ck (along the Beeline Hwy) last week, and dropped off specimens to AVDL and Joyce Longcore.

Mike

Michael J. Sredl  
Arizona Game and Fish Department  
Nongame Branch  
2221 W. Greenway Road  
Phoenix, AZ 85023-4399

Voice: (602) 789-3515  
Fax: (602) 789-3926  
msredl@gf.state.az.us

*Rana yavapaiensis*

Tadpoles Collected at Saguaro National Park

Collected by: Don Swann

Tadpoles sent to Phil Fernandez, Grand Canyon University

10% formalin

5 tadpoles (1-5) from Chiminea Creek at Madrona Ranger Station

All collected from rock pool cluster just above Ranger Station

T15 S, R17E, Section 4 on 3/13/2000

*Rana yavapaiensis*

Tadpoles Collected at Saguaro National Park

Collected by: Don Swann

Tadpoles sent to Phil Fernandez, Grand Canyon University in 10% formalin

5 tadpoles (6-10) from Loma Verde Canyon east of Loop Road

All collected from DES pools 3x, 4, and 5

T14S, R16E, Section 22, NW1/4 on 3/16/2000

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*Rana yavapaiensis*

Tadpoles Collected at Saguaro National Park. Collected by: Don Swann

Tails preserved in 95% ethanol

5 tadpoles from Chiminea Creek at Madrona Ranger Station

All collected from rock pool cluster just above Ranger Station

T15 S, R17E, Section 4 on 3/13/2000

*Rana yavapaiensis*

Tadpoles Collected at Saguaro National Park. Collected by: Don Swann

Tails preserved in 95% ethanol

5 tadpoles (6-10) from Loma Verde Canyon east of Loop Road

All collected from DES pools 3x, 4, and 5

T14S, R16E, Section 22, NW1/4 on 3/16/2000

---

DNA from tails to Nick Benedict:

Tadpoles Collected at Saguaro National Park

Don Swann

**5 tadpoles (1-5)** from Chiminea Creek at Madrona Ranger Station

(2 samples from #1 – same individual)

All collected from rock pool cluster just above Ranger Station

T15 S, R17E, Section 4 on 3/13/2000

**5 tadpoles (6-10)** from Loma Verde Canyon east of Loop Road

(2 samples from #10 – same individual)

Collected from DES pools 3x, 4, and 5

T14S, R16E, Section 22, NW1/4 on 3/16/2000

December 13, 2000

MR MICHAEL J SREDL  
AZ GAME AND FISH DEPARTMENT  
2221 W GREENWAY ROAD  
PHOENIX AZ 85023-4312

Reference number: 00-4782  
Lowland Leopard Frogs

Don,  
Trends +



Dear Mr. Sredl:

The bodies of two lowland leopard frogs "*Rana yavapaiensis*" were received on December 4, 2000.

**Necropsy:**

One of the two frogs has been partially dismembered. In the second frog there is heavy colonization of the skin by cottony white fungal colonies.

**Microscopic description:**

**SAGU 2-01:**

**Skin:** There is diffuse epidermal hyperplasia and hyperkeratosis with colonization of the keratinized layers by sporangia of chytrid fungi. The basal layers exhibit intracellular and intercellular edema with mild, multifocal infiltration of mononuclear cells. There is also colonization of the skin and skin surface by branching fungal hyphae.

**Heart:** There is multifocal mineralization of the wall of the conus arteriosus with infiltration of a few giant cells and macrophages.

**Lung, Liver, Stomach:** unremarkable.

**Testes:** unremarkable

**SAGU 2-02:**

**Skin:** Much of the epidermis is absent due to advanced postmortem decomposition. A small fragment remains. This fragment exhibits epidermal hyperplasia and the presence of a single chytrid sporangium. The remaining tissue is heavily colonized by large caliber, invasive, branching fungal hyphae.

**Lung:** Bronchi contain cross sections of parasites. The parasites have an irregular ovoid outline that fills the bronchus lumen.

**Liver, Heart, Stomach, and Intestine:** unremarkable

Reference Number: 00-4782

December 13, 2000

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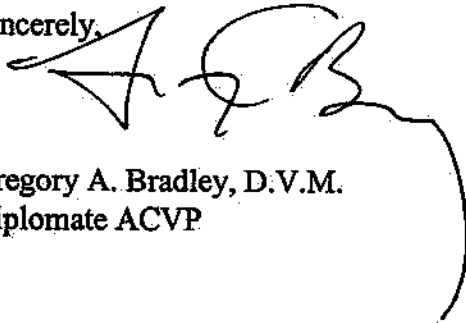
**Diagnosis:**

1. Dermatitis, hyperplastic, with hyperkeratosis and sporangia of chytrid fungi.

**Comment:**

Both frogs had histologic evidence of chytrid fungal infection. Incidental findings included the presence of lung parasites in one animal and localized mineralization of the wall of the conus arteriosus in the other frog. The significance of the latter change is not clear.

Sincerely,

A handwritten signature in black ink, appearing to read 'G. A. Bradley', with a long horizontal flourish extending to the right.

Gregory A. Bradley, D.V.M.  
Diplomate ACVP

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