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HISTOPATHOLOGY OF *Rhabdias* AND *Raillietiella* INFECTED LUNGS OF *Sclerophrys maculata* (HALLOWELL'S TOAD), PORT HARCOURT, NIGERIA

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ABSTRACT – The genera *Rhabdias* (Nematoda) and *Raillietiella* (Pentasomidea) are lung worms infecting anurans and some other vertebrates. They have been associated with several pathologies including reduced growth and death. Here we examine the pathological changes associated with these parasites in infected lungs of *Sclerophrys maculata* from Port Harcourt, Nigeria. Hosts were hand-picked from three locations (Bori in Ogoni, Isiokpo in Ikwerre, and Rivers State University campus, in Port Harcourt). They were euthanized in chloroform vapour and dissected. Histopathological examination of infected and uninfected lungs was done using standard procedures. Pathologies included congested pulmonary vessels and abnormal vascular dilatation in *Rhabdias*-infected lungs. Lungs infected with both parasites also presented with congested pulmonary vessels in addition to areas of pulmonary hemorrhage. We conclude that co-infection of both parasites results in pathological changes that could affect lung function and reduce wild populations of *S. maculata*.

Keywords: amphibians, histopathological changes, lung worm, Raillietiella, Rhabdias

INTRODUCTION

Sclerophrys maculata are bufonid toads very common around human habitations in tropical West Africa and host to several parasites (Rödel, 2000; Amuzie *et al*., 2019). They serve as hosts of the lung helminths, *Rhabdias* and *Raillietiella* species. While *Rhabdias* spp. are nematodes infecting anurans and snakes (Lhermitte-Vallarino *et al*., 2008; Kuzmin, 2013; Nelson *et al*., 2015a,b; Kelehear *et al*., 2019), *Raillietiella* spp. are pentastomids which infect the lungs and air-sacs of reptiles, birds and mammals (Kelehear *et al*. 2011). Among amphibians, only toads (bufonids) are the definitive hosts of *Raillietiella* spp., but they have been found in *Hoplobatrachus occipitalis* (Dicroglossidae) in Rivers State, Nigeria (Amuzie, 2018).

The effect of parasites on their hosts may range from undetectable effects to severe impacts that affect the ability of the hosts to lead normal lives, and even death (Poulin, 2007; Pare, 2008; Kelehear *et al*., 2009; Mihalca *et al*. 2010).

The negative impacts of *Rhabdias* spp. on amphibian hosts have been reported to include reduced growth and survival (Goater and Ward, 1992; Kelehear *et al*., 2009; 2019) and pulmonary lesions (Mihalca *et al*., 2010). They have also been reported to cause pulmonary damage which could be secondarily infected by pathogens leading to death (Poynton and Whitaker, 2001).

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The surface of the lungs of toads is lined by pneumocytes and has few large alveoli and internal septa (Okada *et al*. 1962). Amphibian lungs are characterized by low rate of diffusion of oxygen. This is, however, overcome by cutaneous respiration.

Here, we report on the histology of *S. maculata* lungs infected with *Rhabdias africanus* and *Raillietiella* sp. from Port Harcourt metropolis, Nigeria.

MATERIALS AND METHODS

Study Area and Sample Collection

Amphibian collections were made in three locations in Rivers State, Nigeria: Bori (N4º40', E7º21'; Gokana Local Government Area); Isiokpo (5º03'N, 6º55'E; Ikwerre Local Government Area); and Rivers State University (N4º47', E6º58'; Port Harcourt Local Government Area).

The survey was carried out between May and June, 2019. Adult toads (*Sclerophrys maculata*) were hand captured, at night, between 10 pm and 12 am, using the visual encounter and acoustic survey method (Crump and Scott, 1994). They were transported live to the laboratory in moistened, aerated plastic bottles.

This research was approved by the Academic Board of the Department of Animal and Environmental Biology, Rivers State University, Port-Harcourt, Nigeria.

Parasitic and Histologic Examinations

Host specimens were identified following Rödel (2000) and euthanized by exposure to chloroform vapour in an airtight jar. They were dissected by longitudinal incision on their ventral surface to expose the internal organs. The lungs were excised into Petri dishes containing 0.9% laboratory saline. The apex of each lung was held using forceps and shaken in the solution to release the parasites present. These were picked up using plastic pipettes, counted and the numbers recorded appropriately.

The infected and uninfected lungs were fixed in 10% neutral buffered formalin at room temperature for 24hrs. After serial dehydration steps in alcohol, samples were embedded in paraffin. The blocks of embedded tissue were sectioned at 5µm, and sections were routinely stained with hematoxylin and eosin (HeE) and mounted on DPX. Images were acquired with a Leica DFC280 digital camera attached to a light microscope (Leica 6000B).

The parasites were identified using standard keys (Anderson *et al*., 1974; Riley *et al*., 1988). *Rhabdias africanus* was fixed in 70% alcohol while *Raillietiella* sp. was fixed in 70% alcohol saline.

RESULTS AND DISCUSSION

A total of twenty-eight specimens of *Sclerophrys maculata* were examined from Bori (n=13), Rivers State University (RSU) (n=2) and Isiokpo (n=13). Eleven specimens from Bori were infected with *R. africanus*, one from RSU, and twelve from Isiokpo. Parasite burden in the specimens ranged between one (1) and ten (10) in specimens from Bori, one (1) and eleven (11) in those from Isiokpo and the only infected specimen from RSU harboured 15 *R. africanus* individuals. Five specimens of *Raillietiella* sp. were recovered from same host in RSU co-occurring with *R. africanus*.

Histological observations in the lungs of S. maculata from the study locations

Histological examinations showed that uninfected lungs of amphibians from Rivers State University had normal lungs. These lungs had intact alveolar sacs, alveolar pneumocytes, pulmonary vessels and interstitium. However, lungs infected with *Rhabdias africanus* presented with varying degrees of histopathologic changes. The micrographs are presented in plates 1-6. Plates 1 and 2 show different sections of normal *S. maculata* lungs. Plates 3, 4 and 6 show different sections of *Rhabdias*-infected lungs from the study locations. These were characterized by congested pulmonary vessels and abnormal vascular dilatation. In plate 5, the lung infected with both parasites (*R. africanus* and *Raillietiella* sp.) showed congested pulmonary vessels, abnormal vascular dilatation, and pulmonary hemorrhage.

The normal alveolar sacs are thin walled, well delineated, irregular septae with a polygonal geometry. The normal alveolar pneumocytes have both Type I and type II alveolar cells common in vertebrates, are prismatic in geometry and showed continuity. In the abnormal lungs, the alveolar sacs were broken and either enlarged or shrunken while the pneumocytes were enlarged and lacked continuity. The interstitium was normal in all hosts examined having all cells (such as, alveolar epithelium and basement membrane) intact.

The pulmonary vessels include the pulmonary capillaries, arterioles, and venules. The pulmonary capillaries have a single layer of endothelial cells which connect arterioles to venules. The arterioles are also thin walled; whereas the capillaries lack muscular layers, the arterioles have smooth muscle cells. The venules on the other hand, have three cell layers including a poorly developed middle layer of muscle and elastic tissues. Hemorrhage and vascular congestion were observed in the abnormal lungs. In the case of hemorrhage, there was the extravasation of red blood cells from the pulmonary capillaries leading to the presence of blood in the alveoli. In the case of vascular congestion, a distension of the pulmonary vessels with red blood cells was observed.

Results of the histologic examination of uninfected lungs of *S. maculata* showed normal lung histology. However, *Rhabdias* infected lungs presented with congested pulmonary vessels and abnormal vascular dilatation. This is concurrent to the reports of other researches who reported that *Rhabdias* spp. actually cause histological alterations in amphibian lungs (Goater and Ward, 1992; Kelehear *et al*., 2009; 2019; Mihalca *et al*., 2010). Poynton and Whitaker (2001) reported that heavy infections of *Rhabdias* sp. in captive amphibians caused pulmonary damage such as eosinophilic pneumonia that led to secondary infections and eventually death.

Similar reports have also been made in reptiles. For instance, Jacobson (2007) stated proliferative pneumonia and hypertrophy of pneumocytes as symptoms of *Rhabdias* infection in snakes. Santos *et al*. (2008) found "an infiltrate of granulocytic and mononuclear cells in the lungs" of snakes, *Crotalus durissus terrificus*. Mihalca *et al*. (2010) also examined the lungs of *Rhabdias fuscovenosa*-infected snakes and found that the respiratory epithelium had vacuolar degeneration; cellular debris filled the faveolae, and there was intrafaveolar hemorrhage, smooth muscle degeneration in the interfaveolar septa, collapse of pulmonary structures, and obstruction of faveolae.

In the present research, histologic sections of lungs infected with both *R. africanus* and *Raillietiella* sp. presented with congestion of pulmonary vessels and abnormal vascular dilatation in addition to pulmonary hemorrhage. This implies that co-infection with the pentastomid, *Raillietiella* sp., increases the pathological changes which can alter lung function. There is paucity of information on histopathological changes attributed to *Raillietiella* spp. However, in the alligator, *Alligator*

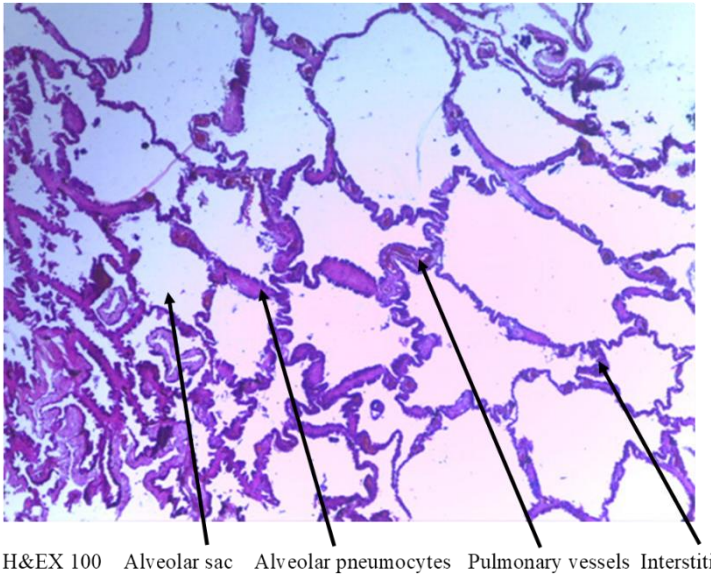

Plate 1: Histologic sections of *Rhabdias*-free lungs of *S. maculata* from Isiokpo showing no histologic change.

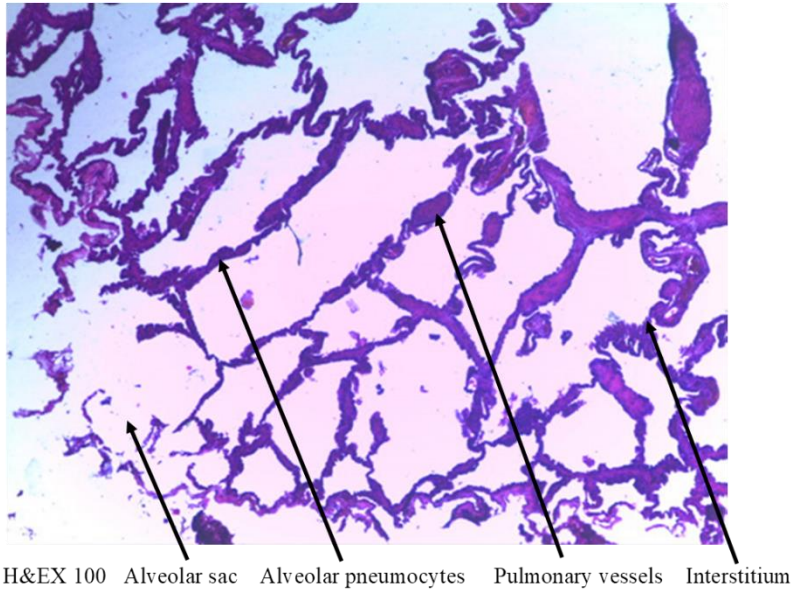

Plate 2. Histologic sections from *Rhabdias*-free lungs of *S. maculata* (RSU) showing no histologic change.

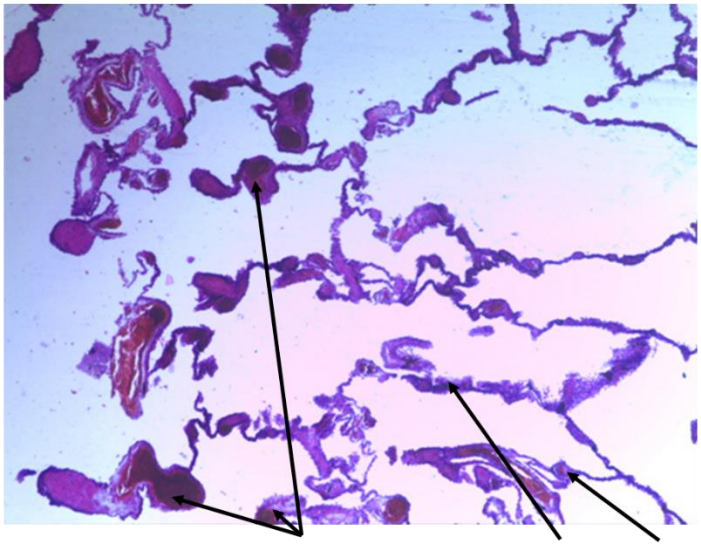

Plate 3. Histologic sections from the lungs of *S. maculata* (Bori) infected with *R. africanus* showing several congested pulmonary vessels and abnormal vascular dilatation.

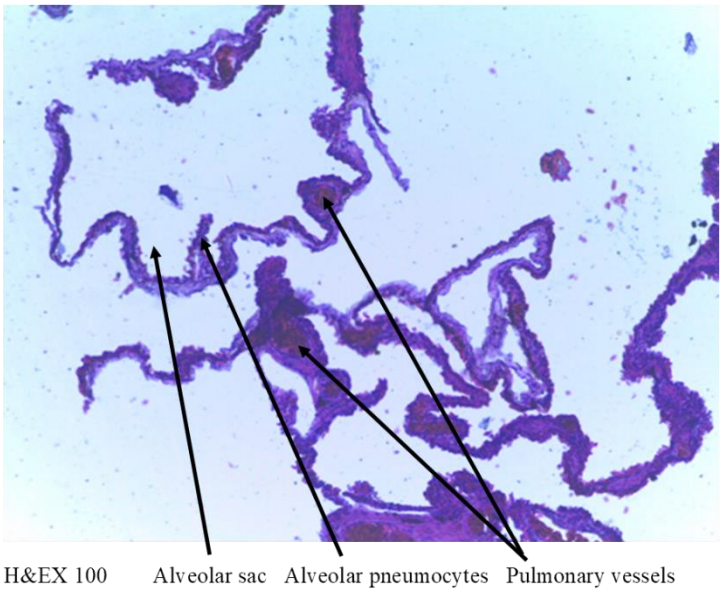

Plate 4. Histologic sections from *Rhabdias*-infected lungs of *S. maculata* (Isiokpo) showing several congested pulmonary vessels and abnormal vascular dilatation.

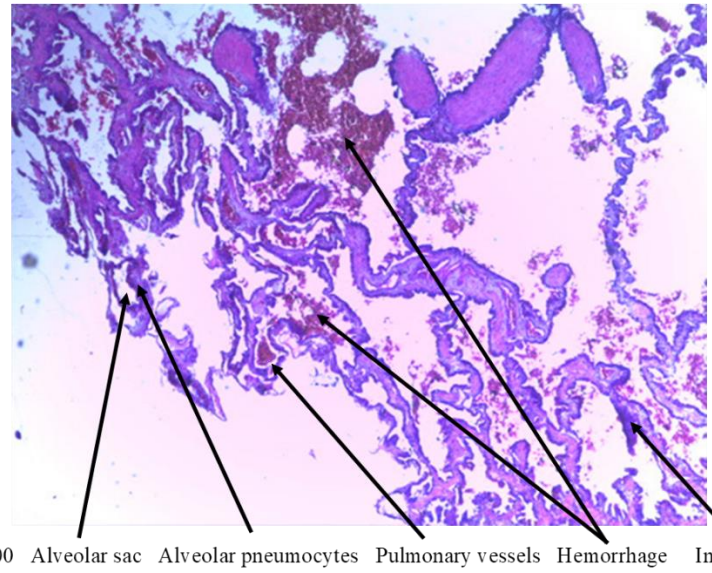

Plate 5. Histologic sections from the lungs of *S. maculata* infected with *R. africanus* (n=15) and *Raillietiella* sp. (n=5), showing several congested pulmonary vessels and abnormal vascular dilatation with areas of pulmonary hemorrhage.

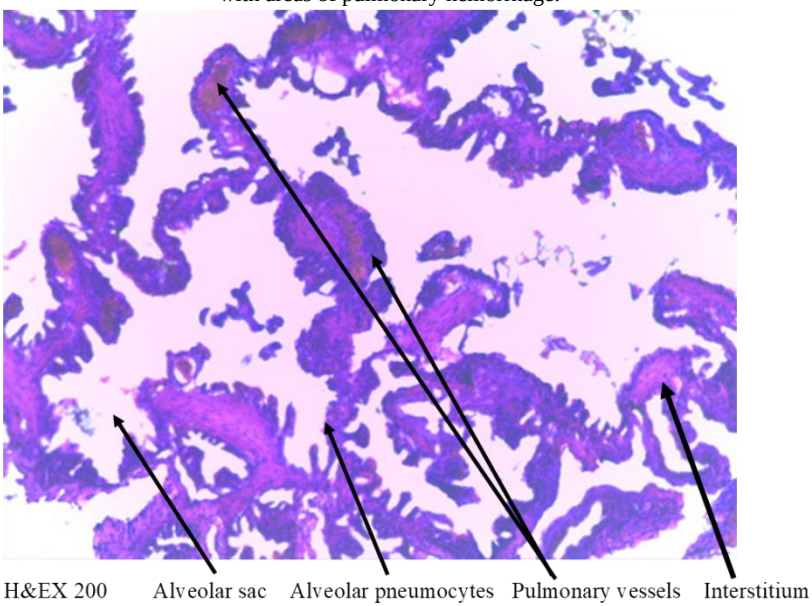

Plate 6. Histologic sections from *Rhabdias*-infected lungs of *S. maculata* (Bori) showing several congested pulmonary vessels and abnormal vascular dilatation.

mississippiensis, Woodyard *et al.* (2019) reported that lungs infected with the pentastome, *Sebekia mississippiensis*, had compressed tissues without inflammation. These authors also reported the presence of a large granuloma, and stated that "larval migration or heavy pentastome burden may cause significant pulmonary injury, particularly in the young".

CONCLUSION AND RECOMMENDATIONS

This study showed that the presence of *R. africanus* and its co-infection with *Raillietiella* sp. caused histopathological changes (such as congestion of pulmonary vessels, abnormal vascular dilatation and pulmonary hemorrhage) in the lungs of *S. maculata*. These pathologic changes could affect lung function thereby leading to death and decrease in the wild populations of the host species.

It is therefore recommended that treatment of these parasites in wild toads be included in amphibian conservation projects as their contributions to host population declines cannot be underestimated.

STATEMENT OF AUTHORSHIP

C.C. Amuzie conceptualized and designed the study as well as prepared the initial write up. F.C. Brown collected the specimens, performed the experiments and gathered data. E.R. Daka supervised the entire experiments. All three authors participated in writing and reviewing the manuscript.

REFERENCES

Amuzie, C.C. (2018). Diversity, parasitic helminths and heavy metal contamination of anurans of Rumuesara and Agbada-2 oil flow station, Rivers State, Nigeria. PhD thesis submitted to Department of Applied and Environmental Biology, Rivers State University, Port Harcourt, Nigeria. 252 pages.

Amuzie, C.C., Akani, G.C. and Davies, E.L. (2019). Diversity of helminth parasites in *Sclerophrys maculata* of Degema and Ogoni, Rivers State, Nigeria. Sokoto Journal of Medical Laboratory Science. 4: 43 - 50.

Anderson, R.C., Chabaud, A.G. and Willmott, S. (1974). CIH keys to the nematode parasites of vertebrates. Great Britain: Headley Brothers Ltd.

Crump, M.L. and Scott Jr, N.J. (1994). Visual encounters surveys. In: Heyer WR, Donnelly MA, McDiarmid RW, Hayek LC, Foster MS, eds. Measuring and monitoring biological diversity-standard methods for amphibians. Washington, USA, Smithsonian Institution Press. pp. 84-92.

Goater, C.P. and Ward, P.I. (1992). Negative effects of *Rhabdias bufonis* (Nematoda) on the growth and survival of toads (*Bufo bufo*). Oecologia. 89: 161–165.

Jacobson, E. R. (2007). Parasites and parasitic diseases of reptiles. In Infectious diseases and pathology of reptiles, E. R. Jacobson (ed.). CRC Press, Boca Raton, Florida, pp. 571–666.

Kelehear, C., Saltonstall, K. and Torchin, M.E. (2019). Negative effects of parasitic lung nematodes on the fitness of a Neotropical toad (*Rhinella horribilis*). Parasitology. 1–9.

https://doi.org/10.1017/S0031182019000106.

Kuzmin, Y. (2013). Review of Rhabdiasidae (Nematoda) from the Holarctic. Zootaxa. 3639: 1–76.

Lhermitte-Vallarino N., Barbuto, M., Ineich, I., Wanji, S., Lebreton, M., Chirio, L. and Bain, O. (2008). First report of *Rhabdias* (Nematoda: Rhabdiasoidea) from lungs of montane chameleons in Cameroon: description of two new species and notes on biology. Parasite. 15: 553-564.

Mihalca, A.D., Micla˘us, V. and Lefkaditis, M. (2010). Pulmonary Lesions caused by the Nematode *Rhabdias fuscovenosa* in a Grass Snake, *Natrix natrix*. Journal of Wildlife Diseases. 46: 678–681.

Nelson, F.B.L., Brown, G.P., Shilton, C. and Shine, R. (2015a). Host–parasite interactions during a biological invasion: The fate of lungworms (*Rhabdias* spp.) inside native and novel anuran hosts. International Journal for Parasitology: Parasites and Wildlife. 4: 206-215.

Nelson, F.B.L., Brown, G.P., Dubey, S. and Shine, R. (2015b). The effects of a nematode lungworm (*Rhabdias hylae*) on its natural and invasive anuran hosts. Journal of Parasitology. 101: 290–296.

Okada, Y., Ishiko, S., Daido, S., Kim, J. and Ikeda, S. (1962). Comparative Morphology of the Lung with special reference to the alveolar epithelial cells: Lung of the Amphibia. Departmental Bulletin Paper Kyoto University. 11: 63-72.

Pare, J.A. (2008). An overview of Pentastomiasis in reptiles and other vertebrates. Journal of Exotic Pet Medicine. 17: 285–294.

Poulin, R. (2007). Evolutionary Ecology of Parasites, second Ed. Princeton University Press, Princeton, NJ.

Poynton, S.L. and Whitaker, B.R. (2001). Protozoa and metazoan infecting amphibians. In: Wright KM, Whitaker BR, eds. Amphibian Medicine and Captive Husbandry. Malabar FL: Krieger Publishing Company. pp. 193-221.

Riley, J., McAllister, C.T. and Freed, P.S. (1988). *Raillietiella teagueselfi* n. sp. (Pentastomida: Cephalobaenida) from the Mediterranean gecko, Hemidactylus turcicus (Sauria: Gekkonidae), in Texas. Journal of Parasitology. 74: 481–486.

Rödel, M.O. (2000). Herpetofauna of West Africa, Vol 1: Amphibia of West Africa Savannah. Chimaera, Frankfurt.

Santos, K.R., Takahira, R.K.; Rall, V.L.M., Calderón, C., Sequeira, J.L. and Silva, R.J. (2008). Pulmonary, microbiological and hematological changes in *Crotalus durissus terrificus* (Serpentes, Viperidae) parasitized by nematodes of the genus Rhabdias (Nematoda, Rhabdiasidae). *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*; volume 60(3), online version. http://dx.doi.org/10.1590/S0102-09352008000300022. Accessed August 24, 2019.

Woodyard, E.T., Baumgartner, W.A., Rosser, T.G., Bodin, E.N., Ferrara, A.M., Noto, T.W., Ford, L.M. and Rush, S.A. (2019). Morphological, molecular, and histopathological data for *Sebekia mississippiensis* Overstreet, Self, and Vliet, 1985 (Pentastomida: Sebekidae) in the American

Alligator, *Alligator mississippiensis* Daudin, and the Spotted Gar, *Lepisosteus oculatus* Winchell. Journal of Parasitology. 105: 283–298.