Host Species and Prevalence of the Invasive Pentastome Parasite *Raillietiella orientalis* Among Snakes in Central Florida, USA

Aguilar, Alexis D. M.; Mentor: Farrell, Terence M.

Abstract

Parasites, and invasive parasite species specifically, are a frequent cause of endangerment and extinction of native species. Spillover is the introduction of an invasive parasite to a native community, brought by a nonnative species (Dunn et al. 2012). *Raillietiella* is the largest pentastome genus, comprised of ~39 species (Ali & Riley, 1983). *Raillietiella orientalis* is believed to have been brought to the southeastern United States by Burmese pythons (*Python bivittatus)*, a species native to Southeast Asia and a known definitive host for the pentastome parasite. We aimed to identify definitive hosts of *R. orientalis* so that the range and the spread of infection rates can be monitored. We collected snakes between October of 2018 to December of 2019 from four main areas: Lake Woodruff National Wildlife Refuge, Lake Monroe Conservation Area, and various roads in the western half of Volusia County and eastern portion of Lake County. Cloacal samples were obtained from live snakes and microscopically examined for pentastome eggs, while live snakes were dissected for infection examination. Six species were found to be infected: *Sistrurus miliarius* (Pygmy Rattlesnake)*, Thamnophis sauritus* (Peninsula Ribbonsnake)*, Coluber constrictor* (Southern Black Racer)*, Pantherophis guttatus* (Eastern Cornsnake)*, Nerodia fasciata* (Florida Watersnake)*,* and *Lampropeltis elapsoides* (Scarlet Kingsnake). There was a significant difference between the infection rates among the four species with the highest sample size, indicating that infection rate varied significantly

among species (Fisher exact test, P=0.00862). *Coluber constrictor* was found to have the highest infection rate and highest infection intensity.

Introduction

Parasites, and invasive parasite species specifically, are a frequent cause of endangerment and extinction of native species. While parasitic infection commonly results in host mortality, parasites can also have subtle effects on their hosts, such as the reduction in host reproduction and survival. The overall impact of parasites to a population is influenced by parasite prevalence, which can be increased due to parasite spillover. Spillover is the introduction of an invasive parasite to a native community, brought by a nonnative species (Dunn et al. 2012). Spillover was observed by Corn et al. (2011) in exotic and native ectoparasites collected from wild-caught, exotic reptiles in Florida. They stated that introduction of the exotic reptiles to Florida brought exotic ectoparasites to native communities and spillover occurred between native and nonnative species.

Pentastomids are endoparasites that infect the respiratory system of vertebrates, with 90% of their definitive hosts being reptiles (Christoffersen & DeAssis, 2013; Kelehear et al. 2014; Riley, 1986). Most pentastome species have an indirect life cycle, meaning they require an intermediate host (Miller et al. 2017; Pare, 2008). However, the intermediate hosts for most species are unknown (Christoffersen & DeAssis, 2013; Kelehear et al. 2014). Once ingested by an intermediate host, the pentasome larvae use their hooked limbs and a peristaltic motion to penetrate the gut wall. After migrating to visceral tissue, the larvae become an infective nymph (Pare, 2008). After the intermediate host is consumed by the definitive host, the nymph leaves the digestive tract for the lungs, which can result in lesions and scars along their path (Jacobson,

2007; Pare, 2008). In the lungs, the nymph matures into an adult (Miller et al. 2017; Pare, 2008; Riley, 1986). The fertilization process can then begin, with male parasites having a short life span, leaving behind fertilized females that can lay up to several millions of eggs (Pare, 2008). Typically, the eggs in the lungs will pass up the trachea, become swallowed, and will end up in the digestive tract and passed with feces (Ali & Riley, 1983). During an adult pentastomes life in the lungs, the parasite will feed on blood from capillary beds which usually causes damage to the pulmonary lining due to the piercing hooks of the parasite. This damage sometimes results in host mortality (Pare, 2008). Pulmonary function may become reduced and the hosts can suffocate due to blockage of the lungs when multiple and/or largely sized pentastomes are present (Riley, 1986).

Three pentastome genera have been found in snakes in North America: *Raillietiella*, *Porocephalus*, and *Kiricephalus* (Christoffersen & DeAssis, 2013; Miller et al. 2017; Riley, 1981; Riley & Self, 1979; Riley & Self, 1980). Globally, *Raillietiella* is the largest pentastome genus, comprised of ~39 species (Ali & Riley, 1983). *Raillietiellia* adults are typically <25 mm, with males usually being significantly smaller than females. *Raillietiella* species typically mature in snakes and small lizards (Ali & Riley, 1983; Ali et al. 1985). In Australia, the intermediate host for introduced *R. orientalis* was suspected to be amphibians (Kelehear et al., 2011; Kelehear et al., 2014). Miller et al. (2017) also focused on *R. orientalis*, which is a pentastome native to Southeast Asia. *Raillietiella orientalis* is believed to have been brought to the southeastern United States in Burmese pythons (*Python bivittatus)*, a species native to Southeast Asia and a known definitive host for the pentastome parasite. Miller et al. (2017) dissected over 800

Burmese python and 498 snakes of 26 species native to Florida were found to be infected with *R. orientalis.*

Though *R. orientalis* appears to have been introduced in southern Florida, infection rates have begun to spread North from the original infection region, leading to the list of definitive hosts for *R. orientalis* to increase. Farrell et al. (2019) reported on three Pygmy Rattlesnakes (*Sistrurus miliarius*) that were infected with *R. orientalis* 160 km North of the known distribution of Burmese pythons in Florida. Farrell et al (2019) increased the known geographic range of and definitive host list for *R. orientalis* in Florida. Consequently, as the range of *R. orientalis* infection grows, the mortality rates of native snake species may also grow. Farrell et al. (2019) found that the largest female pentastomes were about the same diameter as the trachea of the Pygmy Rattlesnake hosts, i.e. the parasites were so large they could have caused airway obstruction and suffocated the hosts.

The spillover of *R. orientalis* from Burmese pythons to native species in Florida and the observations of the parasites beyond the introduced range of the Burmese python causes increasing concern for conservation. We aimed to identify definitive hosts of *R. orientalis* so that the range and the spread of infection rates can be monitored. Identifying definitive hosts for *R. orientalis* also allows the diet of the hosts to provide evidence on what species *R. orientalis* uses as intermediate hosts. We report on four snakes native to Florida: *Sistrurus miliarius* (Pygmy Rattlesnake), *Thamnophis sauritus* (Peninsula Ribbonsnake), *Coluber constrictor* (Southern Black Racer), and *Pantherophis alleghaniensis* (Eastern Ratsnake). Since we are unsure of the intermediate hosts for *R. orientalis*, expectations on which snake species would serve as definitive hosts are difficult to make. We can assume that either frogs or lizards will be the

intermediate hosts (Ali & Riley, 1983; Ali et al. 1985; Kelehear et al., 2011; Kelehear et al., 2014). We hypothesize that if frogs are a key intermediate host, all four snake species will be infected as they all eat frogs. If lizards are a key intermediate host, we expect *Thamnophis sauritus* to have the lowest infection frequency of the four species, as lizards are a less important component of their diet than the other three species (Krysko et al., 2019).

Methods

Specimen collection

We collected snakes between October of 2018 to December of 2019 from four main areas: Lake Woodruff National Wildlife Refuge, Lake Monroe Conservation Area, and various roads in the western half of Volusia County and eastern portion of Lake County. Lake Woodruff National Wildlife Refuge is on the eastern floodplain of the St Johns River and has a variety of habitats such as areas of mesic forest, freshwater marsh, and cypress swamp (Farrell et al. 2011). Lake Monroe Conservation Area is on the Southeast of Lake Monroe and the habitat consists of pine woodlands and mesic hammock adjacent to the marshes of the St. Johns River floodplain. Deceased snakes were collected, bagged, and frozen until dissection. We captured live snakes by using tongs or by hand (if they were nonvenomous). We retrieved fecal samples by using a Dover Urethral PVC Catheter (3.3 mm diameter) attached to a 1cc U-100 Insulin Syringe with the needle removed to deposit approximately 1 ml of saline solution in a snake's cloaca. The water and fecal material that was flushed out was put into an empty labeled 25 ml vial. We released the live snakes at their capture sites.

Determining infection status

Cloacal samples were observed under 100x magnification with a Zeiss Axiostar Plus microscope to determine if an individual had pentastome eggs in their digestive system, which would indicate that there were reproductive pentastomes in the lungs. To identify if the eggs were of the species *R. orientalis*, the collected eggs were compared in size to *R. orientalis* eggs from parasites in which species identification was confirmed by DNA sequencing (Farrell et al. 2019). We dissected dead snakes by using surgical scissors to cut an individual open from the cloaca up to the throat. The snake's ribs were spread open and the lung, as well as the snake's mouth, was examined for the presence of pentastome parasites.

Data Analysis

The focal species were four species that had more than eight individuals sampled: *Sistrurus miliarius* (Pygmy Rattlesnake), *Thamnophis sauritus* (Peninsula Ribbonsnake), *Coluber constrictor* (Southern Black Racer), and *Pantherophis alleghaniensis* (Eastern Ratsnake). We used a Fisher exact test to determine if pentastome prevalence varied among species. For error bars, we used the 95% confidence interval for proportion calculated by the exact binomial method (http://www.sample-size.net/confidence-interval-proportion/). We also looked for patterns of infection intensity among species.

Results

Out of 122 specimens collected, 24.5% of individuals were infected with pentastome parasites (Table 1). Of the 10 species collected, the six species found to be infected were: *Sistrurus miliarius* (Pygmy Rattlesnake), *Thamnophis sauritus* (Peninsula Ribbonsnake),

Coluber constrictor (Southern Black Racer), *Pantherophis guttatus* (Eastern Cornsnake), *Nerodia fasciata* (Florida Watersnake), and *Lampropeltis elapsoides* (Scarlet Kingsnake) (Table 1). The four species not found to be infected were: *Pantherophis alleghaniensis* (Eastern Ratsnake), *Opheodrys aestivus* (Florida Rough Greensnake), *Thamnophis sirtalis* (Eastern Gartersnake), and *Nerodia taxisplota* (Brown Watersnake) (Table 1).

There was a significant difference between the infection rates among the four species with the highest sample size, indicating that infection rate varied significantly among species (Fisher exact test, P=0.0086). Though *Sistrurus miliarius* had the highest number of infected specimens (Table 1), of the four snake species, *Coluber constrictor* was found to have the highest infection rate with the proportion of infected specimens being 0.33 (Figure 1). *Coluber constrictor* also had the highest infection intensity; we observed 8, 10, and 29 pentastome parasites (Figure 2) in three different infected *C. constrictor* individuals. Other species had infection intensities ranging from 1-6 parasites, as observed in a *S. miliarius* individual in Figure 3. The head of a pentastome is visualized in Figure 4.

Pentastome positive snakes were found in five main areas of our study region (Figure 5). There was a diversity of habitat types in which pentastomes were found. Pentagon one represents an area that has a few houses but is mostly sand pine scrub habitat with a few scattered ponds. Pentagon three represents the area of Lake Woodruff Wildlife Refuge which is a mesic hammock habitat. DeLand (pentagon four) is a high density residential urban area. Pentagon five represents Tiger Bay State Forest, a pine flatwoods habitat.

Table 1. *Pentastome infection status among snake species.* Ten central Florida snake species with the number of specimens collected separated by infection status.

Latin name	Common name	# Pentastome positive	# Pentastome negative	Total
Sistrurus miliarius	Pygmy Rattlesnake	16 (20.25%)	63	79
Thamnophis sauritus	Peninsula Ribbonsake	1 (12.5%)	7	8
Coluber constrictor	Southern Black Racer	4 (33.33%)	8	12
Pantherophis alleghaniensis	Eastern Ratsnake	0 (0%)	8	8
Opheodrys aestivus	Rough green snake	0 (0%)	2	2
Pantherophis guttatus	Corn snake	1 (50%)	1	2
Thamnophis sirtalis	Garter snake	0 (0%)	3	3
Nerodia fasciata	Banded water snake	1 (20%)	4	5
Lampropeltis elapsoides	Scarlet king snake	1 (50%)	1	2
Nerodia taxisplota	Brown water snake	0 (0%)	1	1
	Total	24	98	122

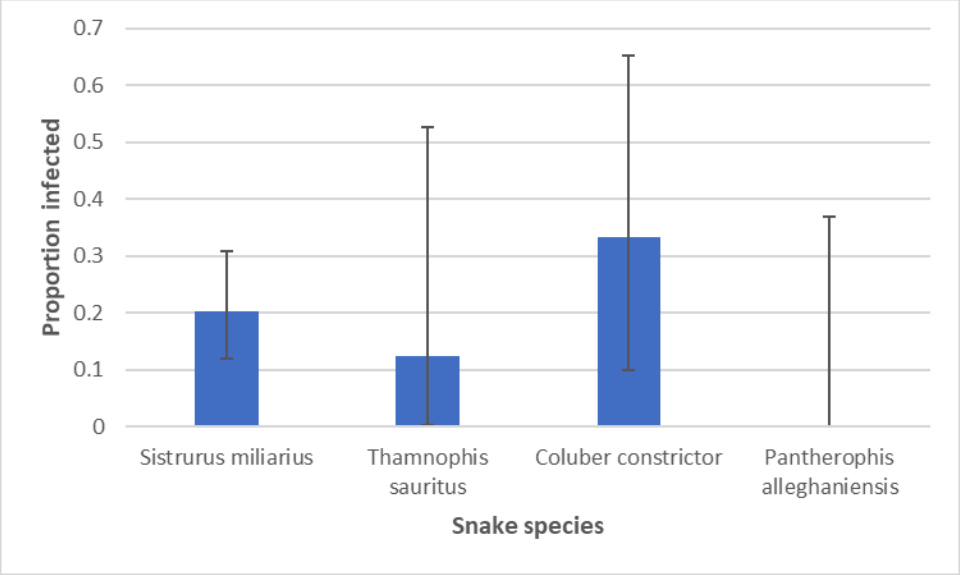

Figure 1. *Infection rate among four snake species.* Proportion of infected individuals (infection rate) per species was calculated by dividing the number of infected specimens by the total amount of specimens collected. Error bars are the 95% confidence interval for proportion.

Figure 2. *Pentastome infection of a* Coluber constrictor *specimen.* A Southern Black Racer collected from DeLand, Florida with the 29 pentastome parasites found in it.

Figure 3. *Pentastome infection of a* Sistrurus miliarius *specimen.* A Pygmy Rattlesnake collected from Lake Woodruff National Wildlife Refuge with six pentastome parasites in the snake's lung.

Figure 4. *Head of a pentastome parasite.* A parasite retrieved from a *Coluber constrictor* specimen was observed under approximately 20x magnification to analyze the head's morphology.

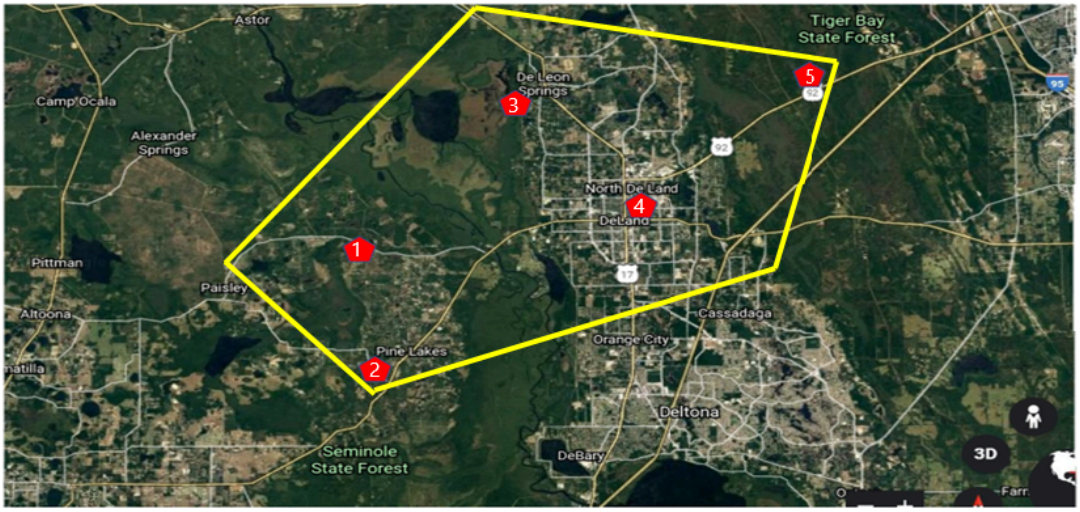

Figure 5. *A map of the Central Florida area where road killed snakes were collected.* The yellow polygon indicates the boundary of the sampled area and the red pentagons indicate sites where pentastome positive snakes were found.

Discussion

Our results support that the range of *Raillietiella orientalis* infection in Florida has spread beyond the introduced range of Burmese pythons (*Python bivittatus)*, the host species responsible for the original parasite spill over. Farrell et al. (2019) found that the pentastome infections had reached Volusia County and our results increase that geographic range throughout a great portion of Volusia County as well as into Lake County. Pentastome infected snakes were found in a variety of habitat types including areas of mesic forest, freshwater marsh, and cypress swamp as found in Lake Woodruff National Wildlife Refuge (Farrell et al. 2011) and areas of pine woodlands, mesic hammock, and marshes as found in Lake Monroe Conservation Area.

Our results support the Farrell et al. (2019) addition of *Sistrurus miliarius* to the definitive host list and also add two new species to that list: *Thamnophis sauritus* and *Lampropeltis elapsoides* (Table 1). The three other species we found pentastome infections in were *Coluber constrictor, Pantherophis guttatus,* and *Nerodia fasciata* (Table 1), species that were already known to serve as definitive hosts for *R. orientalis* (Miller et al. 2017).

Finding new definitive hosts of *R. orientalis* is useful for identifying likely intermediate hosts of the parasite. Scarlet kingsnakes specialize on lizards and rarely eat frogs, so finding a pentastome infected *L. elapsoides* is consistent with lizards acting as an intermediate host. We hypothesized that *Thamnophis sauritus* would have the lowest infection frequency if lizards were the intermediate host, and we found the species to have one of the lowest infection rates of 0.125 (Figure 1). These results could support that lizards are an intermediate host of *R. orientalis*, however, our small sample size was eight individuals (Table 1). As reported by Kelehear et al.

(2011 & 2014), in Australia, the intermediate host for *R. orientalis* was suspected to be frogs. Therefore, we hypothesized that all four of our focal snake species would be infected if frogs were an intermediate host, as they all eat terrestrial frogs. However, *Pantherophis alleghaniensis* is an arboreal species and is less likely to eat terrestrial frogs. This is reflected in our results, as we did not find any pentastome infections in *P. alleghaniensis* specimens. Therefore, our results could support that frogs are an intermediate host of *R. orientalis*. However, we had a small sample size of eight individuals. While we collected 79 *S. miliarius* individuals, only 16 were found to be infected. It is possible that if the sample size of *P. alleghaniensis* was increased, *R. orientalis* infections would be found. Additional research could be done using methods similar to ours to determine intermediate hosts present in the geographic range we focused on. Further research could also expand our geographic range to other regions we did not cover, particularly areas of Northern Florida where *R. orientalis* is likely to be expanding its geographic range.

An interesting finding was that *Coluber constrictor* not only had the highest infection rate but also had the highest infection intensity (Figure 2). While Farrell et al. (2019) found the average of parasite individuals found in *S. miliarius* to be 3.67, we found the average of parasite individuals retrieved from *C. constrictor* to be 12. One *C. constrictor* individual had as many as 29 pentastome parasites; the mean length of the fourteen female parasites removed was 54.1 mm, similar to the 59.0 mm mean length of the nine female parasites removed from *S. miliarius* as reported by Farrell et al. (2019). The largest female we collected from *C. constrictor* was 71 mm. This length, as well as the average length of the parasites that we report, is larger than the average diameter of the *C. constrictor* trachea. Though our specimen was killed from being hit by a car, it is possible that the large size of *R. orientalis* and the number of parasites in one

snake's lung could pose airway obstruction and suffocation as a likely cause of death for other infected specimens.

Overall, we have found that the spillover of *Raillietiella orientalis* has reached native snake species in central Florida and continues to spread throughout multiple regions throughout Florida. Our findings have added two new species to the host list, as well as expanded on the infection prevalence and intensity among already known definitive hosts of *R. orientalis.* Expanding the geographic range, as well as expanding the definitive host list, allows the incidence rates and spread of *R. orientalis* to be monitored. Because parasite infection reaches the definitive hosts through their consumption of infected prey (intermediate hosts), to control the prevalence and spread of *R. orientalis* would require further research on the intermediate hosts of this parasite. Also, the diversity of habitat types is concerning; if pentastomes were found in a single habitat type, conservation efforts could be allocated to snake species present in that certain habitat. However, because there is a variety of habitats, various species are susceptible to infection.

References

Ali, J.H., Riley, J., 1983. Experimental life-cycle studies of *Raillietiella gehyrae* Bovien, 1927 and *Raillietiella frenatus* Ali, Riley and Self, 1981: pentastomid parasites of geckos utilizing insects as intermediate hosts. Parasitology, 86: 147–160.

Ali, J.H., Riley, J., and Self, J.T. 1985. A review of the taxonomy and systematics of the pentastomid genus *Raillietiella* Sambon, 1910 with a description of a new species. Systematic Parasitology, 7(2): 111–123.

Christoffersen, M.L. and De Assis, J.E. 2013. A systematic monograph of the recent pentastomida, with a compilation of their hosts. Zoology of Medicine Leiden, 87: 1-206.

Corn, J. L., Mertins, J. W., Hanson, B., & Snow, S. 2011. First reports of ectoparasites from wild-caught reptiles in Florida. Entomological Society of America, 48: 94–100.

Dunn, A. M., M. E. Torchin, M. J. Hatcher, P. M. Kotanen, D. M. Blumenthal, J. E. Byers, C. A. Coon, V. M. Frankel, R. D. Holt, R. A. Hufbauer, and A. R. Kanarek. 2012. Indirect effects of parasites in invasions. Functional Ecology, 26: 1262–1274.

Farrell, T.M., Pilgrim, M. A., May, P. G., Blihovde, W. B. 2011. The Herpetofauna of Lake Woodruff National Wildlife Refuge, Florida. Southeastern Naturalist, 10: 647-658.

Farrell, T.M., Agugliaro, J., Walden, H.D.S., Wellehan, J.F.X., Childress, A.L., Lind, C.M. 2019. Spillover of Pentastome Parasites from Invasive Burmese Pythons (*Python abivittatus)* to Pygmy Rattlesnakes (*Sistrurus miliarius)*, Extending Parasite Range in Florida, USA. Herpetological Review, 50: 75-78.

Kelehear, C., Spratt, D.M., Dubey, S., Brown, G.P., Shine, R. 2011. Using Combined Morphological, Allometric and Molecular Approaches to Identify Species of the Genus *Raillietiella* (Pentastomida). PLoS ONE, 6(9): e24936.

Kelehear, C., Spratt, D.M., O'Meally, D., Shine, R. 2014. Pentastomids of wild snakes in the Australian tropics. International Journal for Parasitology: Parasites and Wildlife, 3: 20-31.

Krysko, K.L., Enge, K. M., Moler, P. E. 2019. Amphibians and Reptiles of Florida. Gainesville, Florida: University of Florida Press.

Jacobson, E.R. 2007. Parasites and parasitic diseases of reptiles. *Infectious Diseases and Pathology of Reptiles*, 1, CRC Press, pp. 590–592.

Miller, M.A., Kinsella, J.M., Snow, R.W., et al. 2017. Parasite spillover: indirect effects of invasive Burmese pythons. Ecology of Evolution, 8: 830–840.

Paré, J. A. 2008. An overview of pentastomiasis in reptiles and other vertebrates. Journal of Exotic Pet Medicine, 17: 285–294.

Riley, J. 1981. An experimental investigation of the development of *Porocephalus crotali* (Pentastomida: *Porocephalida*) in the western diamond back rattlesnake (*Crotalus atrox*). International Journal of Parasitolology, 11: 127-131.

Riley, J. & Self, J.T. 1979. On the systematics of the pentastomid genus *Porocephalus* (Humboldt, 1811) with descriptions of two new species. Systematic Parasitology, 1: 25–42.

Riley, J. & Self, J.T. 1980. On the systematics and life-cycle of the pentastomid genus *Kiricephalus* Sambon, 1922 with descriptions of three new species. Systematic Parasitology, 1: 127–140.

Riley, J. 1986. The biology of pentastomids. Advances in Parasitology, 25: 45–128.