

11-20-20: Microscopic examination of the tissues was unremarkable/non-diagnostic. Please, note detection of ciliated protozoa in the fecal exam. The gastrointestinal tract of amphibians commonly contains ciliates, and given that there were no associated lesions, I would say that they are commensal in this case. As per our conversations by phone/email, *Mycobacterium marinum* usually causes cutaneous and internal lesions that were not detected gross or histologically in this animal. I am requesting some special stains anyway in attempt to identify acid-fast bacteria. Some bacteriology results are pending and will be included in the final version of this report. Do contact me if you have any question.

11-30-20: All results have been released. *Klebsiella oxytoca* was isolated from the liver and lung. Given that there were no associated lesions, I would say that these are commensal and/or terminal bacteria. Testing is concluded.

Clinical History

Sudden death overnight. Was feeding well, no symptoms.

Frog is part of a colony of 17 *Phyllomedusa sauvagii* (way monkey frog). Arboreal basking frog that prefers low humidity. Husbandry: UVB baking spots immaculate water distilled with small amounts of minerals added back in, diet is mix of crickets and dubia roaches, always dusted with non vit D calcium and twice weekly with multivitamin. History of two other frog deaths in this enclosure during the past 2 months.

These frogs are subject to uroliths and have lost them in the past due to this, thus the tight control of water parameters (they do not drink but soak about once a week). Also in a second enclosure with siblings, 2 frogs have developed lesions over the parotid gland. One suffered with this for nearly a year, another has it past 8 months. Treatment with Baytril unsuccessful. Also topical silvadine and nolvasan ineffective. Finally, in Sept (during the fires but inside) a frog developed a swelling over parotid gland. It was identified as squamous papilloma extends to margins. Within 2 weeks the lesion tripled in size and an attempt was made to debulk/remove it using electrocautery but the frog lived only 48 hours post. Histo and pics of the lesions attached. These frogs are a rare and valuable species and I am looking for as much assistance as possible to identify and pathogens and prevent further deaths. Also, a previous colony from the same source was tested for both ranavirus and chytrid.

Disease or condition suspected: Airborne toxin? Bacterial/mycoplasma?

Gross Observations

Necropsy of a 44.5 g, female waxy monkey frog began at 11.05 am, November 12, 2020.

Carcass is in good nutritional condition, with adequate amount of fat reserves and well fleshed, and in moderate state of postmortem decomposition.

There is an 8.4 g, round, hard, light yellow, 3.2 x 2.5 x 1 cm calculus in the urinary bladder that fills 2/3 of the coelomic cavity. There are no other gross abnormalities in the rest of the carcass. Stomach is empty. There are soft, pasty, dark-brown feces in the caudal large intestine.

Bacteriology

Fecal Direct - Ciliated Protazoa detected

BACTERIAL AEROBIC CULTURE

Animal/Source	Specimen	Specimen Type	Results
314238	S2009123-01.0003	Lung Tissue	Mixed flora Mod# Klebsiella spp. Mod# Klebsiella oxytoca Mod#
314238	S2009123-01.0004	Liver Swab	Mixed flora Mod# Klebsiella spp. Mod# Klebsiella oxytoca Mod#

Biotyper Organism Identification

Animal/Source	Specimen	Specimen Type	Results
314238	S2009123-01.0006	Bacterial Isolate, Liver	Klebsiella oxytoca

Salmonella culture – Avian (non-NPIP)

Animal/Source	Specimen	Specimen Type	Results
314238	S2009123-01.0004	Liver Swab	No Salmonella sp. detected

Histology

Sections of kidney, ovary, stomach, spleen, urinary bladder, lung, skin, liver, and intestines are examined.

There are no significant microscopic abnormalities to note

Addendum 11-30-20

-Ziehl-Neelsen stain is performed in lung sections. No acid-fast organisms are identified

-Four sections of the head (including brain, eyes, and nasal cavity are examined). There are rare, mild foci of lymphocytes in the ear canal.

Parasitology

Test Specific Comments

FECAL EXAM - DIRECT WET SMEAR

* Fecal Direct exam is the only diagnostic option for parasite detection if <1g of feces is submitted; however, false negative results are possible with this method. Fecal flotation requires at least 1g for test accuracy.

FECAL EXAM - DIRECT WET SMEAR

Animal/Source	Specimen	Specimen Type	Results
314238	S2009123-01.0002	Feces	See Discipline Summary



CYSTIC UROLITHIASIS IN CAPTIVE WAXY MONKEY FROGS (*PHYLLOMEDUSA SAUVAGII*)

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Source: Journal of Zoo and Wildlife Medicine, 46(1):105-112.

Published By: American Association of Zoo Veterinarians

DOI: <http://dx.doi.org/10.1638/2014-0086R1.1>

URL: <http://www.bioone.org/doi/full/10.1638/2014-0086R1.1>

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CYSTIC UROLITHIASIS IN CAPTIVE WAXY MONKEY FROGS (*PHYLLOMEDUSA SAUVAGII*)

Kate E. Archibald, D.V.M., Larry J. Minter, M.S., D.V.M., Daniel S. Dombrowski, M.S., D.V.M., Jodi L. O'Brien, D.V.M., and Gregory A. Lewbart, M.S., V.M.D., Dipl. A.C.Z.M.

Abstract: The waxy monkey frog (*Phyllomedusa sauvagii*) is an arboreal amphibian native to arid regions of South America, and it has developed behavioral and physiologic adaptations to permit survival in dry environments. These adaptations include a uricotelic nitrogen metabolism and unique cutaneous lipid excretions to prevent evaporative water loss. Uroliths are a rare finding in amphibians. Six adult, presumed wild-caught waxy monkey frogs housed in a museum animal collection were diagnosed with cystic urolithiasis over a 7-yr period, and a single animal was diagnosed with four recurrent cases. Six cases were identified incidentally at routine physical or postmortem examination and four cases were identified during veterinary evaluation for coelomic distension, lethargy, anorexia, and increased soaking behavior. Calculi were surgically removed from three frogs via cystotomy, and a single frog underwent three cystotomies and two cloacotomies for recurrent urolithiasis. Two frogs died within the 24-hr postoperative period. Two representative calculi from a single frog were submitted for component analysis and found to consist of 100% ammonium urate. In the present report, cystic calculi are proposed to be the result of a high-protein diet based on a single invertebrate source, coupled with uricotelism, dehydration, increased cutaneous water loss, body temperature fluctuations facilitating supersaturation of urine, and subsequent accumulation and precipitation of urogenous wastes within the urinary bladder. Surgical cystotomy represents a short-term treatment strategy for this condition. Preventative measures, such as supplying a diversified and balanced diet in addition to environmental manipulation aimed at promoting adequate hydration, are anticipated to be more-rewarding management tools for cystic urolithiasis in the waxy monkey frog.

Key words: Ammonium urate, amphibian, cystic calculi, cystotomy, *Phyllomedusa sauvagii*, uricotelic.

BRIEF COMMUNICATION

Frogs belonging to the genus *Phyllomedusa*, such as the waxy monkey frog (*Phyllomedusa sauvagii*), are distinguished from other amphibians by a uricotelic nitrogen metabolism and the presence of unique lipid-secreting dermal glands, both adaptations which permit life in arboreal and arid habitats.^{36,42} These frogs are capable of sustaining homeostatic fluid balance in terrestrial environments via the secretion of an extra-epidermal hydrophobic film to reduce evaporative losses through the skin, the excretion of a urogenous metabolite with low osmotic activity,

and by exhibiting periods of behavioral torpor.^{34,35,36} Additionally, waxy monkey frogs utilize fluid maintenance strategies characteristic of other amphibians, such as the capacity to rapidly absorb water transcutaneously and to recover fluid from the urinary bladder in states of dehydration.^{39,47} Reduced evaporative water loss and the production of uric acid by the waxy monkey frog resemble the water conservation mechanisms found in reptiles, a group of uricotelic animals in which urate-based cystic calculi are frequently documented in captivity.^{8,18,29,40}

The formation of urinary calculi is initiated by the precipitation of excretory metabolites in supersaturated urine and, while this is recognized as a significant cause of urinary tract disease in reptiles and domestic mammals, it is uncommonly reported in amphibians.^{7,13,18,24,26,47} Factors credited with stone nidation include dehydration, alterations in urinary pH, abnormalities of urinary tract structure and function, high levels of urolith components in the diet, nutrient deficiencies such as hypovitaminosis A, atypical metabolism of calculus precursors, and the presence of bacteria and other foreign materials within the urinary tract.^{10,14,22,30} Rarely is a single etiology recognized as the inciting cause of urolith development, and a multivariate pathogenesis is gen-

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erally proposed in all species. However, identifying principal factors that contribute to calculi formation can assist in the formulation of strategies to prevent or treat urolithiasis.^{24,43}

This report describes six cases of cystic urolithiasis and four recurrent cases of urolithiasis in captive waxy monkey frogs including clinical and pathologic findings, surgical correction, and urolith component analysis.

Six adult, presumed wild-caught waxy monkey frogs were purchased from a single supplier (Reptile Depot, Chatworth, California 91311, USA) and group-housed as part of a museum animal collection beginning in April 2003. Animals were transferred several times per year between a 95-L, moss-lined exhibit and a smaller, 40-L, paper-lined holding area as part of a collection management and exhibit maintenance routine. Supplemental heat was provided by a 40-watt lamp on a 12-hr light cycle, and natural perching material was supplied spanning the height of the enclosures. Ambient temperatures measured approximately 24°C at the base of the enclosures and 33°C at the highest available perching location. Water was offered in shallow dishes located on the floor of each container. Rarely, intermittent episodes of evaporation of the sole water source within the holding enclosure were noted during the period in which urolithiasis occurred. Frogs were fed one to two adult crickets, dusted with a commercial calcium plus Vitamin D3 supplement (Miner-All Indoor, Sticky Tongue Farms Sun City, California 92586, USA or Rep-Cal Calcium, Rep-Cal Research Labs, Los Gatos, California 95031, USA), three times per week.

The animals in this report were diagnosed with six cases of cystic urolithiasis, and a single animal was diagnosed with four recurrent cases of urolithiasis over a 7-yr period. Six cases were identified incidentally at routine physical or postmortem examination, and four cases were identified during veterinary evaluation for coelomic distension, lethargy, anorexia, and increased soaking behavior. Calculi were surgically removed from three frogs via cystotomy and a single frog underwent three cystotomies and two cloacotomies for recurrent urolithiasis.

In October 2006, a male frog was found dead on exhibit and the whole animal was placed in formalin prior to postmortem examination. Necropsy revealed a single large urolith within the lumen of the urinary bladder. Histologically, the cardiac tissue was characterized by both peri- and epicarditis with no evidence of inflammation in

any additional tissues sampled. The underlying cause of the inflammatory cardiac lesions was not identified, but was proposed to be the result of friction from the closely positioned urolith. The urolith was not measured or submitted for component analysis.

In January 2007, a frog of unknown sex was evaluated for ocular trauma. Upon palpation of the coelomic cavity, two firm, approximately 1-cm spherical masses were identified in the location of the bladder. The animal weighed 16 g at that time and appeared otherwise healthy. While a diagnosis of urolithiasis was suspected, a decision was made to monitor the animal for changes in condition or attitude while developing a treatment plan. The animal was found dead 1 mo later and, due to gross autolysis, no tissues were submitted for histopathology and the urolith was not examined.

During a routine physical examination in March 2007, a male frog weighing 19 g was found to have a firm, approximately 3 cm, palpable mass within the coelomic cavity which was presumed to be located within the urinary bladder. The animal displayed no clinical signs of disease and was observed eating and defecating and appeared to urinate normally. The animal underwent exploratory coeliotomy and cystotomy for urolith removal. Anesthesia was induced by submersion in 2 g of tricaine methanesulfonate (MS-222) (Western Chemical Inc., Ferndale, Washington 98248, USA) per liter of reverse osmosis (RO) water buffered with sodium bicarbonate at a pH of approximately 7.0. Once anesthetized, the animal was placed in dorsal recumbency on a tray lined with absorbent paper moistened with RO water. Anesthesia was maintained by dosing as needed with RO water, or buffered 1–2 g of MS-222 per liter RO water, administered by bathing the animal via a red rubber catheter. The surgical site was prepared with a saline wash and draped with sterile plastic. Using a 15 blade, a roughly 2-cm ventral midline coelomic incision and cystotomy (Fig. 1a) was performed for stone removal. The bladder wall was closed using 5-0 polydioxanone suture (PDS; Novartis Animal Health US Inc., Greensboro, North Carolina 27408, USA) in a simple continuous pattern. The coelomic wall and skin were closed in a single layer using 5-0 PDS in a simple interrupted pattern. Anesthesia and recovery were uneventful. The urolith was tan to white, irregularly shaped, and exhibited a roughened surface (Fig. 1b). It was submitted to the Minnesota Urolith Center (University of Minnesota, St. Paul, Minnesota 55108, USA) and a

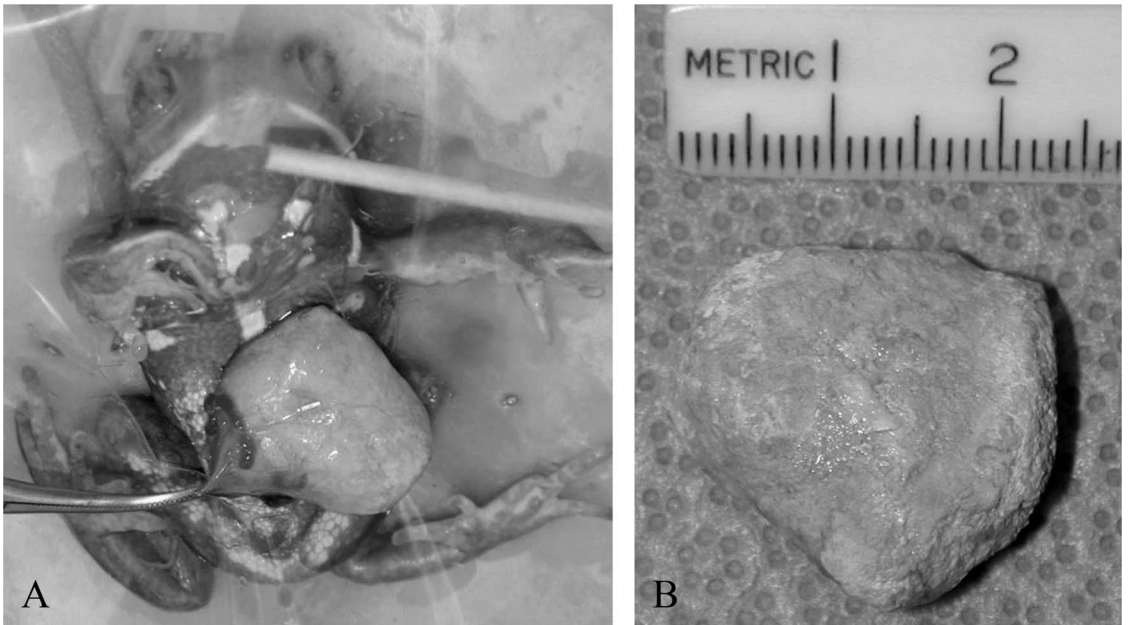


Figure 1. A. Anesthetized waxy monkey frog undergoing cystotomy. The bladder is exteriorized and contains a large cystolith. B. Ammonium urate stone.

composition analysis reported 100% ammonium urate.

In December 2009, a second intracoelomic mass measuring approximately 1.5×0.7 cm was visible through the ventral abdominal wall and was presumed to be a urolith. The frog weighed 26.6 g at that time, and a second cystotomy was scheduled even though the animal showed no signs of clinical disease. Anesthesia was induced and maintained using MS-222 as outlined above. The animal was surgically prepared as previously described and a 2-cm long, right paramedian skin incision was made overlying the mass. Two encircling ligatures of 5-0 PDS were placed around the bladder wall adjacent and proximal to the urolith, allowing complete resection of the bladder wall surrounding the urolith. The skin and coelomic wall were closed in a single layer as previously described. Anesthesia and recovery were uneventful. The urolith was tan to white with an irregular shape and roughened surface and was not submitted for component analysis.

This frog was found to be lethargic on exhibit in June of 2011, and on physical examination a palpable mass was located within the caudoverstral coelomic cavity. The animal weighed 22.6 g at that time, and a partial cystectomy was performed according to the anesthetic and surgical protocol outlined above. The animal recovered well from

this procedure. A section of bladder wall containing an approximately 2×1 -cm urolith was resected and submitted for histopathologic evaluation. Histologically the bladder wall was characterized by a thin layer of urothelium containing an increased number of goblet cells with lymphocytes scattered within the submucosal layer (Fig. 2a). Adjacent to and contained within the bladder wall were numerous, round, discrete crystalline structures of varying sizes, consistent with urate crystals (Fig. 2b). The urolith was not submitted for component analysis.

In September 2012, the animal was examined for a 2-wk history of anorexia and increased soaking behavior. A large, intracoelomic mass was confirmed on palpation and the animal underwent a fourth surgery for cystolith removal. The frog weighed 27 g at that time. Anesthesia was induced using a buffered 3.3 g/L MS-222 solution and maintained as outlined above. A 2-cm long paramedian skin incision was made in the left ventral coelomic wall. A firm, 2-cm mass was identified within the cloaca. The location of this urolith was suspected to be a result of the previous bladder wall resection limiting the volume of the urinary bladder. An incision made into the cloaca allowed for removal of a 3.2-g urolith. The cloacal wall was closed using 5-0 PDS in a simple interrupted pattern. The skin and

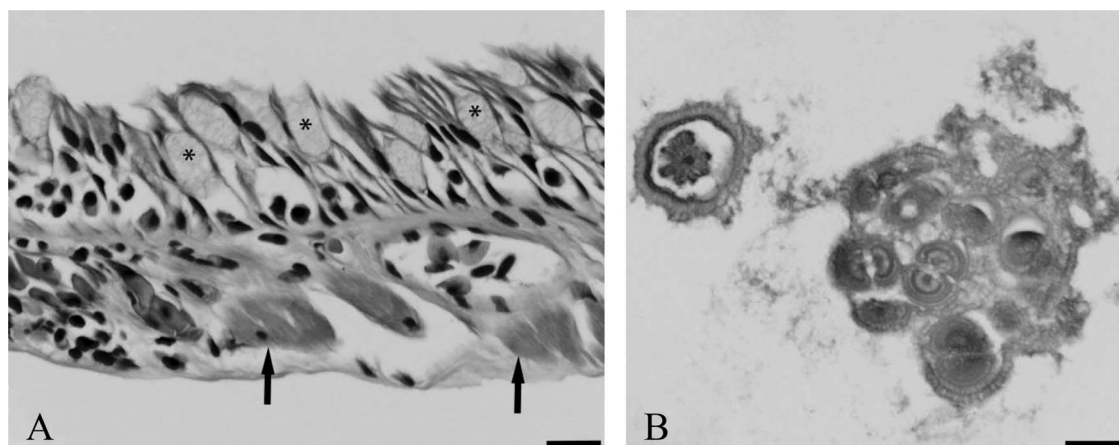


Figure 2. A. Histopathology of the bladder wall from a waxy monkey frog (*Phyllomedusa sauvagii*) with cystic urolithiasis exhibiting multifocal lymphocytic cystitis with goblet cell hyperplasia. The urothelium contains frequent goblet cells (asterisks) and lymphocytes are scattered within the submucosal layer. There is a thin layer of smooth muscle (arrows). B. Mineralized debris with discrete, round, crystalline structures (urate crystals) adjacent to the mucosal surface of the bladder wall. H&E. Bar = 20 μ m.

coelomic wall were closed in a single layer as previously described. The animal recovered well from this procedure. The urolith had a similar appearance to those previously described. It was submitted for component analysis and found to consist of 100% ammonium urate.

Eleven months postsurgery, the frog was reported to have developed another intracoelomic mass measuring approximately 2 \times 2 cm. In October 2013, the animal underwent an exploratory coeliotomy and was anesthetized and surgically prepared according to the procedure outlined above. The animal weighed 23.7 g at that time. A 2-cm long paramedian skin incision was made in the left ventral coelomic wall. A firm mass was identified within the cloaca. An incision made into the cloaca allowed for removal of a 2.4 g urolith. The tissues were closed as previously described and the animal recovered routinely from this procedure. The urolith was not submitted for component analysis.

In November 2007, a male frog was found dead in the exhibit and reported to have a single, large cystic urolith on postmortem examination. No samples were submitted for histopathology and the urolith was not submitted for component analysis.

In January 2008, a frog held in the off-exhibit enclosure was evaluated for lethargy and anorexia. A firm, palpable intracoelomic mass was detected on physical examination and exploratory celiotomy was elected. The frog weighed 12 g at

that time and anesthesia was induced using a buffered, 1 g/L, MS-222 solution and maintained as outlined above. A 4-cm midline incision was made in the ventral coelomic wall. The urinary bladder was noted to be adhered to the body wall at midline. An incision made in the urinary bladder permitted removal of a 1.5 \times 2-cm urolith. The urolith was tan to white with an irregular shape and roughened surface. The tissues were closed as previously described. The animal recovered from anesthesia uneventfully but was found dead the following morning. At necropsy, slight myocardial pallor and mild gall bladder distension were observed. The urinary bladder was determined to be intact based on retention of saline infused into the lumen. Gross examination did not reveal an apparent cause of death and tissues were not submitted for histopathology. The urolith was not submitted for component analysis.

In February 2008, a female frog underwent surgery for cystolith removal. Anesthesia and surgery were performed as outline above. The animal was found dead following recovery from anesthesia. Gross examination did not reveal an apparent cause of death and tissues were not submitted for histopathology. The urolith had a similar appearance to those previously described and was not measured or submitted for component analysis.

Nitrogen-handling strategies in amphibians correlate with niche-specific water availability

and often vary among life stages of a single species if a range of aquatic and terrestrial environments are encountered during development.^{26,47} Following detoxification in the liver, clearance of nitrogenous waste in most amphibians is achieved primarily via renal excretion of urea, or less commonly ammonia, as occurs in aquatic life stages.²⁶ These nitrogen compounds contribute to the osmotic load of urine, drawing water into the urinary tract to facilitate their excretion. This is in contrast with terrestrial birds and reptiles which evolved to rely on poorly soluble uric acid as the primary nitrogen metabolite.⁴⁰ While this metabolite is more-energetically costly to produce, the biologic advantage of uric acid is a significant decrease in the volume of water required to effectively excrete nitrogenous waste. This system supports the maintenance of adequate hydration levels in organisms inhabiting low-moisture environments, such as the waxy monkey frog.

Waxy monkey frogs are an arboreal species native to the dry regions of Bolivia, Paraguay, Brazil, and Argentina and are found in habitats with scarce water resources and in locations where temperatures exceed 40°C during the summer months.³⁷ To sustain life in semiarid environments, the waxy monkey frog employs a variety of physiologic and behavioral adaptations including uricotelism, decreased cutaneous evaporation, recovery of fluid from the urinary bladder, rapid transepithelial water absorption, and minimal energy expenditure during the daytime.^{2,5,35,36,46} We postulate that the mechanisms of urolith development in the present case series consist of a high protein, cricket-based diet coupled with uricotelic nitrogen metabolism, dehydration, increased cutaneous water loss, body temperature fluctuations facilitating supersaturation of urine, and subsequent accumulation and precipitation of urogenous wastes within the urinary bladder.

Compared to similar species within the phyllomedusine genus, waxy monkey frogs produce a significantly larger amount of urate and rely heavily on dietary moisture content as a water source.^{34,36} In circumstances where protein intake is high relative to dietary moisture and water availability, urinary uric acid concentrations increase, resulting in a greater risk of urate-based stone formation.^{25,33,34} The frogs in this case series were reported to have been offered a diet consisting primarily of crickets, consuming one to two crickets three times per week during the period in which urolithiasis was observed.

Among common invertebrates offered to captive insectivores, crickets have the highest protein content on a gram per kilogram basis.¹² The delivery of a diet consisting primarily of this single, high-protein food source is believed to have amplified purine breakdown, leading to increased levels of nitrogen metabolism end products such as uric acid.^{4,25}

Diets based on limited or single live prey species often do not meet the nutrient requirements of captive animals and are considered to be poorly representative of natural diets.^{11,31,32,44,45} A study examining the stomach contents of wild phyllomedusine frogs described a variety of invertebrate prey items, the highest proportion of which included moth and butterfly larvae, spiders, beetles, crickets, ants, and flies, suggesting that offering the waxy monkey frogs a varied diet in captivity may help to mitigate nutrition-related illness.¹⁷ In contrast to crickets, silkworms contain the highest moisture and lowest protein content of commonly fed invertebrates and could prove to be a beneficial addition to the diet of captive waxy monkey frogs. Similarly, fly species are relatively easy to keep in culture and are recommended as a dietary supplement to better approximate the variation found in wild phyllomedusine diets. If an additional food source is not available, offering multiple developmental stages of a single invertebrate can be considered, as nutrient components often differ with the stage of development. For example, cricket nymphs contain a higher moisture and lower protein content on a gram per kilogram basis as compared to adult crickets.¹² Future investigation into the chemical composition of wild phyllomedusine prey items, used in conjunction with component analysis of invertebrate feeder animals, will aid in the formulation of an optimal captive diet.^{3,12,23,32}

In regard to urinary tract disease, low dietary vitamin A is reported to promote urolithiasis in mammalian species²¹ and has been proposed as a mechanism of urolith development occurring in amphibians and reptiles.^{18,28} Diets with insufficient levels of dietary vitamin A are associated with impaired differentiation and squamous metaplasia of respiratory and urinary tract epithelium, resulting in a reduction of mucus-secreting goblet cells within these tissues. This finding has been observed in captive amphibians with hypovitaminosis A,^{27,38} and was initially suspected to be a cause of urolithiasis in the present case series. A portion of urinary bladder wall from a single affected frog was submitted for histologic

examination and was found to have numerous goblet cells (Fig. 2a). Squamous cells were not identified and goblet cell hyperplasia was suspected. Increased mucus secretion by the urinary tract in response to dehydration has been shown to occur in the waxy monkey frog and is associated with the retention of uric acid products in the bladder.³⁴ Consequently, vitamin A deficiency causing squamous metaplasia and loss of mucus-secreting goblet cells in the bladder does not appear to be a factor in the development of urolithiasis in this species.

The animals in this report were maintained within a temperature gradient ranging from 24–33°C and demonstrated preference for locations adjacent to the primary heat source. While rare, intermittent episodes of evaporation of the sole water source within the holding enclosure were noted, thereby periodically limiting the frogs' access to water for transcutaneous absorption or drinking. Previous studies of the waxy monkey frog's response to periods of prolonged water restriction document sudden voiding of large urate masses following immersion in water.³⁴ Dehydration coupled with elevated concentrations of uric acid within the urinary bladder lumen and subsequent retention of excretory products are believed to be contributing factors of calculi formation in the current case series.

The rate of cutaneous evaporation in the waxy monkey frog is approximately 5% of that documented in other anurans and occurs at a level comparable to reptiles.³⁴ Contributing to the waxy monkey frog's ability to limit cutaneous water evaporation and maintain homeostatic fluid balance is the high density of lipid-producing glands present in the frog's epithelium which generate a protective, wax-like secretion.^{5,42} Following discharge onto the skin surface, the frog uses all four limbs to spread the secretory product in a stereotypic manner, creating a transparent hydrophobic film.² Previous investigation has documented a significant escalation of evaporative water loss in waxy monkey frogs, with and without lipid films, when ambient temperatures reached above 35 and 20°C, respectively.¹⁹ The animals presented in the current report were rarely observed displaying typical body wiping motions, behavior that occurs predictably following handling and in response to elevated temperatures.⁵ Ineffective or reduced lipid film generation due to dietary imbalance or the absence of appropriate environmental cues is proposed to have exacerbated dehydration in these animals.

Two representative calculi from a single frog were submitted for analysis and found to be composed of 100% ammonium urate. Calculi from the remaining cases were similar in physical appearance and therefore presumed to be ammonium urate; however, this was not confirmed with stone analysis. The development of ammonium urate stones requires oversaturation of urine with both ammonium ions and the anionic form of uric acid (urate) and is strongly influenced by urinary pH and temperature.^{6,33,40} Relationships between the lithogenesis of uric acid-based stones and urinary pH are complex.^{16,33} Uric acid uroliths form in acidic urine, and the basis of treatment is stone dissolution via urine alkalinization.^{25,33} In contrast, ammonium urate urolith development is promoted by a relatively higher urine pH (slightly acidic to alkaline), a property that is attributed to the overabundance of both urate and ammonium in this pH range.^{6,9,15} Urate readily forms salts with cations filtered by the kidney such as potassium, sodium, or ammonium. Ammonia (NH₃) comprises approximately 5% of urinary nitrogen excretion products in the waxy monkey frog and is found primarily bound to urate.³⁶ Once filtered or secreted by the kidney, ammonia acts as a buffer, reacting with H⁺ in urine and forming ammonium ions (NH₄). This enables formation of insoluble uric acid salts.⁴⁰ Previous investigations of uric acid solubility and ion binding properties found that the addition of NH₄ rapidly reduced the solubility of urate, with little dependency on NH₄ ion concentration, suggesting that the formation of ammonium urate salts is less dependent on NH₄ levels as compared to urate levels.²⁰ Still, elevated NH₃ excretion also likely occurred in the cases presented here due to high levels of nitrogen-containing compounds in the diet, further supporting the development of ammonium urate stones. Unlike uric acid uroliths, ammonium urate uroliths do not readily dissolve in response to manipulation of urinary pH.^{1,15} Though it may not represent a therapeutic target, measurements of urine pH in affected waxy monkey frogs may help further characterize the pathogenesis of ammonium urate urolithiasis in this species.

The ambient temperature within the frogs' enclosures differed by 9°C between the substrate and the highest perching area. Animals in this case series assumed a low-activity state while perching in close proximity to the heat source. Investigations of urate-based urolith characteristics report increased solubility of urate at higher

temperatures and also highlight the ease with which supersaturation of body fluids can occur.^{20,41} It is postulated that an increase in the frog's body temperature when perching close to the heat lamp supported urine supersaturation with urate, a process aggravated by a high-protein diet. Following descent to the enclosure floor, the cooling effect in conjunction with supersaturated urine is suspected to promote urolith nidation and development. Over time, this process could potentiate urolith growth if repeated temperature fluctuations occur, as is common in waxy monkey frog activity patterns.

Routine physical examination, including thorough coelomic palpation or ultrasonographic evaluation, is warranted in captive waxy monkey frogs to aid in the diagnosis and management of cystic urolithiasis prior to the onset of significant sequelae. Supplying a diverse, balanced diet is recommended to mitigate protein overload. To better represent the diet of their wild counterparts, it is recommended that captive waxy monkey frogs be offered varied invertebrate protein sources such as adult and nymphal crickets, flies, and silkworms. Maintaining adequate hydration is vital for the prevention of urine supersaturation and retention of nitrogen metabolites in the urinary tract. In addition to the provision of an easily accessible water source, waxy monkey frogs with existing or previous urinary tract disease are expected to benefit from periodic soaking to ensure adequate transcutaneous fluid absorption and to promote the release of urate from the urinary bladder. Manipulating temperature gradients within the enclosures to limit chronic or periodic episodes of dehydration, and the utilization of altered light cycles to encourage night-time soaking behavior, may serve to prevent or lessen the severity of disease. Surgical cystotomy represents a viable, short-term treatment strategy for cystic urolithiasis in the waxy monkey frog; however, further characterization of the etiopathogenesis is necessary to provide acceptable, long-term management of this disease.

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Received for publication 7 May 2014