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Cutaneous Acariasis in the African Clawed Frog (*Xenopus laevis*)

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Increased mortality was observed in a single colony of 50 *Xenopus laevis*. The frogs were used as oocyte donors in developmental biology studies. Necropsy findings included dermal erythema and petechiation consistent with red leg syndrome; dermal ulcerations and white, filamentous growths on the skin were consistent with *Saprolegnia* sp. Microscopic evaluation of the skin and fungus revealed an astigmatid mite similar to those of the genus *Rhizoglyphus*. The mite was also found in the water and the biological filter of the tanks housing the frogs. This mite is considered not to be a parasite of *X. laevis*; instead, it feeds off moss, fungi, and detritus. Subsequent evaluation of the sphagnum moss used for shipping the frogs from the supplier revealed the same mite in the moss. Our hypothesis is that the mite was introduced into the tank with the shipment of new frogs in sphagnum moss. The mites lived within the biological filter, and were only found after the growth of *Saprolegnia* sp. attracted the mites to the frogs. Laboratory animal care and veterinary personnel should consider non-pathogenic species of mites in the differential diagnosis of acariasis in *Xenopus* frogs.

The African clawed frog, *Xenopus laevis*, is a freshwater aquatic species found in numerous bodies of water in sub-Saharan Africa. It is one of the most widely used vertebrate species in a research setting (11). The frogs are used principally in molecular, cellular, and developmental biological studies, commonly as oocyte donors (11).

Xenopus laevis is hardy in the laboratory environment and is affected with only a few diseases and infections. Common conditions include bacterial septicemia (red leg syndrome), salmonellosis, mycobacteriosis, Lucke's renal adenocarcinoma, chlamydiosis, phycomycosis, saprolegniasis, chromomycosis, and parasitism (1). More than 25 genera of parasites have been described (15). The most common laboratory parasite is *Pseudocapillaroides xenopodis*, a nematode with a direct life cycle in the frog epidermis (15). *Xenopacarus africanus* is the only mite described for the *Xenopus* frog, and is found in the nasal cavity and Eustachian tubes (1, 15). We describe herein another acarid on the integument and in the housing environment of *Xenopus laevis*.

Clinical History

Five adult, female *Xenopus laevis* from two tanks, used as oocyte donors in a pharmacologic research protocol, were found moribund or dead during a 4-week period. All frogs had white, filamentous, fungal growths on the epidermis. These developed predominantly in the axillary region, on the medial aspect of the thigh, or over the entire dorsum. Some frogs also had ulcerative skin lesions associated with the fungal growths. The skin on the distal portion of the extremities, ventral aspect of the abdomen, and adjacent to external suture lines from previous oocyte harvests was erythematous. Microscopic evaluation of the epidermal fungal growths revealed numerous mites atypical of the common *Xenopus* parasites, parasites of other common laboratory animals, and parasites of the indoor environment.

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Materials and Methods

Colony management. The non-specific pathogen-free *Xenopus* frogs were supplied by Nasco (Fort Atkinson, Wis.) in a container filled with wet sphagnum moss (Mosser Lee Company; Millston, Wis.). On arrival, frogs were allowed to adjust to room temperature (17 to 18°C) while in the shipping container. After 3 h, the frogs were sent through a two-bath process to allow acclimation to water temperature (15 to 17°C) and to clean the moss from their bodies. A small amount of moss was still associated with the frogs when they were placed in the group tank; the remaining moss was skimmed off the water surface with a net.

The frogs were housed in a single room in an AAALAC-accredited animal facility. The room contained two tanks, each containing approximately 25 *Xenopus* frogs. The fiberglass housing tank was slightly angled lengthwise to facilitate water changes, and measured 75 × 34 × 18 in. The tanks were serviced by a Magnum aquarium pump (Marineland Aquarium Products, Moorpark, Calif.) and were connected via a Little Giant submersion pump (Little Giant Pump Co., Oklahoma City, Okla.) with a detached filter to a 36 × 22 × 18-in. reservoir. The filtration system was operated by re-circulating the water in the tanks. In each tank, two containers were filled with aquarium rocks that were colonized with denitrifying bacteria and served as the biological filter to prevent build up of ammonia and nitrite (Fig. 1). Water flowed through the biological filter to an external canister filter (Magnum pump/filter) and back to the tank. Water was supplied by dechlorinating and aerating DeKalb County, Georgia tap water, maintaining pH of 6.4 and water temperature between 15 and 18°C.

Frogs were observed, and debris was removed from the tanks

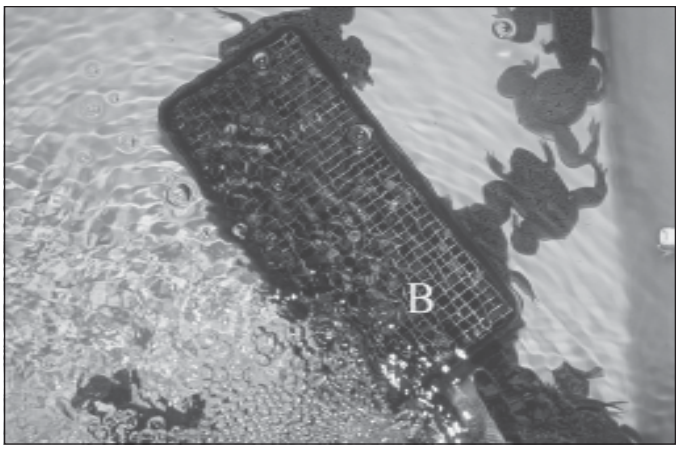

Figure 1. Biological filter (B) in tank with *Xenopus laevis*.

daily; water quality analysis (pH; ammonia, nitrite, and chlorine contents; specific gravity; and hardness) was performed weekly. Evaporated water was replaced weekly, and a 50% freshwater change was performed every 2 weeks. The frogs were fed three times a week, twice with frog brittle and once with beef heart. Uneaten food and debris were removed 30 min after food was offered. All frogs were treated humanely, and all procedures using the frogs were approved by the Emory University Animal Care and Use Committee.

Surgical procedures. The frogs were used as oocyte donors in a pharmacologic research protocol. The oocyte harvest was a survival procedure, performed on frogs under MS-222 (Finquel, Argent Chemical Laboratories; Redmond, Wash.)-induced anesthesia. A 2-cm, paramedian, ventral abdominal incision allowed oocytes to be removed, and the coelom was closed in a single layer, using a continuous pattern of a braided, absorbable suture.

Necropsy. Four of the five frogs were found dead, and the bodies were submitted for necropsy. One frog was moribund and was euthanized by induction of anesthesia with MS-222 (Finquel, Argent Chemical Laboratories; Redmond, Wash.) and subsequent decapitation. The following tissues were evaluated histologically: heart, lung, kidneys, liver, gastrointestinal tract, spleen, skeletal muscle, nasal cavity, and skin. Tissues were fixed in 10% formalin, cut into 5-µm-thick sections, treated with hematoxylin and eosin and Kinyoun's acid-fast bacilli (liver and skin) stains, and evaluated via light microscopy. The acid fast-stained sections were compared with positive-control tissues. Specimens of muscle and liver tissue were sent to Antech Diagnostics (Farmingdale, N.Y.) for bacteriologic culture.

Skin scrapings. Skin lesions on the five frogs were scraped by use of a No. 10 scalpel blade that was dulled on a stainless steel table to prevent incising the skin during the procedure. The epithelium remained intact during this procedure, with only the slime layer and its associated fungi and debris removed.

Twelve normal frogs were caught, and using manual restraint and a blunt No. 10 scalpel blade, the slime layer was scraped from the proximal portion of the hind limbs in broad superficial strokes. Only minimal epithelium was removed.

Tank evaluation. Water samples were collected in 50-ml conical tubes from both tanks. One tube was filled with surface water from each tank, and another tube was filled with bottom water. These samples were spun in a Jouan C312 centrifuge (Winchester, Va.) at 1360 ×*g* for 10 min. The supernatant was removed, and the remaining liquid was pipetted onto dry microscope slides for evaluation.

One biological filter from each tank was brought to the surface for visual inspection. Aquarium rocks used in the filter and any loose material in the biological filter from each tank were placed in conical 50-ml tubes. The surface algae on the rocks were scraped off by use of a No. 10 scalpel blade. The algae and loose material from the filter were placed on a dry microscope slide for evaluation.

Sphagnum moss evaluation. Sphagnum moss was donated by Nasco and Mosser Lee Company (Millston, Wis.) for evaluation. Two-hundred fifty micrograms of moss was placed in a 50-ml conical tube with 30 ml of neutral-buffered 10% formalin. The tube was vortexed to separate any organisms and foreign material from the moss. The vortexed material in the tube was then allowed to settle overnight. The tubes were centrifuged at 1360 ×*g* for 10 min, and the supernatant was removed. The remaining liquid was pipetted onto a dry microscope slide for evaluation.

Approximately 1 g of moss was placed in a 1-liter container of dechlorinated water. The moss was rehydrated and placed in a clean 1-liter container. White gauze was cut to fit a Tissue-Tek cassette (Sakura Finetek U.S.A., Inc., Torrance, Calif.), and the gauze was saturated with peanut oil (used as an attractant for the mites, since they have been described to thrive on water-soaked peanuts [9]), placed in the tissue cassette, and positioned among the moss. The moss was allowed to sit for 18 h, and the gauze was evaluated for live mites every 24 h. After the initial 48 h, 200 ml of dechlorinated water was added to the container of damp moss. Every 72 h, new peanut oil-saturated gauze was placed in the Tissue-Tek cassette. Daily gauze evaluation continued for seven days, then evaluation was done every three days. After 18 days, the water was drained from the container into 50-ml conical tubes. The tubes were centrifuged at 1360 ×*g* for 10 min. The supernatant was removed, and the remaining liquid was evaluated for live mites.

A Baermann's examination (16) was then performed on the remaining moss. Ten grams of wet sphagnum moss was wrapped in a double layer of cheesecloth, a plastic champagne glass was filled with luke-warm tap water, and the cheesecloth was suspended in the water. This suspension was allowed to sit for 22 h when water from the bottom of the stem of the glass was pipetted and placed on a dry microscope slide for evaluation of live mites. The unstained skin scraping and moss specimens were evaluated by use of light microscopy.

Results

Gross pathologic findings. All frogs (A–E) had a history of recent celiotomy for oocyte harvest. All had erythema ranging from petechiation to diffuse reddening of a region of the body that was evident adjacent to external suture lines and in the distal portion of the hind limbs. All frogs also had white, filamentous material on the skin. Three (B, C, and E) frogs had brown to white granules diffusely distributed within the opaque, white slime layer. Two (A and E) had dermal ulcerations associated with the white, filamentous material (Fig. 2). Frog B had subcutaneous edema, and frog D had bloated abdomen. The liver of frog D had multifocal, white lesions. Frog E had intense reddening of the musculature of the caudal portion of the thigh. All other organs were grossly normal.

Culture of muscle and liver specimens from frog D yielded

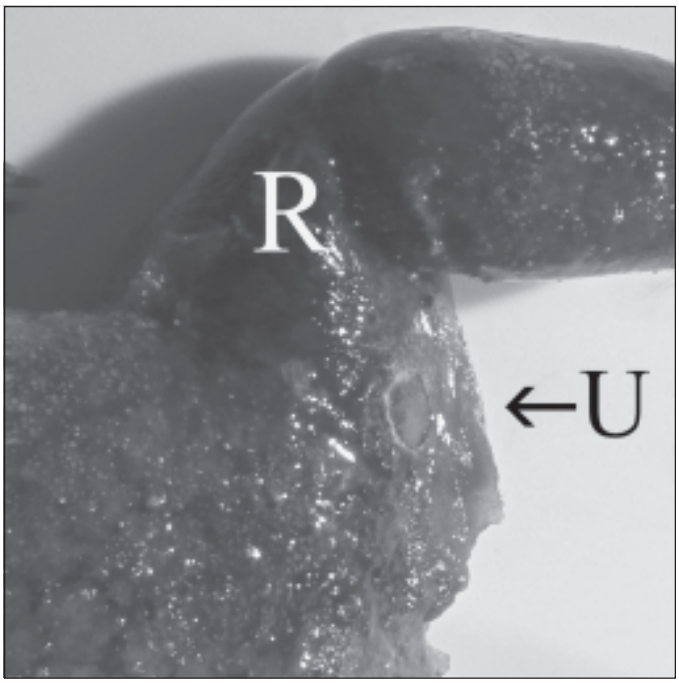

Figure 2. Pelvic limbs of *X. laevis*. Caudal portion of the right thigh (R) has dermal ulceration and *Saprolegnia* sp. (U). The skin contains multiple brown and white granules in the surface slime layer.

Aeromonas hydrophilia/caviae, γ (non-hemolytic)-*Streptococcus* species, and β-hemolytic *Streptococcus* species in the muscle, and *Aeromonas hydrophilia/caviae*, methicillin-resistant *Staphylococcus aureus* in the liver. Culture of muscle specimens from frog E yielded *Aeromonas hydrophilia/caviae* and α-hemolytic *Streptococcus* sp. Requests for mycobacterial culture were not made.

Skin scrapings. Examination of the skin scrapings from the control frogs did not reveal any fungal elements or external parasites; however, specimens from all subject frogs had fungal hyphae, identified as *Saprolegnea* sp., and numerous mites. The mites were characterized by piercing mouthparts and eight legs composed of four segments, each terminating with a sickle-shaped claw (Fig. 3). Larval stages with six legs also were observed. Several mites were gravid. A few eggs were seen free within the fungal mat. In frog A's scrapings, the mites appeared dead; scrapings from all other frogs had live mites. This mite was identified as an Astigmatid that feeds on plants, moss, and fungi, and is similar to *Rhizoglyphus* sp. (3), of which the bulb mite is the most widely known. *Collembola* sp., a wingless insect found in moist habitats (5), and free-living nematodes also were observed within the fungal hyphae.

Histopathologic findings. Four of five frogs (A, C, D, and E) had myositis and necrosis of the skeletal muscle. Many erythrocytes surrounded the myocytes in frogs A and E. In frog D, multifocal clusters of coccoid bacteria were associated with the myocytes.

Mites were observed microscopically in all sections of skin. The mites were located in the keratin layer of the skin or among debris external to the keratin layer; however, in one frog specimen, a mite was observed within the epidermis (Fig. 4). The mite had a highly chitinized body wall, striated musculature, jointed appendages, and eggs (8). Mites were not found in the nasal cavity.

Three of five frogs (A–C) had a mononuclear dermatitis characterized by inflammatory cells at the dermal-epidermal junction. The inflammation correlated with the gross skin lesions, but was not in direct association with the mites. Two frogs (A and E) had hyperkeratosis of the epidermis, two (A and B) had acanthosis, and one (E) had parakaratosis.

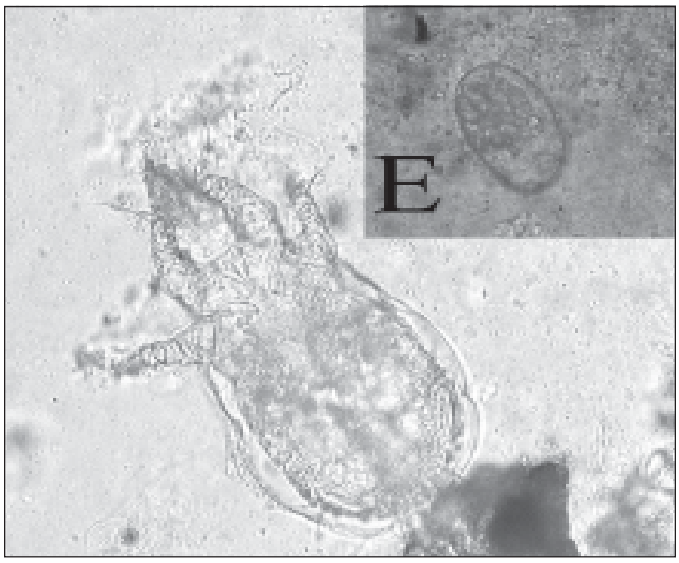

Figure 3. Mite from *X. laevis* skin scraping (magnification, ×20). Only the cranial appendages are in focus. Inset: mite eggs (E) at ×40 magnification. Mites found in the tank and the moss were identical.

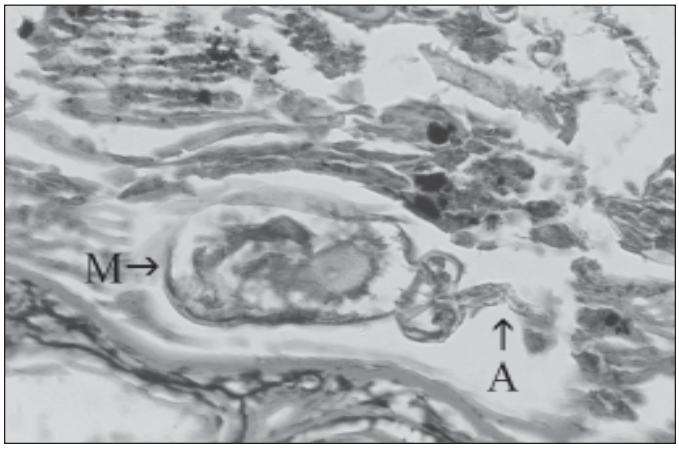

Figure 4. Photomicrograph of a section of frog epidermis. The mite had a chitinous exoskeleton (M) with jointed appendages (A). H&E stain; magnification, ×40.

Coccoid bacteria were observed in the liver and the heart of frog D. Coccoid bacteria were observed in the hepatic vasculature, splenic mononuclear cells, and cardiac myosites of frog E. Splenic subcapsular hemorrhage also was observed in frog E (Table 1).

No mycobacteria were found by use of acid-fast staining of liver and skin specimens from all frogs. Furthermore, granulomatous inflammation to suggest mycobacterial infection was not evident in the liver or skin.

Tank evaluation. Superficial water samples yielded one live larval mite in tank 1, as well as *Collembola* sp. and numerous protozoa. Mites were not observed in the superficial water sample from tank 2; however, numerous protozoa and *Collembola* sp. were found. The deep-water sample yielded a

Table 1. Histologic findings and their corresponding frequency

Lesion	Frequency
Mites associated with skin	5/5
Myositis and muscular necrosis	4/5
Mononuclear dermatitis	3/5
Hyperkeratosis	2/5
Acanthosis	2/5
Hemorrhagic myositis	2/5
Bacterial septicemia	2/5
Parakeratosis	1/5
Splenic subcapsular hemorrhage	1/5

single live adult mite from each tank, and moderate numbers of *Collembola* sp. and protozoa.

The biological filter in tank 1 did not contain gross contaminants; the aquarium rocks had only a light coating of algae on the surface. Microscopic evaluation of the algae on the rocks did not reveal mites or other organisms. The biological filter in tank 2 contained algae and 10 2-cm spheres of brown, filamentous material on top of the aquarium rocks. Microscopically, each sphere was composed of fungal hyphae and numerous *Collembola* sp., free-living nematodes, numerous live mites in various life stages, and eggs. Microscopic evaluation of the algae did not reveal relevant findings.

Moss evaluation. Microscopic evaluation of the moss revealed mites anatomically identical to those observed on the frogs and on the fungal mats within the biological filter. Several dead mites were found in the moss; however, live mites were not obtained from the moss at any time.

Discussion

Each of the frogs had three common skin findings; mites on the skin, either gross or histologic evidence of red leg syndrome, and *Saprolegnea* growth. Parasitic mites have not been described on the skin of *X. laevis*; however, an acarid parasite, *Xenopacarus africanus*, is found in the nasal cavity and Eustachian tubes of *X. laevis*. *Xenopacarus africanus* is of the family Ereynetidae (6), the same family as that of the chigger, and feeds off blood obtained from vessels in the head of the frog (2). *Xenopacarus africanus* is in the differential diagnosis for a mite on a *Xenopus* frog; however, the microscopic characteristics of it do not correlate with those of the mite observed in these frogs, nor were mites found in the nasal cavity of these frogs.

Similar lesions are found in frogs and other amphibians from North and South America with infestation by *Hannemania dunni* (7). This mite also is from the chigger family, and it attaches in the skin of its host. Only the larval form is found in frog skin. The mite observed in these frogs has larger, piercing mouth parts and different leg morphology. The *Hannemania* larva is also nearly 1 ml long, and thus would be nearly visible to the naked eye (7).

Astigmatid mites of the genus *Rhizoglyphus* are often found in moist, humid habitats, with highest recovery from decaying vegetation, fungi, and soils with abundant organic matter (4). The most likely source of the mite, based on its dietary and morphologic descriptions, is the sphagnum moss used to transport *X. laevis*. Microscopic evaluation of the moss confirmed that the mite observed on the frog skin also was in the moss. The moss is naturally grown in wetlands in western Wisconsin, with no barriers to prevent pest contamination (mammalian, insect, avian). The moss is cut and dried on fan beds, and large debris is removed; then, the moss is packaged (12). Mosser Lee Company estimates that their product contains 30 to 40% other organic material, mainly plants, but no hazardous organisms (12).

Our attempts to recover live mites from the sphagnum moss were not successful. Our hypothesis is that the mite eggs are attached to the moss and survive the drying, packaging, and shipping process. Once the moss is rehydrated, prior to shipment with the frogs, mite eggs are stimulated to develop and hatch. Since there is a small amount of moss that gets into the main tank despite the two-bath method, the mite eggs have access to the environment of the frogs, or the eggs may adhere to the frogs from the moss during transport from the supplier. The biofilter in the tanks provide a suitable habitat for the eggs to hatch and for the mites to mature and reproduce. The mites remain unnoticed unless problems develop with the *Xenopus* colony.

The mites were observed in this *Xenopus* colony due to red leg syndrome, a bacterial septicemia caused by gram-negative organisms, usually *A. hydrophilia* (13). This syndrome is usually associated with temperature fluctuations, trauma, infrequent water changes, and stress (10). *Aeromonas caviae* has been found in zebrafish from the same institution (14). *Saprolegnea* sp., which was observed in conjunction with the red leg syndrome, is a ubiquitous fungus in fresh water. In many amphibians, ulcerative lesions develop on the dermis and epidermis. Lesions typically start at the head and progresses caudad (1). The skin is only colonized in frogs with immunosuppressed states or when the skin is diseased or traumatized (13).

The increased mortality observed in the colony correlated with oocyte-harvesting surgery. Surgical harvesting of oocytes is stressful, as well as traumatic to the skin, which may predispose these animals to red leg syndrome. This was followed by *Saprolegnea* sp. growing on the diseased skin of the frogs and allowed the mite, due to its attraction to the fungi, to colonize the frog skin.

Consultation with the investigators led to a change in surgical technique. Plans were established to remove the aquarium rock biofilter from the tanks and to set up an external biofilter with a mesh prefilter to remove gross debris.

To the author's knowledge, this is the first report of astigmatid mites in a *X. laevis* laboratory colony. Using appropriate quarantine procedures for new arrivals and proper cleaning of tanks with their associated filters, these mites should not survive in the established enclosures. Although this astigmatid mite is considered to be non-pathogenic to *X. laevis* in the laboratory setting due to generalizations that can be made about the genus of mite found, one must consider its parasitic potential with parasitic and free-living forms. However, controlled experimentation is needed to offer proof that this may indeed be a parasite rather than an incidental finding.

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