Invasive Parasites: A survey of endoparasites in *Salvator merianae* populations in Florida

Marisa Fonseca

Advisor: Dr. Gabriel Langford

Florida Southern College

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Abstract

The Argentine Black and White Tegu (*Salvator meriane)* is a large lizard native to South America that has been introduced to central and south Florida. With successful breeding populations in at least two locations, and the ability to acclimate to many temperate climates of the other southeastern states, it is becoming increasingly more important to monitor all biological aspects of this invasive lizard, including their parasites. No detailed published records exist on the endoparasites from these invasive populations. Thirty-one Argentine Black and White Tegus from central and southern Florida were necropsied to perform an extensive parasite examination of these invasive populations. The necropsies revealed that the tegus brought three species of exotic nematode to Florida from South America (*Physaloptera tupinambae, Diaphanocephalus galeatus, Cruzia lauroi*) and were also infected with *Raillietiella orientalis,* a pentastome introduced to Florida via invasive Burmese Pythons (*Python bivittatus*). The invasive pentastome is known to infect native snakes and cause harm to their respiratory tracts. It is unknown how the exotic parasites from the tegus will impact native Florida reptiles, however, the ability for *R. orientalis* to infect the Argentine Black and White Tegu could lead to the rapid spread of this invasive parasite throughout the southeastern United States.

Introduction

Non-native species are considered majors threats to biological diversity and there are more species of non-native lizards reproducing in Florida than native species (Pernas, et al., 2012). Global trade has contributed to the spread of non-native species, with access points often being coastal ports (Wood, et al., 2018). Although not all non-native species threaten the new regions they are brought into, many become invasive and outcompete the native habitants. Often, these

species do not have natural enemies (predators and parasites) in the new environment and can become invasive by negatively impacting the new environment through competition, predation, or disease (Wood, et al., 2018). Florida, with its tourism industry and habitable climate, is especially at risk of being taken over by these invasive species that cost the United States an estimated $120 billion per year in economic damages (USFWS, 2012).

In addition to outcompeting the native species within a region, these invasive species can pose serious health risks to the native species by introducing and spreading new pathogens or parasites. A previous study found that introduced Burmese Pythons, which are native to Southeast Asia, have brought along with them a new lung parasite: *Raillietiella orientalis* (Miller, et al., 2017). This pentastome was previously unknown to inhabit North America and is now transferring to native species that have no defenses against this parasite (Miller, et al., 2017). This occurrence has been documented in Everglades National Park and private lands in southern Florida and is known as parasite spillover (Miller, et al., 2017). Parasite spillover is defined as the transmission of a parasite from one host species to another host species, whether further transmission is successful or not, and can lead to outbreaks (Borremans, et al., 2019).

By studying the parasites of invasive species, conclusions can be drawn about the origin of the species and its history in the invaded region. One such example of this is *Anolis sagrei* populations in southwestern Taiwan. By examining the helminth community of these anoles in Taiwan, researchers were able to identify the emigration point of this reptile (Norval, et al., 2011). Identifying *Cyrtosomum penneri*, which has been previously reported in Floridian populations of *A. sagrei,* in the Taiwanese population of brown anoles supported the notion that the Taiwan population of the brown anole originated from Florida (Norval, et al., 2011).

The Argentine Black and White Tegu (*Salvator merianae*) is a large, diurnal lizard native to South America and lives in both natural and disturbed habitats (Kiefer and Sazima, 2002; McEachern, et al, 2015). It is a fecund lizard with a varied omnivorous diet that includes vegetation, snails, arthropods, eggs, small mammals, reptiles, and carrion (Wood, et al., 2018). Juveniles typically need more animal protein and eat less plant material, whereas adults eat a lot more plants and are important seed dispersers (Kiefer and Sazima, 2002). They also eat various invertebrates such as millipedes as well as carrion, which is indicated by the often-found maggots in their stomach content (Kiefer and Sazima, 2002). This species exhibits dormancy during winter temperatures and droughts, allowing it to live in a variety of ecosystems such as fields, rocky areas, and forests (Wood, et al., 2018; Viera, et al., 2016). There are 7 known nematodes (*Cruzia travossi, Cruzia lauroi, Physaloptera retusa, Physaloptera tupinambae, Physaloptera bainae, Diaphanocephalus galeatus, Spinicauda spinicauda*) and 1 known cestode (Oochoristicaca sp.) that infect *S. merinae* in South America (Viera, et al., 2016).

From 2000-2002, over 7,700 live Argentine Black and White Tegus were transported to the United States for the pet trade, with the first free-ranging tegu in Florida being documented in 2002 (McEachern, et al, 2015). Since then, their population throughout Florida has increased drastically with the earliest established breeding populations being documented in 2006 (Wood, et al., 2018). Today, there are at least two known breeding populations in Florida; one in Hillsborough county and one in Miami-Dade county (Wood, et al., 2018). However, these reptiles can be found as far north as northern Georgia and sightings in South Carolina were reported in 2020 (FWC, 2021). It is unknown what their full diet in Florida is and they are suggested to be a threat to sensitive ground nesting species like gopher tortoises, birds, and alligators (Wood, et al., 2018).

The diet of an animal can influence the parasites they have, as many parasites utilize other species as intermediate hosts. By identifying the parasites in the invasive populations of Argentine Black and White Tegus in Florida, we can make inferences as to what the tegus are eating based on what the intermediate hosts of the parasites are. It is also possible to identify if the tegus have introduced a new parasite species to the Florida reptiles, which could lead to inferences regarding where the tegus originated from as well as determine if monitoring of native parasite populations for novel parasites is necessary. This study is also important because it can help address how severe the threat of these invasive lizards is to the native Florida ecosystems.

Methods

Thirty-one Argentine Black and White Tegus were collected from traps and humanely euthanized in central and southern Florida from efforts to control the spread of the tegus. Specimens were placed on ice, transported to the laboratory, then frozen until dissection was possible. Each specimen's weight, snout-tail length, and snout-vent length were recorded along with any outstanding observation. A full necropsy was performed on each tegu using a dissecting microscope. Any parasites found were counted, preliminarily identified, and stored in 70% or 95% ethyl alcohol. After all the dissections were completed, parasites were mounted on slides and cleared in glycerol. The parasites were then examined under a light microscope and notable features were recorded and photographed. Morphological traits were then compared to the primary literature to identify species.

Results

Lungs

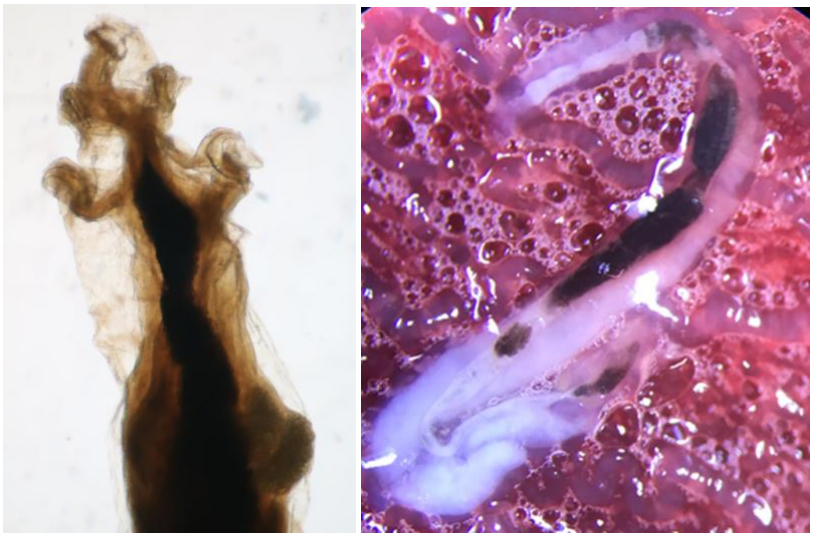

Figure 1. *Raillietiella orientalis* (left: cephalic end. Right: pentastome in lung tissue) found in *Salvator merianae* from Florida.

The lung pentastome *Raillietiella orientalis* (Figure 1) was found to be in 5/31 tegus with a mean intensity of 3.8 parasites per individual tegu. This parasite was confidently identified by morphology based on a description provided by Miller, et al., 2017. The matching hooks, buccal cadre, and spicules of the males confirms that the specimens found in this study are *R. orientalis*.

Stomach

Figure 2. *Physaloptera tupinambae* (cephalic end) found in *Salvatore merianae* from Florida.

The species in Figure 2 was identified as *Physaloptera tupinambae*, a species of nematode found in the stomach of *Salvator merianae* from Brazil. They were found with a prevalence of 6/31 and mean intensity of 3.17.

Figure 3. *Oswaldocruzia* sp. found in *Salvator merianae* from Florida. Cephalic end on left, posterior end on right.

This hookworm (Figure 3) found in the stomach has been identified as *Oswaldocruzia* sp. These specimens are being sent off for molecular identification due to morphological identification of species being inconclusive. They were found with w prevalence of 1/31 and mean intensity of 42.

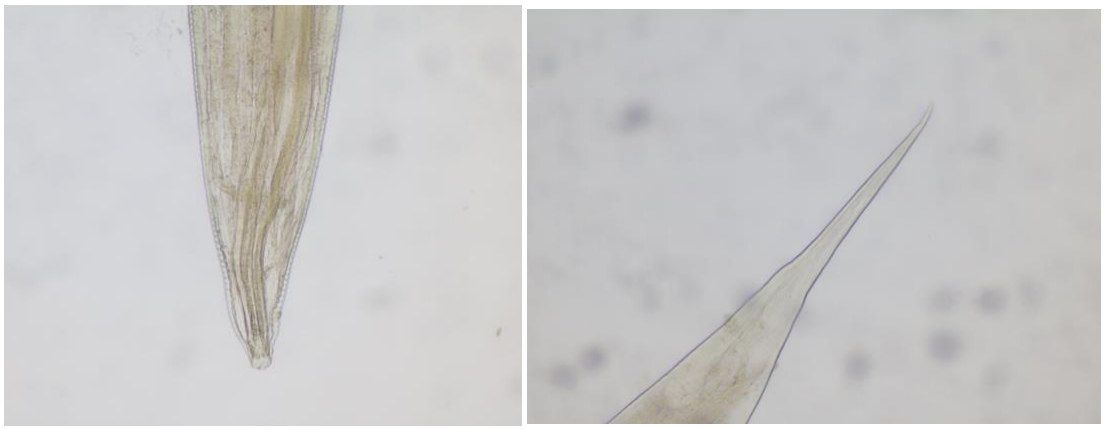

Figure 4. Pinworm found in *Salvator merianae* from Florida. Cephalic end on left, posterior end on right.

This pinworm (Figure 4) found in the stomach is presumed to be from the Pharyngodonidae family based on morphology, but species identification has been inconclusive. These specimens are being sent off for molecular identification. They were found with a prevalence of 1/31 with a mean intensity of 989.

Small and Large Intestine

Figure 5. *Centrorhynchus* spp. found in *Salvator merianae* from Florida.

The acanthocephalan (Figure 5) found in the small intestine could not be identified down to species. However, it was decided that it was likely a *Centrorhynchus* sp.

Figure 6. *Diaphanocephalus galeatus* (cephalic end) found in *Salvator merianae* from Florida.

The nematode shown in Figure 6 was identified as *Diaphanocephalus galeatus*. It was found with a prevalence of 15/31 and mean intensity of 7.73 in the small intestine and a prevalence of 7/31 and mean intensity of 9.14 in the large intestine.

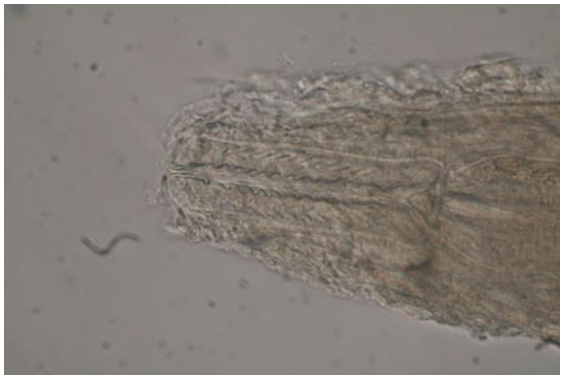

Figure 7. *Cruzia lauroi* (cephalic end) found in *Salvator merianae* from Florida.

The species shown in Figure 8 has been identified to be *Cruzia lauroi* from the Kathlaniidae family based on the specimens having 12 tooth-like structures, which is an identifying feature of this species. In the small intestine, it was found with a prevalence of 1/31 and mean intensity of 29. In the large intestine, it was found with a prevalence of 3/31 and mean intensity of 64.33.

Muscle

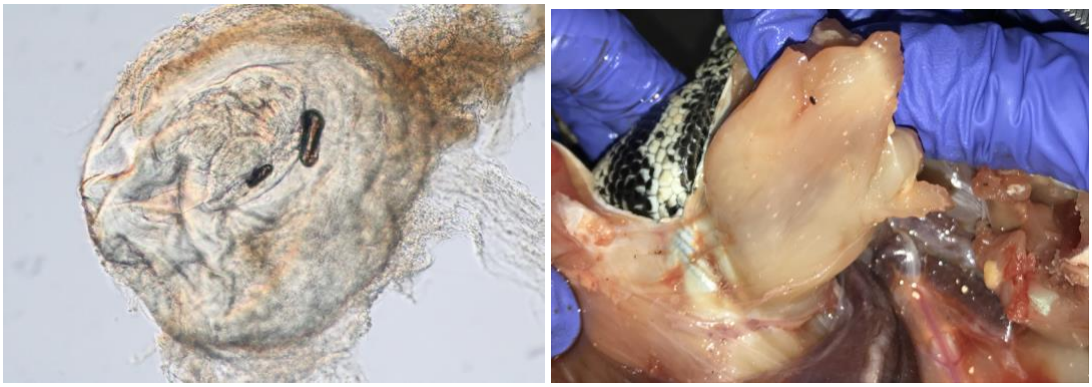

Figure 8. Possible *Mesocestoides* spp. Found in *Salvator merianae* from Florida. Encysted nematode shown on the left, muscle tissue infected with cysts shown on the right.

Figure 8 shows parasites found in the muscle of the tegus in this study. These cysts are potentially *Mesocestoides* spp., possibly *Mesocestoides tertrathyridia*. They were found with a prevalence of 1/31.

Foodstuffs

In the stomach content, I have found a wide variety of foodstuff including a gopher tortoise, a snake, other tegus, terrestrial snails, grasshoppers, crickets, fur, nuts, plant material, raspberries and other berries, and eggshells.

Discussion

Nine different parasitic species were found to infect the thirty-one Argentine Black and White Tegus that were necropsied. Of those nine, at least three (*Diaphanocephalus galeatus, Physolaptera tupinambae, Cruzia lauroi*) are species that have not been previously found in Florida but have been noted in Brazilian populations of tegu, making this the first documentation of these species in Florida. All three of these species have been noted specifically in Brazilian

populations of the Argentine Black and White Tegu, which could imply that the tegus imported from South America are from Brazil or that other tegu populations in South America are infected with these parasites.

Diaphanocephalus galeatus has been described infecting *S. merianae* from southern Brazil (Vieira, et al, 2016). It is known to feed on the tissue in the gastrointestinal tract of the host, causing lesions on the intestinal mucosa without causing ulcers (Viera, et al., 2016). Not much is known about the life cycle of diaphanocephalids, but it is known that eggs deposited by females develop rapidly in feces and water (Anderson, 2000). Snakes are able to be experimentally infected by oral inoculation and it is suggested that larvae might infect snakes when they test the environment with their tongue, which is a behavior that tegus also exhibit (Anderson, 2000; Schad, 1956). With the eggs of these parasites likely having the ability to stay viable while in the environment, it could be possible for other reptile species in Florida that exhibit tongue-flicking behavior to become infected with *Diaphanocephalus galeatus*.

Physaloptera tupinambae has been described in Argentine Black and White Tegus from southeastern Brazil (Pereira, et al., 2012). Physalopterine eggs can survive in the environment for long periods of time (Anderson, 2000). Definitive hosts can become infected via ingestion of larvae-infected insects and it is believed that paratenic hosts are widely used by these parasites (Anderson, 2000). With 6 individual tegus being infected with this parasite in this study, it can be assumed that these nematodes have paratenic hosts here in Florida, whether they were brought from South America as well or are able to infect insects that already inhabit Florida. It can also be speculated that other reptile species in Florida that may ingest these larvae-infected insects are at risk of becoming infected with *Physaloptera tupinambae*.

Cruzia lauroi is a nematode from the Kathlaniidae family that has been described infecting the intestinal tract of Argentine Black and White Tegus in Brazil (Vieira, et al., 2020). Very little is known about the life cycle of Kathlaniidae parasites, but it is possible that they utilize invertebrates as paratenic hosts (Anderson, 2000). As with *P. tupinambae,* it can be hypothesized that a viable paratenic hosts exists in Florida and there is a risk of this parasite infecting other Florida reptiles.

It is unknown if these three parasites are able to spillover into other reptiles in Florida and their populations in tegus and in other Florida reptiles should be monitored closely. With little known about the pathology of these parasites, it is important to monitor Florida reptiles for infection in the case that these parasites pose a deadly risk to Florida species.

While the introduction of invasive parasites is important, it is vital to recognize the importance *Raillietiella orientalis* in *Salvator merianae* in Florida. This parasite is hypothesized to have been brought to Florida by the invasive Burmese Python (Miller, et al., 2017). Parasitic infections of native Pygmy Rattlesnakes has lead to death of those individuals (Farrell, et al., 2019). Infection of hosts by *R. orientalis* often lead to damage of the lung parenchyma (Farrel, et al., 2019). Pygmy Rattlesnakes (*Sistrurus miliarius*) found infected with *Raillietiella orientalis* had obvious gross lesions in the lung and trachea and the largest pentastomes were approximately the same diameter of the Pygmy Rattlesnake trachea, which could potentially cause airway obstruction if infection was severe (Farrell, et al., 2019). It was previously believed that these pentastomes would remain in Florida due to the range of the Burmese Python being restricted to Florida, however, a study conducted by Goetz, et al, 2021 concluded that these tegus can survive the environments of much of the southeastern United States, including Georgia and Alabama. Tegus have already been sighted in Georgia beginning in early 2019, and have also

been reported in South Carolina as of 2020 (FWC, 2021). With these findings, it is highly likely that the Argentine Black and White Tegu could facilitate the northward spread of *R. orientalis*, subsequently infecting other native US reptiles in the southeast. In 2018, pygmy rattlesnakes in Georgia were found to be infected with *R. orientalis* more than 160km north of the range of Burmese Pythons in Florida (Walden, et al, 2020). This indicates that these pentastomes have already begun their northward spread, and tegus may be contributing to this.

In addition to presenting as a risk of disease for Florida reptiles, it is implied that these tegus will have detrimental effects to the ground-nesting organisms based on their diet in South America. Florida does not have an equivalent native lizard, which likely means native animals are naïve to such a large lizard predator. The identification of a juvenile Gopher Tortoise (*Gopherus polyphemus*) in the stomach content of a tegu is alarming because Gopher Tortoises are endangered and could be heavily impacted by the predation of the tegu. They also appear to eat a variety of other plants and animals, which could cause damage to several different populations.

Further research is being conducted to identify the parasites from this study that could not be identified to species based on morphology. Other research efforts should include the continued monitoring of parasitic infections in Argentine Black and White Tegu populations in Florida as well as monitoring the parasitic population in other Florida reptiles. With these tegus expanding their population northward, it is critical to conduct research on population density in other states as well as parasitic infection of other United States populations. It will be especially important to monitor the spread of the three invasive parasites found in this study and the spread of *R. orientalis* to determine how they will effect the southeastern United States ecosystem.

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