Journal of Aquatic Animal Health 33:44–52, 2021

ISSN: 0899-7659 print / 1548-8667 online
DOI: 10.1002/aah.10119

ARTICLE

Nephroblastoma in a Common Mudpuppy *Necturus maculosus* simultaneously Present with a Mollicute Bacterium of the Genus *Acholeplasma*

Isaac Standish*, Eric Leis, Sara Erickson, and Ryan Katona
U.S. Fish and Wildlife Service, La Crosse Fish Health Center, Onalaska, Wisconsin 54650, USA

Wes Baumgartner
College of Veterinary Medicine, Diagnostic Laboratory, University of Illinois, Urbana, Illinois 61802, USA

Kevin Hanson
Iowa Department of Natural Resources, Guttenberg Fish Hatchery, Guttenberg, Iowa 52052, USA

Iman Ibrahim
Department of Pathology, College of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt

Tony Goldberg
Department of Pathobiological Sciences, University of Wisconsin–Madison, Madison, Wisconsin 53706, USA

Abstract

In March 2017, a wild-caught female common mudpuppy *Necturus maculosus* from Iowa, USA, with an enlarged posterior abdomen was submitted for diagnostic assessment. The cause of the abdominal distension was a large fluid-filled abdominal mass, diagnosed as a nephroblastoma. Parasites and numerous bacteria were isolated and identified from the mudpuppy but were determined to be incidental. Samples of the neoplasm inoculated onto an American toad *Anaxyrus americanus* cell line (BufoTad) yielded cytopathic effect during several passages. However, standard molecular testing of the cell culture supernatant failed to identify any viruses. Next-generation sequencing identified the replicating agent as a bacterium of the genus *Acholeplasma*. Immunohistochemistry confirmed the presence of *Acholeplasma* within the nephroblastoma, including within tumor cells. This is the first report of nephroblastoma and the second report of neoplasia in this species. The results also suggest that certain bacteria of the genus *Acholeplasma* might be oncogenic.

The common mudpuppy *Necturus maculosus* is a large, fully aquatic salamander. Common mudpuppies are a slow-growing, long-lived species (up to 30 years), reaching sexual maturity in 7–10 years (Matson 2005). Common mudpuppy populations are inconspicuous, found in cold lentic and lotic waters throughout the Great Lakes region, the Midwest, and the northeastern United States (McKercher 2019). The conservation status of this species

*Corresponding author: sirisaac_standish@fws.gov
Received August 4, 2020; accepted November 14, 2020

is not well known, although they were historically abundant (King et al. 1997; Holman 2012). In recent decades, populations have declined (Davis et al. 1998; Minton 1998; Minton 2001; Holman 2012). Common mudpuppy populations have experienced mortality events (Faisal 2006), threats from lampricide use (Matson 1998; Boogaard et al. 2003), overharvest and persecution, habitat loss, competition from invasive species, and water quality declines due to nutrient addition and toxic algal blooms (King et al. 1997; Casper 1998; Davis et al. 1998; Lannoo 2005; Holman 2012).

Notably, the common mudpuppy is the only known host species for the salamander mussel *Simpsonaias ambigua*, a species listed as endangered or threatened by several Midwestern states (Bogan et al. 2017). The U.S. Fish and Wildlife Service (USFWS) has developed a recovery program for the salamander mussel that involves supplementing natural populations. Efforts to propagate this mussel involve bringing common mudpuppies into hatchery facilities to serve as glochidia (larval mussel) hosts. These same efforts are predicated upon healthy wild salamander mussel and common mudpuppy populations (Hoffman et al. 2014; Standish et al. 2019). As part of this effort, the La Crosse Fish Health Center performs routine health inspections of common mudpuppy populations prior to incorporating them into hatchery operations, as well as conducting diagnostic evaluations of wild mudpuppies as needed. These efforts have demonstrated that wild common mudpuppies can harbor *Yersinia ruckeri* (Standish et al. 2019), a bacterium pathogenic to a wide range of fish species.

A variety of neoplasms have been reported in other amphibian species (Balls 1962). Cases of cutaneous tumors are common (Balls 1962), but renal neoplasia is uncommon (Green and Harshbarger 2001). Neoplasia has been reported once in a common mudpuppy, a renal adenocarcinoma (Schlumberger 1958). However, Lucké renal carcinoma, caused by ranid herpesvirus 1 (McKinnell 1973), has been widely noted in North American leopard frogs *Rana pipiens* (Lucké 1934; McKinnell 1984). Moreover, there are case reports of nephroblastoma in a clawed frog *Xenopus laevis* (Meyer-Rochow et al. 1991), a Japanese fire-bellied newt *Cynops pyrrhogaster* (Zwart 1970), and a Japanese giant salamander *Andrias japonicas* (Kawasumi et al. 2012). Herein, we present the first report of a common mudpuppy with a nephroblastoma, including its association with a bacterium of the *Acholeplasma* genus.

METHODS

Mudpuppies.— Wild common mudpuppies were captured as part of a population monitoring program conducted by the Iowa Department of Natural Resources. Mudpuppies were captured using baited minnow traps in a side channel of the Mississippi River near Guttenburg, Iowa (Figure 1A). One mature female mudpuppy presented with an enlarged posterior abdomen (Figure 1B, C). The female mudpuppy was held alive in aerated river water prior to transport to the La Crosse Fish Health Center (Onalaska, Wisconsin). The mudpuppy was euthanized by immersion in 2 g/L of buffered tricaine methanesulfonate (MS-222; Western Chemical, Ferndale, Washington).

Tissue sample collection.— Skin swabs were collected following Hyatt et al. (2007) and processed as described by Standish et al. (2019). Blood smears were examined, and the mudpuppy was inspected for external parasites. The mudpuppy was aseptically necropsied, and kidney samples were collected for bacterial, viral, histological, and molecular analyses. Samples of the neoplastic kidney, liver, and spleen (KSL) as well as a separate sample of the intratumor interstitial fluid (IF) were collected and placed into Hank's balanced salt solution (HBSS; Sigma-Aldrich, St. Louis, Missouri), transport media containing penicillin (500 U/mL), streptomycin (500 µg/ mL), gentamycin (20 µg/ mL) and nystatin (25 units per mL) at a 1:10 ratio (weight : volume).

Tissue culture.— Three cell lines, epithelioma papulosum cyprini (EPC), Chinook Salmon embryo (CHSE), and a recently developed cell line (BufoTad) derived from American toad *Anaxyrus americanus* tadpoles (Vo et al. 2019) were used for pathogen isolation. All cells were grown to confluence in minimum essential medium with Hank's balanced salts (Sigma-Aldrich) and maintained at 20°C. The EPC and CHSE cells were supplemented with 10% fetal bovine serum (VWR International, Radnor, Pennsylvania), and BufoTad cells with 20% fetal bovine serum.

For pathogen isolation, KSL and IF samples were then homogenized using a Seward Stomacher 80 Biomaster (Seward Laboratory Systems, Bohemia, New York) and centrifuged ($1{,}832 \times g$ for 15 min at 4°C) to precipitate solids. Then the supernatant was diluted at a 1:1 (volume : volume) in transport media and incubated overnight at 4°C. Following incubation, samples were pelleted at $1{,}832 \times g$ for 15 min at 4°C, and 0.1 mL of supernatant was placed, in replicate, onto a confluent 24-well cell culture plate containing either EPC, CHSE, or BufoTad cells. Plates were gently rocked for 1 h at room temperature and then overlain with 0.5 mL of minimum essential medium supplemented with 5% fetal bovine serum and gentamycin (200 µg/mL; Sigma-Aldrich) and incubated at 15°C (EPC and CHSE) and 20°C (EPC and BufoTad). Cells were monitored three times per week for signs of cytopathic effect (CPE). After 2 weeks, samples underwent a blind passage. Cell monolayers were then scraped, removed, centrifuged ($1{,}832 \times g$ for 15 min at 4°C), diluted 1:5, and reinoculated onto confluent cells. The original sample

FIGURE 1. Common mudpuppy capture and gross images, including **(A)** a baited minnow trap used to capture mudpuppies, **(B)** a wild-caught mature female mudpuppy with an enlargement of the posterior abdomen, **(C)** a view from the ventral side of the female mudpuppy, **(D)** a vascular neoplasm involving ovarian and renal tissues, and **(E)** turbid fluid contained with the neoplastic mass. [Color figure can viewed at afsjournals.org.]

tubes and supernatants from all wells from the original inoculation, including negative controls, were archived at −80°C.

Bacteriology.— For bacterial isolation, kidney samples were collected using a sterile disposable 1-μL inoculating loop and inoculated on trypticase soy agar (TSA; Becton Dickinson, Franklin Lakes, New Jersey) plates and incubated at 22°C for 96 h. Individual colonies were subcultured onto TSA slants for up to 48 h for molecular and biochemical testing in accordance with the USFWS and American Fisheries Society Fish Health Section Blue Book (USFWS and AFS–FHS 2014). Additional biochemical analyses were performed using a Biolog MicroStation (Biolog, Hayward, California) and the GEN III MicroPlate (Biolog) following the manufacturer's instructions.

Histology.— The remaining portion of the neoplasm was excised and fixed whole in Davidson's fixative, serially sectioned and embedded in paraffin wax blocks. Ribbons of 5-μm thickness were cut from the blocks, placed on glass slides and stained with hematoxylin and eosin and periodic acid–Schiff (PAS); stained sections were examined using light microscopy.

Immunohistochemistry.— BufoTad KSL and IF culture media was pelleted by ultracentrifugation at 150,000 rpm for 15 min. A concentrated cell culture supernatant

containing *Acholeplasma laidlawii* was used as the positive control. The pellet was washed with phosphate buffered saline (PBS), and then 0.5 mL of molten 1% agarose gel was added. The mixture was left to solidify at room temperature. Then 500 mL of 10% neutral buffered formalin was added to preserve the live cells. The preserved cells in agarose were embedded and cut by microtome. Five-μm sections of paraffin-embedded tissue blocks were deparaffinized in an oven (70–75°C) for 20 min and rehydrated for 2 min in xylene and a graded series of ethanol (100% and 70%). Antigens were retrieved by incubating slides in 10× Dako target retrieval solution, pH 9 (Dako, Denmark) in a pressurized food steamer (Oster, China) for 30 min and then allowed to cool at room temperature for 10 min. Endogenous peroxidase activity was inactivated through incubation with hydrogen peroxide for 30 min at room temperature. To block nonspecific antibody binding, the slides were incubated in serum-free Dako protein block (Dako North America, Carpinteria, California) for 1 h and then rinsed with PBS. Slides were then incubated overnight in a humid chamber at 4°C in a 1:500 dilution of an *Acholeplasma*-specific antibody gifted from N. Ferguson-Noel (Mycoplasma Research Lab, Poultry Diagnostic and Research Center, University of Georgia). Next, slides were rinsed in PBS before being incubated for another hour in the secondary antibody (Dako Envision + Dual link system) horseradish peroxidase (Dako North America). After incubation with the secondary antibody, the slides were first rinsed with PBS followed by 10-min incubation in DAKO liquid 3,3'-diaminobenzidine-horseradish peroxidase + substrate chromogen system (Dako) in a dark, humid chamber at room temperature followed by a rinse with tap water. Finally, the slides were counterstained with hematoxylin for 5 min (Lecia Biosystem, Richmond, Illinois), rinsed in distilled water, washed in acid ammonia water, dehydrated in an ascending alcohol series, cleared by xylol, and mounted with coverslips. Slides were analyzed and photographed using an Olympus BX43 microscope. Slides incubated with PBS instead of primary antibody were used as a negative control.

Molecular analyses.— Extracted DNA from skin swabs was used to screen for *Batrachochytrium dendrobatidis* (*Bd*), *B. salamandrivorans* (*Bsal*), and the ranavirus frog virus 3 (FV3). Multiplex quantitative PCR was conducted as described by Standish et al. (2018).

To identify the agent causing CPE in the BufoTad cell line, PCR was initially conducted for viral hemorrhagic septicemia virus and spring viremia of carp virus (SVCV) in accordance with the American Fisheries Society *FHS Blue Book* (AFS–FHS 2014). Samples were then subjected to next-generation sequencing for pathogen discovery as previously described (Sibley et al. 2016; Goldberg et al. 2018, 2019). Briefly, total nucleic acids were extracted from supernatants using the QIAamp MinElute virus kit (Qiagen, Hilden, Germany), RNA was reverse transcribed, and libraries were prepared for sequencing on an Illumina MiSeq instrument using the Nextera XT DNA sample kit (Illumina, San Diego, California). We then used CLC Genomics Workbench version 12.0 (CLC Bio, Aarhus, Denmark) for de novo assembly of sequences and resulting contiguous sequences were compared at the translated-protein level to GenBank databases of known agents using the blastx algorithm (Altschul et al. 1990). Contiguous matching sequences of known agents ("hits") were then confirmed manually at the nucleotide and amino acid sequence levels by comparison to the full GenBank database using the blastn and blastx homology searching algorithms, respectively (Altschul et al. 1990).

Based on the results of next-generation sequencing (see below), *Acholeplasma*-specific PCRs were conducted on all supernatants and negative controls. The 16S-23S ribosomal RNA (rRNA) intergenic transcribed spacer (ITS) and *rpoB* and *gyrB* genes were amplified using primer sets and cycling conditions described by Volokhov et al. (2007). A portion of the 16S rRNA gene was also amplified using universal primers 8F and 1492R (Turner et al. 1999) with the following cycling conditions: 95°C for 4 min, and 35 cycles of 95°C for 60 s, 50°C for 60 s, and 72°C for 2 min, and concluding with 72°C for 7 min. All *Acholeplasma* genes were amplified using 50 μL reactions containing 200 nM of each primer and Platinum PCR SuperMix (Thermo Fisher Scientific, Waltham, Massachusetts). Amplicons were visualized using the E-Gel electrophoresis system (Thermo Fisher Scientific) and compared to bands of the TrackIt 100 bp ladder (Thermo Fisher Scientific). Amplicons were sequenced by Eton Biosciences (San Diego, California), and resulting sequences were analyzed using Geneious v11.1.5 (Auckland, New Zealand).

RESULTS

Clinical Observations

Upon arrival at the La Crosse Fish Health Center, the adult female common mudpuppy presented in good body condition. The uterus appeared gravid, although eggs were immature. The gills were heavily infested with *Trichodina* sp., which are common commensals of amphibians, fishes, and invertebrates (Mitchell 2007; Collymore et al. 2013). The swelling of the posterior third of the abdomen was a vascularized, multicystic, thin-walled, 2–4-cm-wide, fluctuant, pink/tan mass that was attached to the mesonephroi and contained abundant watery, tan, turbid fluid (Figure 1D, E). The mass encompassed the mesonephroi and appeared to be neoplastic (Figures 1, 3). Feeding did not appear to be impaired by the mass, as the animal was well conditioned, with a partially digested Fathead Minnow *Pimephales promelas* in its stomach and a juvenile

Freshwater Drum *Aplodinotus grunniens* in its lower gastrointestinal tract. Two unspecified acanthocephalan parasites were found in the intestine. No other lesions were observed.

Tissue Culture

No CPE was observed in CHSE and EPC cells. However, a slowly developing CPE was observed in BufoTad cells (Figure 2), associated with both KSL and IF samples on day 20 after the initial inoculation, 12 d after first reset, and 10 d after the second reset. Typical monolayers of the BufoTad cell line display a homogenous, cobblestone appearance with apparent contact inhibition (Vo et al. 2019). The BufoTad monolayers inoculated with samples from the mudpuppy contained numerous detached cells and a loss of contact inhibition, which was especially interesting given that the samples were derived from a tumor. Despite the large numbers of floating cells, plaque formation was limited as spaces in the monolayer were seemingly repopulated quickly by cells reproducing at an increased rate and lacking cellular organization. Individual infected cells were granular in appearance, often with conspicuous vacuolization. Cell culture supernatant also became acidic and turned the pH indicator bright yellow. This CPE was dissimilar to that observed for common fish viral pathogens (AFS–FHS 2014).

Bacteriology

No growth was observed on TSA slants collected from KSL. Minimal growth of two colony types was observed on TSA slants collected from IF. Biochemical testing of two isolates were inconsistent with *Aeromonas salmonicida*, *Yersinia ruckeri*, and *Edwardsiella ictaluri*. Biolog was used to identify the two isolates as *Pseudomonas fluorescens* and *Aeromonas* sp.

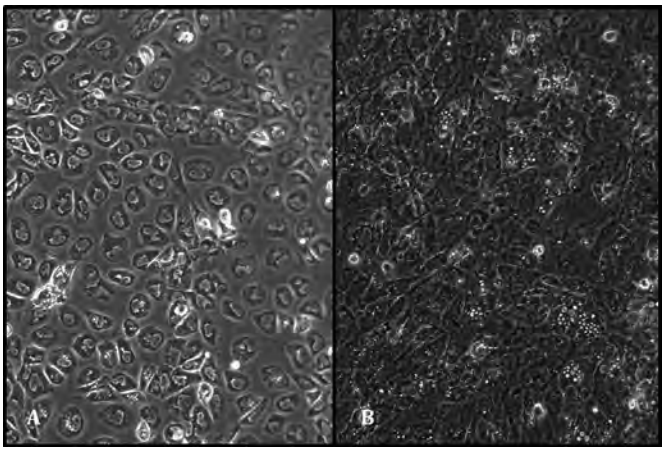

FIGURE 2. Cytopathic effect (CPE) on the BufoTad cell line derived from American toad tadpoles. Negative control BufoTad cells are shown on the left. The image on the right shows slowly developing CPE following inoculation of neoplastic mass homogenate from a common mudpuppy. The monolayer remains intact, though cells become increasingly dense and develop numerous vacuoles as the CPE progresses.

Histopathology

The mass was a multicystic renal neoplasm composed of a mixture of blastema, epithelium, and stroma (Figure 3A). Blastemal cells had large, ovoid nuclei that were tightly packed such that they were deformed against each other, with coarsely aggregated chromatin, parachromatin clearing, obscured nucleoli, and inconspicuous cytoplasm. Aggregates of these cells formed clusters and sinuous cords that mingled imperceptibly with the epithelial component, which was composed of haphazard cysts and small tubules. Epithelial cells were similar to blastemal cells but were larger, columnar, and more uniform; they were closely regimented, lacking polarity, along basal laminae that were highlighted with PAS stain (Figure 3B). Occasional embryonic glomeruloid structures were evident where the tubules were invaginated by tufts of stromal tissue (Figure 3C). The stromal component was loose and mucinous with fine collagen and fibroblasts (Figure 3A). One mitotic figure was seen.

Immunohistochemistry

Fine, coccoid, monomorphic positive signal reactions were evident, consistent with specific staining of the organism (Figure 3D). Within the neoplasm, a few cells contained positive granular signals (Figure 3E, F).

Molecular Analyses

During multiplex quantitative PCR, amplification was observed in wells containing synthetic gene block (gblock) standards, but no amplification was observed in duplicate wells containing extracted DNA from the skin swab. All reactions were negative for the amphibian pathogens *Bd*, *Bsal* and FV3. Next-generation sequencing yielded 113,439 sequence reads, of which 32,844 mapped to *American toad*, from which the BufoTad cell line was derived. The remaining 80,595 reads (71.05%) mapped to bacteria of the genus *Acholeplasma*. No reads mapped to any other viral, bacterial, or eukaryotic parasitic taxa.

The 16S rRNA, ITS, *rpoB* and *gyrB* genes of *Acholeplasma* were successfully PCR-amplified and sequenced from supernatant of the primary inoculation sample wells displaying CPE. Additionally, negative control supernatants from the original plate of the BufoTad cell line were evaluated with 16S rRNA gene PCR and products were not visualized, suggesting that the presence of *Acholeplasma* was not a result of contaminated cultures. A blastn search of resulting sequences against the GenBank database showed the sequences to match most closely sequences of *A. laidlawii*. Specifically, a 1,397-bp region of 16S gene (GenBank accession number

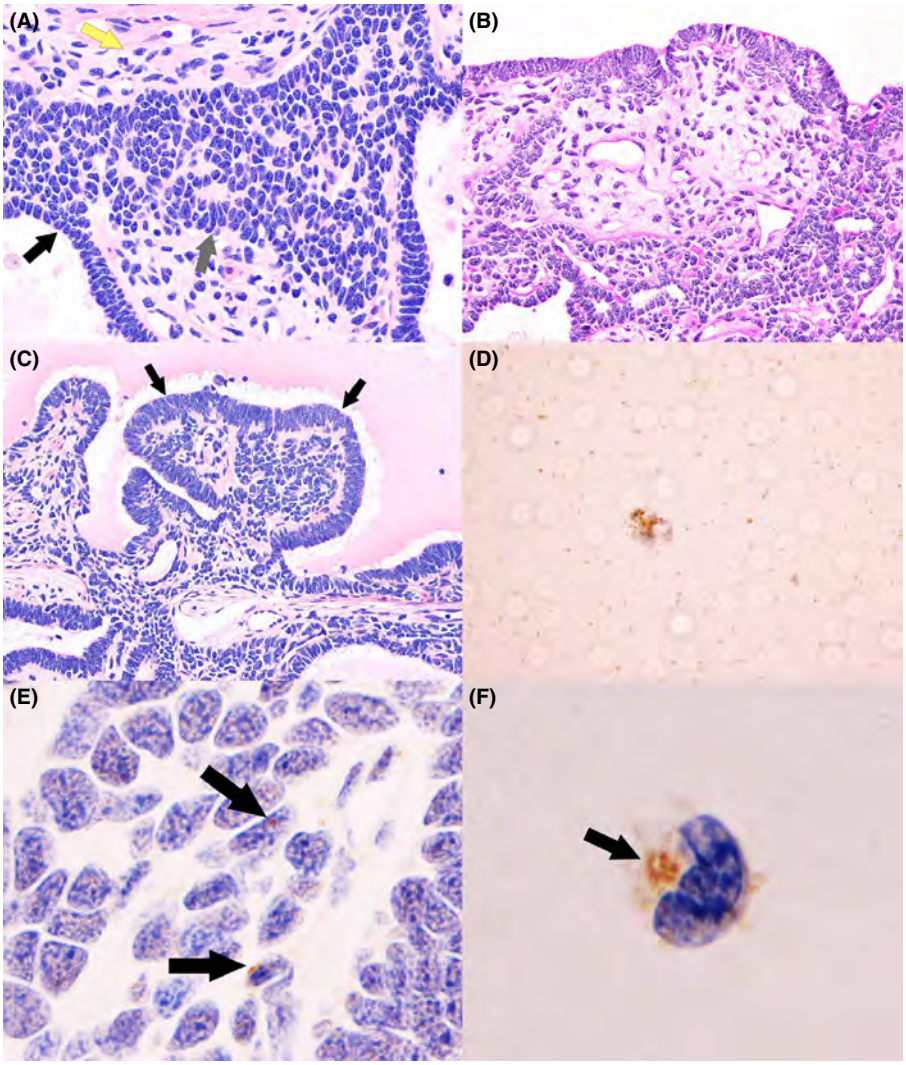

FIGURE 3. Nephroblastoma from wild-caught female mudpuppy showing **(A)** blastemal cells (gray arrow) in serpentine arrays merging with the epithelial component, which forms embryonic tubules and cysts (black arrow). Plump mesenchymal cells are present in the stroma (yellow arrow; 100× hematoxylin and eosin [HE]). **(B)** The stromal component is loose/areolar and mildly PAS positive (mucin). A delicate brightly PAS positive basement membrane delimits the epithelium from the stroma (100× PAS stain). **(C)** A glomeruloid structure is evident (black arrows), formed by the invagination of stroma and blastemal cells into a cystic space (100× HE). **(D)** *Acholeplasma laidlawii*-specific antibody stain of positive control cell culture supernatant is shown. Many fine punctate, monomorphic positive signals, consistent with *Acholeplasma* sp. are present (600×). The immunohistochemistry (*Acholeplasma laidlawii*-specific antibody) of the nephroblastoma is shown, **(E)** with black arrows pointing to cells with internal punctate positivity (400×) and **(F)** sloughed intraluminal nephroblastoma cell containing granular positive material (400×). [Color figure can viewed at afsjournals.org.]

MT237181) was 99.79% similar to the *A. laidlawii* type strain (GenBank accession number LS483439). A 1,651-bp region of the *rpoB* gene (GenBank accession number MT237178) was 98.85% similar to this same *A. laidlawi* type strain, a 1,574-bp region of the *gyrB* gene (GenBank accession number MT237179) was 94.86% similar to the *A. laidlawi* type strain, and a 965-bp region of the ITS gene (GenBank accession number MT237180) was 99.2% similar to the *A. laidlawi* type strain. The *Acholeplasma pleciae* type strain was the next most closely related species for the 16S, *rpoB*, and *gyrB* genes at 98.35%, 87.67%, and 84.23% similarity, respectively (GenBank accession numbers NR113898, DQ234658, DQ217917). The *Acholeplasma granularum* type strain (GenBank accession number AY786572) was the next closest relative (96.27% similar) for the ITS gene sequence.

DISCUSSION

We present the first description of a nephroblastoma in a common mudpuppy. Moreover, comprehensive diagnostics for infectious agents identified only a bacterium within the

genus *Acholeplasma* to be associated with the tumor. A nephroblastoma, also known in human pathology as Wilms' tumor, is a neoplasm composed of renal blastema (primordial cells), embryonic epithelium, and myxomatous stroma admixed in varying amounts (Meuten and Meuten 2016). They derive from embryonic stem cells that develop into epithelial, blastema, and stromal cells. The blastemal cells are arranged in sheets, have scant cytoplasm, and blend into areas of cyst and tubule formation (epithelial component). The most definite character for diagnosis is the formation of structures resembling glomeruli, where epithelial tufts invaginate into lumens. The stroma is loose and mucinous, the evidence of which can be seen with PAS or Alcian blue stains. In this case, these features were evident. Nephroblastomas have been reported in many species, including mammals, fish, and amphibians; they are often expansile and cystic and, in some mammalian species, frequently metastatic (Green and Harshbarger 2001; Lombardini et al. 2014; Meuten and Meuten 2016). In amphibians, spontaneous nephroblastomas have been reported in the African clawed frog, a Japanese fire-bellied newt, and a Japanese giant salamander (Green and Harshbarger 2001; Kawasumi et al. 2012). The only other report of neoplasia of any kind in common mudpuppy is a renal adenocarcinoma (Schlumberger 1958).

Neoplasms in aquatic animals can be caused by infectious organisms. For example, the viral pathogens ranid herpesvirus 1 (Lucké renal adenocarcinoma of frogs; Lucké′, 1934), *Oncorhynchus masou* virus (SalHV2; Kimura et al. 1981; Yoshimizu et al. 1987), cyprinid herpesvirus 1 (CyHV1; Sano et al. 1985), and Atlantic Salmon papillomatosis (SalHV4; Carlisle and Roberts 1977) have each been associated with neoplasias. Retrovirus infections can also cause tumors (Coffee et al. 2013), such as Walleye dermal sarcoma (WDSV; Rovnak and Quakenbush 2010), which can seasonally affect more than 20% of adult Walleye *Sander vitreus* (Martineau et al. 1992). However, such associations have rarely been reported for nonviral agents, however.

The link between mollicutes and oncogenicity has been hypothesized for more than 50 years, when Macpherson and Russell (1966) observed the "transformation" of the BHK21 cell line derived from hamster fibroblast cells infected with several mycoplasma species in vitro. The authors noted a range of "remarkable" altered cells ranging from multinucleated giant cells, epithelioid cells, round cells, and spindle-shaped cells growing in disarray (Macpherson and Russel 1966). Other previous in vitro studies have identified potential mechanisms for mycoplasmas to influence oncogenesis. For example, potential mechanisms include activation of nuclear factor NF-κB, reduced activation of p53 protein, inhibition of apoptosis (Logunov et al. 2008), induction of bone morphogenetic protein linked to human lung cancer (Rogers 2011), and influencing the bioavailability and toxicity of nucleoside analogues (Vande Voorde et al. 2014). A link has also been reported between intestinal neoplasms and a related species of *Mycoplasma*, *M. penetrans*, in Zebrafish *Danio rerio* intestines (Burns et al. 2018). The presence of an *Acholeplasma* sp. within sections of the neoplasm indicate a possible association with the nephroblastoma.

Mollicutes are also common in the environment and can be saprophytic, commensal, or pathogenic (Simecka et al. 1992). *Acholeplasma laidlawii* specifically has been reported from aquatic animals, including the mud crab *Scylla serrata* during a clearwater disease epizootic, showing ascites, a white carapace, and weak grip strength of pincers (Chen et al. 2011). In another report, *A. laidlawii* was isolated from centrarchid fishes (Largemouth Bass *Micopterus salmoides*, Black Crappie *Pomoxis nigromaculatus*, Bluegill *Leopomis macrochrius*, and Redear Sunfish *L. microlophus*) in Florida; Largemouth Bass displayed emaciation and inflammation and ecchymotic hemmorrage in the swim bladder (Francis-Floyd et al. 1998). However, inability to fulfill Koch's postulates in Largemouth Bass in this case suggested that this bacterium was not the causative agent (Francis-Floyd et al. 1998). The *Acholeplasma* sp. reported herein is most closely related to *A. laidlawii*, although its *gyrB* gene sequence differed from that of the *A. laidlawii* type strain by over 5%. This finding agrees with a previous study (Volokhov et al. 2007), which reported lower interspecies similarity rates and phylogenetically deeper branch resolution of the *gyrB* and *rpoB* gene sequences compared to 16S rRNA gene. It is therefore unclear whether the bacterium is a novel species within the *Acholeplasma* genus or a divergent variant.

Notably, the Iowa Department of Natural Resources has observed other common mudpuppies with enlarged abdomens similar to the female described herein. One tagged individual with an enlarged abdomen was recaptured 329 d later, and the enlargement had resolved and her weight had dropped from 419 g to 308 g. It is noteworthy that the clinical signs we have described here were observed in other individuals in the population and, in at least one instance, appear to have resolved. Additional study of such individuals for the nature of the lesion and its etiology should be prioritized.

ACKNOWLEDGMENTS

The authors thank the USFWS staff and coworkers at the Midwest Fisheries Center and the National Conservation Training Center (NCTC) for their assistance and motivation, John Fisher (USFWS, NCTC) for his assistance in obtaining literature and publications, and Christopher Dunn for assistance with next-generation sequencing. This research was funded in part by the

University of Wisconsin–Madison John D. MacArthur Fellowship Program. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the USFWS or U.S. Government. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the USFWS or U.S. Government. There is no conflict of interest declared in this article.

ORCID

Isaac Standish https://orcid.org/0000-0003-2846-1872
Eric Leis https://orcid.org/0000-0001-8379-3285
Tony Goldberg https://orcid.org/0000-0003-3962-4913

REFERENCES

AFS–FHS (American Fisheries Society–Fish Health Section). 2014. FHS blue book: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 2016 edition. AFS–FHS, Bethesda, Maryland.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. Journal of Molecular Biology 215:403–410.

Balls, M. 1962. Spontaneous neoplasms in amphibian: a review and description of six new cases. Cancer Research 22:1142–1154.

Bogan, A. E., D. Woolnough, and M. B. Seddon. 2017. *Simpsonaias ambigua*. The IUCN Red List of Threatened Species 2017: e.T20247A62905797.

Boogaard, M. A., T. D. Bills, and D. A. Johnson. 2003. Acute toxicity of TFM and a TFM/niclosamide mixture to selected species of fish, including Lake Sturgeon (*Acipenser fulvescens*) and mudpuppies (*Necturus maculosus*), in laboratory and field exposures. Journal of Great Lakes Research 29:529–541.

Burns, A. R., V. Watral, S. Sichel, S. Spagnoli, A. V. Banse, E. Mittge, T. J. Sharpton, K. Guillemin, and M. L. Kent. 2018. Transmission of a common intestinal neoplasm in Zebrafish by cohabitation. Journal of Fish Diseases 41:569–579.

Carlisle, J. C., and R. J. Roberts. 1977. An epidermal papilloma of the Atlantic Salmon I: epizootiology, pathology and immunology. Journal of Wildlife Diseases 13:230–234.

Casper, G. S. 1998. Review of the status of Wisconsin amphibians. Pages 199–205 *in* M. J. Lannoo, editor. Status and conservation of Midwestern amphibians. University of Iowa Press, Iowa City.

Chen, J.-G., D. Lou, and J.-F. Yang. 2011. Isolation and identification of Acholeplasma sp. from mud crab, *Sylla serrata*. Marine Biotechnology 2011:209406.

Coffee, L. L., J. W. Casey, and P. R. Bower. 2013. Pathology of tumors in fish associated with retroviruses: a review. Veterinary Pathology 50:390–403.

Collymore, C., J. R. White, and C. Lieggi. 2013. *Trichodina xenopodus*, a ciliated protozoan, in a laboratory-maintained *Xenopus laevis*. Comparative Medicine 63:310–312.

Davis, J. G., P. J. Krusling, and J. W. Ferner. 1998. Status of amphibian populations in Hamilton County, Ohio. Pages 155–165 *in* M. J. Lannoo, editor. Status and conservation of Midwestern amphibians. University of Iowa Press, Iowa City.

Faisal, M. 2006. Aquatic animal health laboratory: preliminary laboratory report. Michigan Department of Natural Resources, Fisheries Division, Lansing.

Francis-Floyd, R., P. Reed, P. Gibbs, E. Shotts, B. Bolon, W. Coleman, and R. Klinger. 1998. Isolation of *Acholeplasma laidlawii* from centrarchids in a central Florida lake. Journal of Aquatic Animal Health 10:252–258.

Goldberg, T. L., V. L. Clyde, A. Gendron-Fitzpatrick, S. D. Sibley, and R. Wallace. 2018. Severe neurologic disease and chick mortality in crested screamers (*Chauna torquata*) infected with a novel gyrovirus. Virology 520:111–115.

Goldberg, T. L., S. D. Sibley, M. E. Pinkerton, C. D. Dunn, L. J. Long, L. C. White, and S. D. Strom. 2019. Multidecade mortality and a homolog of hepatitis C virus in bald eagles (*Haliaeetus leucocephalus*), the national bird of the USA. Scientific Reports [online serial] 9:14953.

Green, D. E., and J. C. Harshbarger. 2001. Spontaneous neoplasia in amphibian. Pages 335–400 *in* K. M. Wright and B. R. Whitaker, editors. Amphibian medicine and captive husbandry. Krieger Publishing Company, Malabar, Florida.

Hoffman, A. S., J. R. Robb, and B. E. Fisher. 2014. Recent records for mudpuppies (*Necturus maculosus*) in Indiana with notes on presumed declines throughout the Midwest. Proceedings of the Indiana Academy of Science 123:1–6.

Holman, J. A. 2012. The amphibians and reptiles of Michigan: a quaternary and recent faunal adventure. Wayne State University Press, Detroit, Michigan.

Hyatt, A. D., D. G. Boyle, V. Olsen, D. B. Boyle, L. Berger, D. Obendorf, A. Dalton, K. Kriger, M. Heros, H. Hines, R. Phillot, R. Campbell, G. Marantelli, F. Gleason, and A. Coiling. 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. Diseases of Aquatic Organisms 73:175–192.

Kawasumi, T., T. Kudo, and Y. Une. 2012. Spontaneous nephroblastoma in a Japanese giant salamander (*Andrias japonicas*). Journal of Veterinary Medical Science 74:673–675.

Kimura, R., M. Yoshimizu, and M. Tanaka. 1981. Studies on a new virus (OMV) from *Oncorhynchus masou*—I. Characteristics and pathogenicity. Fish Pathology 15:143–147.

King, R. B., M. J. Oldham, W. F. Weller, and D. Wynn. 1997. Historic and current amphibian and reptile distributions in the island region of western Lake Erie. American Midland Naturalist 138:153–173.

Lannoo, M. J. 2005. Amphibian declines: the conservation status of United States species. University of California Press, Berkeley.

Logunov, D. Y., D. V. Scheblyakov, O. V. Zubkova, M. M. Shmarov, I. V. Rakovskaya, K. V. Gurova, N. D. Taraova, L. G. Burdelya, B. S. Naroditsky, A. I. Ginzburg, and A. V. Gudkov. 2008. Mycoplasma infection suppresses p53, activates NF-κB and cooperates with oncogenic Ras in rodent fibroblast transformation. Oncogene 27:4521–4531.

Lombardini, E. D., G. C. Hard, and J. C. Harshbarger. 2014. Neoplasms of the urinary tract in fish. Veterinary Pathology 51:1000–1012.

Lucké, B. 1934. A neoplastic disease of the kidney of the frog, *Rana pipiens*. The American Journal of Cancer 20:352–379.

Macpherson, I., and W. Russel. 1966. Transformation in hamster cells mediated by mycoplasmas. Nature 210:1343–1345.

Martineau, D., P. R. Bowser, R. R. Renshaw, and J. W. Casey. 1992. Molecular characterization of a unique retrovirus associated with fish tumor. Journal of Virology 66:596–599.

Matson, T. O. 1998. Evidence for home ranges in mudpuppies and implications for impacts due to episodic applications of the lampricide TFM. Pages 279–287 *in* M. J. Lannoo, editor. Status and conservation of Midwestern amphibians. University of Iowa Press, Iowa City.

Matson, T. O. 2005. *Necturus maculosus* (Rafinesque, 1818) mudpuppy. Pages 870–871 *in* M. J. Lannoo, editor. Amphibian declines: the conservation status of United States species. University of California Press, Berkeley.

McKercher, E. 2019. *Necturus maculosus* (Rafinesque, 1818). Nonindigenous Aquatic Species [online database]. U.S. Geological Survey, Reston, Virginia. Available: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=156. (December 2019).

McKinnell, R. G. 1973. The Lucké frog kidney tumor and its herpesvirus. American Zoologist 13:97–114.

McKinnell, R. G. 1984. Lucké tumor of frogs. Pages 581–605 *in* G. L. Hoff, F. L. Frye, and E. R. Jacobson, editors. Diseases of amphibians and reptiles. Springer, Boston.

Meuten, D. J., and T. L. K. Meuten. 2016. Tumors of the urinary system. Pages 632–688 *in* D. J. Meuten, editor. Tumors in domestic animals, 5th edition. Wiley-Blackwell, Hoboken, New Jersey.

Meyer-Rochow, V. B., M. Asashima, and S. D. Moro. 1991. Nephroblastoma in clawed frog *Xenopus laevis*. Journal of Experimental Animal Science 34:225–228.

Minton, S. A. 1998. Observations on Indiana amphibian populations: a 45-year overview. Pages 217–220 *in* M. J. Lannoo, editor. Status and conservation of Midwestern amphibians. University of Iowa Press, Iowa City.

Minton, S. A. 2001. Amphibians and reptiles of Indiana. Indiana Academy of Science, Indianapolis.

Mitchell, M. A. 2007. Parasites of amphibians. Pages 118–171 *in* D. G. Baker, editor. Flynn's parasites of laboratory animals, 2nd edition. Blackwell Publishing, Ames, Iowa.

Rogers, M. B. 2011. Mycoplasma and cancer: in search of the link. Oncotarget 2:271–273.

Rovnak, J., and S. L. Quackenbush. 2010. Walleye dermal sarcoma virus: molecular biology and oncogensis. Viruses 2:1984–1999.

Sano, T., H. Fukuda, and M. Furukawa. 1985. *Herpesvirus cyprini*: biological and oncogenic properties. Fish Pathology 20:381–388.

Schlumberger, H. G. 1958. Krankheiten der fische, amphibien, und reptile. [Diseases of amphibians and reptiles.] Pages 733–746 *in* P. Cohrs, R. Jaffe, and H. Meesen, editors. Pathologie der laboratoriumstiere, volume 2. Springer, Heidelberg, Germany.

Sibley, S. D., M. A. Finley, B. B. Baker, C. Puzach, A. G. Armien, D. Giehtbrock, and T. L. Goldberg. 2016. Novel reovirus associated with epidemic mortality in wild Largemouth Bass (*Micropterus salmoides*). Journal of General Virology 97:2482–2487.

Simecka, J. W., J. K. Davis, M. K. Davidson, S. E. Ross, C. T. Stadtlander, and G. H. Cassell. 1992. *Mycoplasma* diseases of animals. Pages 91–394 *in* J. Maniloff, R. McElhaney, L. Finch, and J. Baseman, editors. Mycoplasmas: molecular biology and pathogenesis. American Society for Microbiology, Washington, D.C.

Standish, I., S. Erickson, E. Kleman, R. McCann, R. Katona, and E. Leis. 2019. *Yersinia ruckeri* isolated from common mudpuppy *Necturus maculosus*. Journal of Aquatic Animal Health 31:71–74.

Standish, I., E. Leis, N. Schmitz, J. Credico, S. Erickson, J. Bailey, J. Kerby, K. Phillips, and T. Lewis. 2018. Optimizing, validating, and field testing a multiplex qPCR for the detection of amphibian pathogens. Diseases of Aquatic Organisms 129:1–13.

Turner, S., K. M. Pryer, V. P. W. Miao, and J. D. Palmer. 1999. Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. Journal of Eukaryotic Microbiology 46:327–338.

Vande Voorde, J., J. Balzarini, and S. Liekens. 2014. Mycoplasmas and cancer: focus on nucleoside metabolism. EXCLI Journal 13:300–322.

Vo, N. T. K., L. C. Moore, E. Leis, and S. J. DeWitte-Orr. 2019. Class A scavenger receptors mediate extracellular dsRNA sensing, leading to downstream antiviral gene expression in a novel American toad cell line BufoTad. Developmental and Comparative Immunology 92:140–149.

Volokhov, D. V., A. A. Neverov, J. George, H. Kong, S. X. Liu, C. Anderson, M. K. Davidson, and V. Chizhikov. 2007. Genetic analysis of housekeeping genes of members of the genus *Acholeplasma*: phylogeny and complementary molecular markers to the 16S rRNA gene. Molecular Phylogenetics and Evolution 44:699–710.

Yoshimizu, M., M. Tanaka, and T. Kimura. 1987. *Oncorhynchus masou* virus (OMV): incidence of tumor development experimentally infected representative salmonid species. Fish Pathology 22:7–10.

Zwart, P. 1970. A nephroblastoma in a fire-bellied newt, *Cynops pyrrhogaster*. Cancer Research 30:2691–2694.