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Detecting Snake Fungal Disease (Ophidiomyces ophiodiicola) in the Lower Rio Grande Valley

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DETECTING SNAKE FUNGAL DISEASE (OPHIDIOMYCES OPHIODIICOLA) IN THE LOWER RIO GRANDE VALLEY

A Thesis

by

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ABSTRACT

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Emerging diseases such as Snake Fungal Disease (SFD) caused by the fungus *Ophidiomyces ophiodiicola* (*Oo*) have caused population declines in various snake species in the United States which play a crucial role in the ecosystem as a natural pest control. This fungus targets the scales as a medium to thrive on which can lead to facial disfiguration and respiratory infections. We examined snakes to see if SFD was present in the LRGV and if other fungal species pose a threat to the various snake species population. The data for this study consisted of 14 live snakes captured in the wild and released after being swabbed, 2 deceased snakes and 4 sheds. The swabs were then cultured and isolated and a total of 29 isolates were sent to MIDI Labs for 28S rRNA PCR assays. The DNA sequence report from MIDI Labs did not identify *Oo* as being present in any of the samples but other fungal species were present in 15 of the total isolates. Seeing that harsh cold snaps and high moisture levels are rare in the LRGV, this lowers the likelihood that snakes use communal dens to maintain thermoregulation; the typical infection route for *Oo* to find hosts and thrive. The newly discovered fungi may have implications for the agriculture industry and public health as snakes could serve as a possible vector.

Keywords: emerging diseases, Snake Fungal Disease, epidemiology, South Texas

DEDICATION

This research would not be possible from the love and support from my family and friends who were there with me the entire way. To my mom and dad, thank you for always believing in me and always pushing me to do the best I can in everything I do and thank you for never discouraging my love for reptiles and amphibians since I was a kid. To my grandparents, aunts, uncles and cousins thank you for supporting me and my decisions all these years leading up to this. To my close friends Noe, Jerry, Eddie, Jesus, Cris, Gilbert and Dorian for always being there for me and proving that we can always keep each other on the right path in life. Lastly, I would like to dedicate this to my beautiful girlfriend Maria. She has been there through all my struggles in college and has always helped me to accomplish my goals. Through all those sleepless study nights before exams, to all those late nights searching miles upon miles for snakes she never complained. She was my rock through this whole experience, and I wouldn't want it any other way.

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I would also like to thank Texas Parks and Wildlife Department for granting me the scientific research permit and Wildlife Management Area use permit so that I could go about collecting samples in accordance with the wildlife laws of the state.

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CHAPTER 1

INTRODUCTION

Statement of the Problem

Given the current state of the planet, it is evident that the increase in urbanization, trophy hunting, the pet trade (legal and illegal), traditional medicine, along with many other factors, has led many animal species to near extinction with a grim chance for their populations to recover. When one adds the issue of emerging pathogens that affect targeted species, conservation efforts to re-establish said communities become much more difficult, especially for those that are endemic to a particular geographical area. One such example where an animal group is facing such a decline is snakes. Their natural ranges are more restricted due to the increasing amount of urbanization. They are becoming much more difficult to find, and when encountered, they face death due to a human's fear and being misidentified. Aside from this, there is a silent killer among many snake species, and that is an emerging fungal pathogen known as *Ophidiomyces ophiodiicola* (*Oo*).

Significance of the Study

Seeing how unique the biodiversity is in the Lower Rio Grande Valley, it would be disheartening to see snake populations decline like the Texas Indigo Snake, Western Diamondback Rattlesnake, Texas Coral Snake, and Coachwhip snake, to name a few. They are already dropping in numbers due to the increase in urbanization and climate change and is only getting worse with the added factor of *Ophidiomyces ophiodiicola* in the environment. Some of

these species are considered keystone species, such as the Texas Indigo Snake. They are an essential predator in their domain and their presence indicates a healthy ecosystem. It is known that farmers and ranchers have a profound appreciation for this specific species since they are known to prey on rattlesnakes by simply overpowering them without having any venom and without constricting them. Rattlesnakes can potentially inject a lethal dose of venom with one or two bites to their livestock if they feel threatened. Aside from being among the largest non-venomous snakes in the country, Texas Indigo snakes have an inherited resistance to rattlesnake venom, making it one of nature's heartiest predators leaving it with only a few natural predators of its own.

For this reason, ranchers and homeowners will make a specific area of their land hospitable by placing fallen brush, hollow logs, aluminum, or plywood sheets next to a permanent water source to keep them around. Its home range is quite extensive, extending from the southern portion of the Edward's Plateau down to Veracruz, Mexico, with their breeding season being in the late fall/early winter when they are sighted more frequently as opposed to other species that might brumate during the same time if certain weather conditions are met. However, the primary reason for their population decline today is again due to habitat loss, getting run over while crossing or basking on roads, and being misidentified and killed out of fear. When this emerging fungal pathogen is added to the list as a contributing factor to their decline, one can only wonder how much more they can handle before they are pushed to near extinction in the wild which goes for all snake species.

CHAPTER II

LITERATURE REVIEW

Concerning Snake Fungal Disease

Within the past two decades, there has been a growing concern among scientists regarding snake fungal disease (SFD) caused by the fungus *Ophidiomyces ophiodiicola* (*Oo*), which thrives on the scales of snakes, thus being termed a keratinophilic fungus that is very closely related to the *Chrysosporium* anamorph *Nannizziopsis vriesii* (CANV) complex which is also the cause of severe dermal lesions in other reptile species (Allender et al., 2015). Most prominent clinical signs associated with this fungus compose of scabs, crusty scales, superficial pustules, subcutaneous nodules, dysecdysis, and ocular cloudiness, which can lead to facial disfiguration, sometimes respiratory infections, and low body conditions that could be fatal (Guthrie et al. 2015) & (Lind, McCoy, & Farrell, 2018).

It has already been seen that fungal pathogens have decimated bat and anuran populations throughout the planet. The first southernmost confirmed case of SFD, from Louisiana in 2015, indicates the spread of the disease (Glorioso, Waddle, E, & Jeffrey, 2016). Overall, this fungus has been confirmed in the majority of the United States and Great Britain, and the Czech Republic, given reason to believe that it is present in mainland Europe (Franklinos et al., 2017). More troubling are the reports that it has been affecting many if not all terrestrial and semiaquatic snakes regardless of species, whether they are captive or wild, thus throwing off balance in the ecosystem by removing a predator that acts as a natural form of pest control and

causing problems to snake breeders along with pet owners which one can call a wildlife epidemic (Allender et al., 2015). The generalist nature of the fungus gives it the potential to wipe out endangered wild snake populations such as the Mangshan pit viper, Burmese python in its natural range, King cobra, Chinese cobra, or the Roatan coral snake to name a few (Pare & Sigler, 2016).

On the topic of population decline, it has been suggested that this emergent disease has the capability of inducing mortality either directly or through sublethal effects when it comes to hosting reproduction by compromising testosterone or estradiol levels, which are crucial during the breeding season (Lind et al., 2018). Adding to this, there has been evidence of possible vertical transmission of the disease from parent to offspring whether the female gives birth to live young or to eggs, which could lead to postnatal mortality (Stengle et al., 2019; Britton et al., 2019). From this possible evidence of vertical transmission, breeding snakes for conservation purposes can become more complicated than it already is when trying to replicate the best conditions that would be found in their natural ranges to induce a breeding cycle. Preventative measures must be taken to reduce this emerging fungal pathogen's spread so that a detrimental ecological chain reaction does not occur due to these serpents' loss.

Methods of Detection and Treatment

Diagnosis of the disease is primarily done through histology, microbiology, and conventional polymerase chain reaction. A real-time PCR (qPCR) assay to detect a region of a ribosomal RNA gene was developed to increase the sensitivity and accuracy of detection

(Allender et al., 2015). This now means that detection will be much more efficient, all with a simple swab of a snake and/or some tissue samples.

When trying to culture this fungus in a controlled laboratory setting, the isolates will reproduce asexually through arthroconidia into yellow-white powdery colonies. The underside will seem like a pale-yellow color (Allender et al., 2015). Growth also occurs over a vast pH range that is 5-11. Still, the temperature does seem to impact development, with temperatures of $14°C$ or less inhibiting growth, whereas $25°C$ proved to be optimal temperature, and anything higher than $35°C$ again inhibited growth. Unfortunately, there is no immediate cure for the infected snakes since the disease's progression can be amplified by favoring environmental conditions.

The best way to combat this fungus is to take strict preventative measures at large zoos, aquariums, local herpetariums, traveling reptile exhibits, and snake breeding facilities. Any personnel handling snakes must practice routine disinfection techniques to prevent the spread of this opportunistic fungus. One study showed that 3-10% bleach, 70% ethanol, quaternary ammonium disinfectant, Lysol products, CLR Bath & Kitchen Cleaner, and 409 disinfectants were effective at inhibiting the growth of this fungus when exposed to contact times of five to ten minutes minimum (Rzadkowska et al., 2016). By merely utilizing these inexpensive and common household cleaning chemicals on handling equipment and even housing for all snakes kept as recreational pets and commercial breeding groups, along with those used for research and conservation purposes, can greatly reduce the risk of snake fungal disease transmission in the collection.

Ecological Importance of Snakes

It doesn't make much sense to kill snakes on sight when spotted in one's yard which could be a rare occurrence depending where one lives or when a person is simply going for a walk and happens to stumble upon them. The chances of being bitten is significantly lowered if they are left alone, this is especially is directed at organizers of rattlesnake roundups which is incredibly inhumane and unethical, religious snake handlers who whole heartedly believe their faith is all they need to survive a bite from a venomous snake, and venomous snake keepers that have little to no experience handling them. Instead, the public can educate themselves on the different species of snakes that live in their area, work on being able to identify them safely and clean up the yard so that favorable hiding spots are eliminated causing the snake to migrate elsewhere. As for the average person going out for walks, wear closed-toed shoes and if a snake is encountered either back away safely or go around it safely giving it enough space so that it may retreat if it wishes to (Steen, 2020).

To add to this, the likelihood of encountering any of the three venomous snakes in the LRGV with the most common being the Western Diamondback Rattlesnake are still slim unless one lives out in an area that is dense with thornforest or scrubland type habitat. It is more likely that one would encounter a non-venomous Dekay's brown snake, garter snake or patchnose snake which are beneficial to keep around since they predate on certain invertebrate species along with the invasive Mediterranean gecko and brown anole. With this being said, given the climate in the LRGV, most snakes will be primarily active during the early morning or in the evening year-round to hunt and to thermoregulate themselves. If they were to be active in the

middle of day where temperatures easily peak over $37°C$ this could be fatal to them if their ectothermic body reaches their critical upper maximum temperature range. All in all, a person's chance of coming in contact with a snake is extremely rare unless they are actively seeking them out.

Snakes may be very secretive creatures, but they do indeed play an essential role in the ecosystem wherever they are located across the world once taken the time to understand them. Most terrestrial snakes' primary diet consists of small mammals, while others specialize in predating on fish or even other reptiles along with amphibians and birds. By consuming these prey items, they can keep those populations in check from exploding exponentially and disrupting the trophic systems. One point can be made that since various snake species prey on different types of rodents or even other prey items, this keeps their populations in check from increasing dramatically, leading to an increase in the transmission of zoonotic diseases to other wildlife or even humans.

Also, an excessive population of birds along with rodents can cause significant damage the agriculture industry in the LRGV by consuming the young/immature crops before it gets to be harvested or defecating in the general area, which in turn contaminates them before being supplied to consumers and end up being thrown away. Having said this, it would be a beneficial practice to leave pieces of deadwood, aluminum sheets, or even plywood by the borders of the various crop fields to create a habitat for the many non-venomous species of snakes that can be found while agriculture fields will provide the food source being these agricultural pests mentioned previously.

Summary and Conclusion of the Literature

To understand the importance of snakes as a whole, it is necessary to first understand them on a behavioral, cultural, and evolutionary level. They have been here as early as the Middle-Jurassic-Lower Cretaceous period, but maybe not as how they look now since it is said that the limbless body feature evolved before the snakehead (Caldwell et al., 2015). Adding to this, snakes have been depicted in much different folklore, legends, and myths across different cultures and eras. They have had a significant impact on the varying views the human population has about them. Snakes have never been man-eating or super aggressive to the point they actively seek out to hurt humans as depicted in movies such as "Anaconda." They simply fear us and wish to be left alone and only display defensive behaviors if they feel they have no other option; otherwise, they will simply notice our presence, rattle their tails amongst leaf litter to mimic a rattlesnake's warning signal or hiss so loud like bullsnakes or Indigo snakes that it sounds like a rattlesnake rattling their tail. Some will even go as far as playing dead like hognose snakes if encountered off-guard (Steen, 2020).

Ecologically they hold a significant role by their diet alone. Their predation strategies and the evolution of venom in some species make them efficient predators in their own right. By predating on mammals, fish, birds, amphibians, and even other reptiles, they can keep these populations in check that may have the potential to transmit zoonotic diseases directly or indirectly to other wildlife or even humans.

Seeing that is impossible to cure every free-ranging snake that has contracted this fungus, all that can be done now is to fully understand this fungus in depth down to the very last detail

and halt the spread in areas that are possible such as aquariums, herpetariums, zoos, snake breeding facilities, traveling reptile shows and with the average reptile owner. This can easily be accomplished by ensuring a proper and routine sanitization and quarantine process of all specimens. This is just a small step that can potentially save the lives of many snakes that live in captivity and are essential for conservation breeding projects to help re-establish and strengthen their populations in their natural geographic ranges out in the wild. Fortunately, thanks to recent studies, the detection of snake fungal disease has become more efficient using a simple swab of a potentially infected snake and Taqman real-time polymerase chain reaction assays (Bohuski et al., 2015).

Purpose of the Study

The purpose of this study is to determine if *O. ophiodiicola* is present in the Lower Rio Grande Valley and if the native snake species are being affected by it. Specifically, the study would like to focus on Texas Indigo Snakes (*Drymarchon melanurus erebennus*), which are a protected/keystone species in the state of Texas. Additionally, the study would like to determine if the snakes present in the LRGV play host to other fungal species that might be found in the environment and the effects they have on them.

Hypothesis

Given the fact that most snakes will not travel long distances and are limited in their travels by natural barriers and tend to brumate in mass numbers in the more northern states where the temperatures can reach freezing levels, it is highly unlikely that SFD will be present in the southernmost tip of Texas, but other fungal species already present in the area will likely be

found using the snakes as a host. If results indeed show a presence of SFD, then it would be followed-up by determining what factors attributed to the spread of this disease, seeing that it has become an international issue. Possible factors include but are not limited to the pet trade (legal and illegal), traveling reptile shows, unintentional spread through the soles of people's shoes, and if habitat fragmentation amplifies the prevalence of this fungus. This will be done by collecting swab samples from live and deceased snakes and sheds from local nature centers, wildlife management areas (WMAs), some urbanized areas, and perhaps some ranches and agriculture fields with permission from the landowners.

CHAPTER III

METHODOLOGY

Locations and Timeline

This project took place in various parts of Cameron, Hidalgo, Starr, and Willacy county to cover the vast majority of the Lower Rio Grande Valley during 2018-2020. Permission to conduct sampling in WMA's belonging to Texas Parks and Wildlife was sought and obtained to maximize sampling from the four different counties. Permission to access private ranches and agricultural fields was also sought, but no response was given to my requests. As a result, searching for snakes was limited to these WMAs that were not in use during the various hunting seasons, canals, and public roadways using a road herping. The unpredictable weather experienced these past two years in the Lower Rio Grande Valley also played a significant factor in locating these serpents.

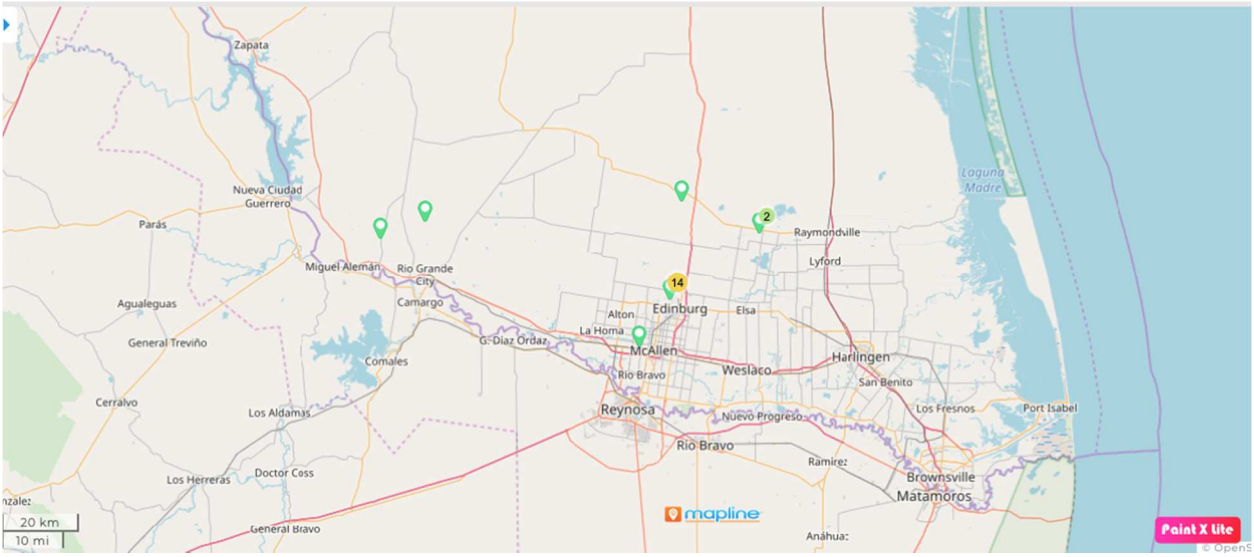

Figure 1. Map of the LRGV with pins showing sample collection locations.

Equipment and Data Collection Technique

Once required permits were obtained from Texas Parks and Wildlife collection of samples commenced. A standard snake hook (DocSeward COPPERHEAD SERIES™ STD43), snake tongs (IC ICLOVER 52 INCH) and sterile disposable gloves (GLOVEWORKS HD Industrial Black Nitrile Gloves with Diamond Grip) were used to handle each specimen safely to reduce the risk of cross contamination and injury for the snake. After handling a snake, new gloves were worn and a cleaning spray containing 10% bleach was used to disinfect the snake hook and tongs. As for sampling, a three-inch MEDLINE Cotton Tipped Applicator, was used to rub along the dorsal and ventral side starting at the head along the body to the tail of any non-venomous snake found in the wild to obtain any possible DNA belonging to microorganisms using the animal as a host. Once the snake had been swabbed the cotton swabs were placed in a Fisherbrand® sterile 50 mL disposable centrifuge tube for transport back to the laboratory. In

addition, if any past sheds for snakes were found, they were collected and taken back to the laboratory and rehydrated, lightly centrifuged and swabbed as well. As for deceased snakes that were found a swab sample was also collected for testing.

A total of 19 samples were taken back to the laboratory, they were then inoculated onto agar plates containing sabouraud dextrose agar (SDA). From there any colonies that resemble the physical characteristics of the targeted fungus were isolated to grow on a separate set of agar plates. After all the samples that had colonies that resembled the physical characteristics of *Oo*, they were shipped to MIDI Labs to undergo 28S ribosomal RNA (rRNA) PCR assays and DNA sequencing to determine if the targeted fungus was indeed present in the samples.

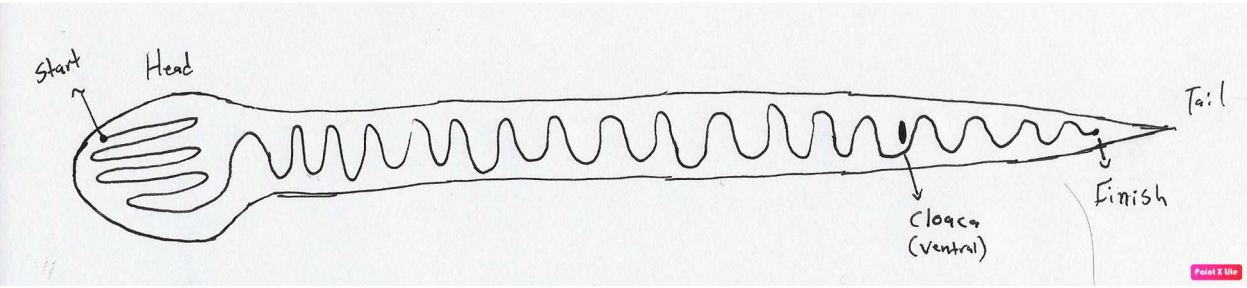

Figure 2. Depiction of swab technique done on both the dorsal and ventral side of a snake.

Table 1. Spreadsheet of samples collected across the LRGV

Sample	Date	Time	Temperature(F)	Humidity(%)	Sample Type	County	Latitude	Longitude	Scientific Name	Common Name	Notes
1	5/12/18	10:30	80	76	Shed	Hidalgo	26.3119	-98.1786	Drymarchon melanurus erebennus	Texas Indigo Snake	Mcallen Nature Center
2	4/21/19	13:00	90	77	Shed	Starr	26.497	-98.8183	Masticophis flagellum	Western Coachwhip	
3	6/6/19	21:20	91	58	Swab	Hidalgo	26.3195	-98.1878	Nerodia rhombifer rhombifer	Diamondback Water Snake	
4	9/18/19	21:13	85	71	Swab	Hidalgo	26.3154	-98.1886	Nerodia rhombifer rhombifer	Diamondback Water Snake	
5	9/18/19	21:13	85	71	Swab	Hidalgo	26.3154	-98.1886	Nerodia rhombifer rhombifer	Diamondback Water Snake	
6	9/18/19	21:13	85	71	Swab	Hidalgo	26.3154	-98.1886	Storeria dekayi texana	Texas Brown Snake	
7	9/27/19	22:04	85	71	Swab	Hidalgo	26.3154	-98.1886	Nerodia rhombifer rhombifer	Diamondback Water Snake	Physical SFD signs
8	9/27/19	22:12	85	71	Swab	Hidalgo	26.3154	-98.1886	Nerodia rhombifer rhombifer	Diamondback Water Snake	
9	2/15/20	17:35	79	46	Swab	Hidalgo	26.3119	-98.1786	Drymarchon melanurus erebennus	Texas Indigo Snake	Mcallen Nature Center
10	3/1/20	19:37	73	64	Swab	Hidalgo	26.3156	-98.1886	Nerodia rhombifer rhombifer	Diamondback Water Snake	
11	3/2/20	9:00	79	46	Shed	Hidalgo	26.3119	-98.1786	Salvadoran grahamiae lineata	Texas Patchnose Snake	Mcallen Nature Center
12	3/14/20	10:21	75	75	Swab	Hidalgo	26.206	-98.266	Drymarchon melanurus erebennus	Texas Indigo Snake	Mcallen Nature Center
13	5/10/20	20:59	72	70	Swab	Hidalgo	26.5455	-98.1587	Sonora semiannulata	Western Ground Snake	No physical signs
14	5/27/20	0:02	73	90	Swab	Cameron	26.4574	-97.9618	Storeria dekayi texana	Texas Brown Snake	
15	6/8/20	22:09	88	71	Swab	Starr	26.4572	-98.9333	Pituophis melanoleucus sayi	Bullsnake	Deceased >1hour
16	7/5/20	21:30	90	65	Swab	Hidalgo	26.3154	-98.1886	Nerodia rhombifer rhombifer	Diamondback Water Snake	
17	7/5/20	21:30	90	65	Swab	Hidalgo	26.3154	-98.1886	Nerodia rhombifer rhombifer	Diamondback Water Snake	
17-A	7/5/20	21:30	90	65	Swab	Hidalgo	26.3154	-98.1886	Nerodia rhombifer rhombifer	Diamondback Water Snake	Specific Location from 17
18	9/1/20	21:54	84	75	Swab	Cameron	26.4855	-97.9509	Thamnophis proximus	Western Ribbon Snake	Deceased

CHAPTER IV

RESULTS

It was determined from the finalized DNA sequence reports from MIDI Labs found in the Appendix which was based on the samples that were collected and isolated as shown in Table 1 above that *Oo* was not present in any of the isolates sent even though some samples displayed physical signs of SFD. This sampling only represents a small fraction of the snakes present in the LRGV. Given that a serpent's method of movement is limited to either lateral undulation, rectilinear movement, concertina movement and sidewinding on the ground it is safe to say they will pick up many different fungi across their lifetime and play host to them without them being affected or being mildly affected. This was the case with some of the samples that came back positive for different types of fungi as shown in Table 2.

Of the 18 original snakes sampled, there was 29 isolates sent to MIDI Labs for 28S rRNA PCR assays and only 14 returned negative for any fungus. The other 15 returned containing some percent identity for other fungal species that was not *Oo*. The isolates that returned negative for any fungal species were 3-1X, 5, 5-1X, 6, 6-1X, 7, 8, 8-2X, 9, 9-2X, 14, 17-1, 17-A. Sample 13 did not meet the preliminary requirement to be sent to MIDI Labs as there was no significant microbial growth on the SDA plate. Sample 17-A was a specific location on the snake the resembled some of the physical characteristics of SFD. The isolates that returned containing another fungal species were: 1-1x, 18 and 18-1x with a zero percent genetic difference for *Fusarium chlamydosporum/Fusarium incarnatum,* 16-1 with a 0.31% genetic difference for

Fusarium spp.*,* 2 with a 0.93% genetic difference for *Aspergillus versicolor,* 4-1X with a zero percent genetic difference for *Aspergillu flavus,* 7-1X with a 0.62% genetic difference for *Paecilomyces variotii,* 7-2X having a 0.62%, 8-1X having a zero percent and 11-1X having a 0.63% genetic difference for *Cladosporium* spp./*Davidiella allicina/Mycosphaerella* spp*,* 9-1X with a zero percent genetic difference for *Penicillum oxalicum,* 10-1X with a 1.25% genetic difference for *Pseudallescheria ellipsoidea,* 12-1X with zero percent genetic difference for *Papilotrema laurentii,* 15 with 1.64% genetic difference for *Mucor* irregularis and 16-2 with a zero percent genetic difference for *Alternari alternata/Cochliobolus carbonum/Stemphylium vesicarium*. From this sample population it equates to 63% playing host to some sort of fungal species with the other 37% being clear. Of the isolates that indicated a presence of some sort of fungus, isolates 1-1X, 2, and 11-1X came from shed skins that were found. Isolate 15, 18 and 18-1X were obtained from freshly deceased specimens found on the road. The remaining isolates were obtained from living specimens that were released shortly after being swabbed.

Table 2.: Results obtained from MIDI Labs sequencing report. *The number alone denotes the original sample taken while a number followed by a hyphen, a number and the letter X (#-#X) denotes an isolate of the original sample.

Sample	Closest DNA Match
1-1X	*Fusarium chlamydosporum/Fusarium incarnatum*
2	*Aspergillus versicolor*
3-1X	No Fungal Growth
4-1X	*Aspergillus flavus*
5	No Fungal Growth
5-1X	No Fungal Growth
6	No Fungal Growth
6-1X	No Fungal Growth
7	No Fungal Growth
7-1X	*Paecilomyces variotii*
7-2X	*Cladosporium* spp/*Davidiella allicina* /*Mycosphaerella* spp
8	No Fungal Growth
8-1X	*Cladosporium* spp/*Davidiella* allicina/*Mycosphaerella* spp
8-2X	No Fungal Growth
9	No Fungal Growth
9-1X	*Penicillium oxalicum*
9-2X	No Fungal Growth
10-1X	*Pseudallescheria ellipsoidea*
11-1X	*Cladosporium* spp/*Davidiella allicina* /*Mycosphaerella* spp
12-1X	*Papiliotrema laurentii*
13	No Fungal Growth
14	No Fungal Growth
15	*Mucor irregularis*
16-1X	*Fusarium* spp
16-2X	*Alternaria alternata/Cochliobolus carbonum/Stemphylium vesicarium*
17-1	No Fungal Growth
17-A	No Fungal Growth
18	*Fusarium chlamydosporum/Fusarium incarnatum*
18-1X	*Fusarium chlamydosporum/Fusarium incarnatum*

CHAPTER V

DISCUSSION

After reviewing the MIDI lab report that determined there was no *Oo* in any of the isolates sent, it was interesting to see the isolates that did indeed return positive for some fungal species. Fungal species that have been noted to cause varying degrees of mycotic diseases in reptiles can come from the genus *Aspergillus, Fusarium, Geotrichium, Mucor, Paecilomyces,* and *Penicillium* (Jacobson & Maxwell, 2000).

The fungal species present in isolates 1-1X, 18-1X, 16-1X, and sample 18 are of extreme importance since the *Fusarium* genus can cause similar interactions as SFD necrotizing mycotic dermatitis, which can be fatal to the animal if left untreated (Barber et al., 2016). Another critical piece of information regarding the genus *Fusarium* is that they are a field fungus that affects crops through mycotoxins called fumonisins in subtropical and temperate regions responsible for vomiting and diarrhea in humans (Gupta, 2017).

In isolates 7-2X, 8-1X, and 11-1X, the genus *Cladosporium* and its anamorphs *Davidiella allicina* and *Mycosphaerella* were most closely identified, which happen to be very prevalent in the environment and can be found on all kinds of hosts. This particular group is critical to research since it has a broad ecological range, affects not only plants but also animals and humans, causing allergies or even diseases (Bensch et al., 2012).

The next sample of concern was sample 2 and isolate 4-1X where *Aspergillus versicolor* and *Aspergillus flavus* were most closely identified, respectively commonly known as

black mold. This genus can cause crusting lesions with granuloma formation, necrosis, respiratory infections in reptiles if left untreated, opportunistic pathogens of immune-compromised animals and humans termed aspergillosis. These infections could become fatal for captive reptiles kept in an immune-compromising condition, such as extremely poor husbandry and hygiene conditions (Seyedmousavi et al., 2015). If *Aspergillus versicolor*is is transmitted to humans, it can potentially cause allergic states, toxicosis, and other invasive infections. One other important factor concerning the *Aspergillus* genus is that it can tolerate temperatures upwards of 37°C and can tolerate pH ranges from 2 to 9, making it a hearty fungus in tropical and subtropical regions (Seyedmousavi, Aspergillosis in Humans and Animals, 2019).

Specifically, *Aspergillus flavus* is a widely spread food-borne fungus and is also commonly found in the soil. Fungi in this genus are known to produce aflatoxins, which contaminates food crops and destroys around 25% annually of the world's food crop supply pre- and post-harvest (World Health Organization: Department of Food Safety and Zoonoses, 2018). When looking at this species in a public health concern setting, it can cause what is known as aflatoxicosis in both humans and animals that have consumed infected crops. If there is repeated exposure, then cancers may potentially develop due to the carcinogens found in aflatoxins, which can very well lead to immunosuppression and be life-threatening.

Isolate 7-1X most closely identified *Paecilomyces variotii,* another cosmopolitan fungus found across the world in soil, drinking water, food, and airborne. There is no literature suggesting this species affects snakes in any way, but in humans, it has the potential to cause endocarditis, fungemia, osteomyelitis, peritonitis, and can lead to immune-compromising

situations. What's more is that this fungal genus seems resistant to antifungal agents, which is extremely important in the medical field (Steiner et al., 2013).

Isolate 9-1X most closely identified a presence of *Penicillium oxalicum,* a fungus commonly found in the environment. It is known that the genus *Penicillium,* along with *Aspergillus,* is associated with respiratory tract infections in captive reptiles, which can be fatal if left untreated (Freire, et al., 2019). In particular, this species is known for the devastating effects it has on crops such as corn, causing ear rot just like the *Fusarium spp.* and *Aspergillus* spp*; however*, it tends to favor colder climates (Munkvold et al., 2019).

Isolate 10-1X most closely indicated a presence for *Pseudallescheria ellipsoidea,* which is part of a group of fungal species in the *Psuedallescheria boydii* species complex found in the soil and contaminated drinking water. This complex is of importance in clinical settings since it has been reported that species in this group such as *P. ellipsoidea* are an opportunistic fungus that has been the cause of some infections in immunocompromised patients diagnosed with cystic fibrosis and immunocompetent patients (Gilgadoet al., 2005)

Isolate 12-1X most closely indicated a presence for *Papiliotrema laurentii,* which was formerly known as *Cryptococcus laurentii*. This fungus is commonly cultured from bird droppings such as the invasive rock pigeon adapted to thrive in highly urbanized areas allowing it to be easily transmitted unknowingly. Typically, *Cryptococcus neoformans* is a common opportunistic pathogen that can cause a variety of life-threatening infections in humans. Typically, diseases due to *C. laurentii* are relatively rare. Still, due to the increasing number of immunocompromised patients worldwide, the rate of infections by this species is starting to

increase. Researches have been able to identify the diseases caused by *C. laurentii,* such as cutaneous infections, fungemia, keratitis, and pulmonary infections (Mattsson, Haemig, & Olsen, 1999). From what I could gather, the literature does not show any implications that snakes are affected by this particular species considering birds and reptiles are closely related in the evolutionary tree compared to mammals, fish, or invertebrates.

Sample 15 most closely indicated a presence of *Mucor irregularis,* an emerging fungal pathogen that can cause cutaneous and subcutaneous invasive infections in humans that can be life-threatening. It is known that the genus *Mucor* does affect captive reptiles, causing some form of dermatitis that can also prove fatal if not treated properly (Bertelsen, et al., 2005). Again, this fungus is commonly found in the environment, making it highly plausible that snakes will come in contact with it. In humans, it turns out that mucormycosis caused by this genus is the third common life-threatening fungal disease falling slightly behind aspergillosis caused by the genus *Aspergillus* and candidiasis caused by the genus *Candida (Xu et al., 2017)*. It has been reported to cause severe ulceration and tissue necrosis even in immunocompetent patients showcasing how infectious it is even with a healthy immune response. Not only that, but it has been shown to cause food spoilage in soy-based products (Lu et al., 2013).

Isolate 16-2X most closely indicated a presence for *Alternaria alternata, Cochilobolus carbonum,* and *Stemphylium vesicarium*. Starting with *A. alternata*, like some of the fungi mentioned previously, it is a cosmopolitan fungal species typically found in the soil or plants. It doesn't seem to affect the snakes that carry this species' spores; however, it can cause allergies in humans, which can be severe for those already who have asthma or allergic rhinitis (Salo et

al., 2005). This fungus had also become of more importance to medical researchers since more people have been developing severe allergies and asthma-like symptoms by being exposed to *A. alternata* antigens at home when it was previously thought that it was mainly associated as an outdoor allergen during the summer and fall months. Thus, it is essential that homeowners, specifically those with family members that might have asthma or severe allergies, practice thorough cleaning procedures to prevent any further growth of mold or fungi due to excess moisture that might trigger these conditions (Salo et al., 2006). In produce, *A. alternata* is a common pathogen that thrives in moist conditions and causes black rot and produces mycotoxins before and after harvest causing significant losses in the agriculture industry (Tronocoso-Rojas & Tiznado-Hernandez, 2014).

The next fungal species identified in isolate 16-2X *Cochliobolus carbonum* are also associated with affecting crops and grasses by producing a toxin known as HC toxin. In maize, it is referred to as the northern corn leaf spot. This fungal species is reported only to infect inbred corn species, while hybrids are not affected at all. There have also been reports that the affected plants can eventually develop a type of resistance to the toxins produced by this species (Agrios, 2005).

The last fungal species identified in isolate 16-2X was *Stemphylium vesicarium,* commonly associated with necrosis of the leaf tissue, otherwise known as brown spot disease in crops, such as pears and onion asparagus, to name a few, and grasses as well. Given the information that this is a saprophytic fungus meaning it can grow on dead fallen

leaves, it makes it that much more challenging to eliminate, making preventive measures only partly successful (Kohl et al., 2009).

Additionally, efficient ways to cope with mycotoxins affecting crops has been done through either chemical, physical, biological control or irradiation techniques to prevent further transmission. To date measures to decrease the transmission of fungal species such as *Aspergillus flavus* to crops are being developed such as genetic engineering various crop species to be resistant and inoculating crops with non-toxic versions of the fungus so that it may compete and displace the naturally occurring infectious version which is a favorable solution that does indeed have potential (World Health Organization: Department of Food Safety and Zoonoses, 2018).

Limitations were experienced throughout this study, mainly being weather conditions. The LRGV is known for having very erratic weather, with most days averaging daily highs of around 37°C, limiting searching for snakes from sunset throughout the night to sunrise. Other days there will be heavy scattered thunderstorms that displace snakes from their homes, searching for homes that have not been flooded, making these areas almost inaccessible. Another limitation encountered was the fact that the most frequently encountered species was, in fact, *Crotalus atrox,* commonly known as the western diamondback rattlesnake. Due to my inexperience working with venomous snakes and to ensure my safety, this species was omitted from the study. As there is not much literature regarding SFD in Texas, this study could act as a preliminary status report to agencies like Texas Parks and Wildlife (TPWD) and US Fish and Wildlife Service (USFWS) tasked with the conservation of wildlife.

CHAPTER VI

CONCLUSION

This study aimed to determine that SFD was present in the valley and if the native snake populations played host to other fungal species already present in the environment and to what extent are, they affected by it. Seeing that *Oo* was not present in the samples sent and after reviewing the fungal species that were present and examining their potential effects on the snakes themselves, public health and the agriculture industry we now know that snakes can be an unintentional carrier of these fungi after picking them up in already contaminated agriculture fields or contaminated urban areas in search of prey. Although the sample population used in this study was not large enough due to the limitations experienced to give a definitive answer as to whether the fungus responsible for SFD is present in the LRGV, it would be in the best interest of TPWD and USFWS to collect a more extensive sample population in Texas along with rest of the United States. This way, these government agencies could confirm a more accurate geographic range of *Oo* and develop a plan to prevent further spread that applies to everyone that houses snakes, such as facilities that use them for educational and conservation purposes and especially for those that breed, sell and import wild caught snakes for the pet trade.

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APPENDICES

APPENDIX A

APPENDIX A

Institutional Animal Care and Use Committee (IACUC)
The University of Texas Rio Grande Valley
UTRGV.edu

AMENDMENT/MODIFICATION APPROVAL NOTICE

February 28, 2020

Principal Investigator Name: Frederic Zaidan, PhD.
IACUC Protocol Number: AUP-19-03
Proposal Title: "Detecting Snake Fungal Disease (Ophiodiomyces ophiodiicola) in the Lower Rio Grande Valley"

Dear Investigator,

A modification request for the AUP protocol referenced above has been reviewed and approved. Approval has been granted for the following modification(s):

-Removing Collection of Soil samples.

IACUC Approval Period from: IACUC Approval Period from February 21, 2020 through February 20, 2022

Please be notified that the UTRGV Institutional Animal Care and Use Committee (IACUC) approved the amendment/modification to your above-referenced protocol. The approval of this protocol is limited to the animal model(s) and procedures described in the application. You must obtain prior approval from IACUC if any other modification or addition to this protocol is initiated.

All training must be maintained current for all employees/personnel working with animals on this protocol. Any changes in status will need to be reported to the IACUC for approval.

ANIMAL WELFARE ASSURANCE AND ACCREDITATION

The Executive Vice President for Research, Graduate Studies & New Program Development at The University of Texas Rio Grande Valley received confirmation from the NIH Office of Laboratory Animal Welfare (OLAW) that our Animal Welfare Assurance (AWA) became effective September 25, 2019 and will expire on August 31, 2023. The UTRGV Animal Welfare Assurance identification number with the Department of Health and Human Services is: A4730-01. UTRGV's USDA Certification Number is: 74-R-0216.

Christopher Vitek, Ph.D.
IACUC Chair and Animal Assurance Officer
Associate Professor

Institutional Animal Care and Use Committee (IACUC)
The University of Texas Rio Grande Valley
UTRGV.edu

APPROVAL NOTICE

February 28, 2020

Principal Investigator Name: Frederic Zaidan, PhD.
IACUC Protocol Number: AUP-19-03
Proposal Title: "Detecting Snake Fungal Disease (Ophiodiomyces ophiodiicola) in the Lower Rio Grande Valley"

IACUC Approval Period from February 21, 2020 through February 20, 2022 – First annual report Approval.

Please be notified that the UTRGV Institutional Animal Care and Use Committee (IACUC) approved your above-referenced protocol. The approval of this protocol is limited to the animal model(s) and procedures described in the application. You must obtain prior approval from IACUC if any modifications or additions to this protocol are initiated. The approval of your project includes:

ANIMAL SUBJECT(S): The grand total number of animal by species approved for this protocol is:

Total Animals Approved: **5 Heterodon**
5 Lampropeltis
5 Leptodeira
5 Nerodia
5 Pituopnis
5 Sonora
5 Tantilla

ANIMAL WELFARE ASSURANCE AND ACCREDITATION

The Senior Vice President for Research, Innovation, and Economic Development at The University of Texas Rio Grande Valley received confirmation from the NIH Office of Laboratory Animal Welfare (OLAW) that our Animal Welfare Assurance (AWA) became effective September 25, 2019 and will expire on August 31, 2023. The UTRGV Animal Welfare Assurance identification number with the Department of Health and Human Services is: A4730-01. UTRGV's USDA Certification Number is: 74-R-0216.

Christopher Vitek, Ph.D.
IACUC Chair and Animal Assurance Officer
Associate Professor

Institutional Animal Care and Use Committee (IACUC)
The University of Texas Rio Grande Valley
UTRGV.edu

APPROVAL NOTICE

February 05, 2019

Principal Investigator Name: Frederic Zaidan, PhD.
IACUC Protocol Number: AUP-19-03
Proposal Title: "Detecting Snake Fungal Disease (Ophiodiomyces ophiodiicola) in the Lower Rio Grande Valley"

IACUC Approval Period from February 05, 2019 through February 04, 2022 - with annual reports to show progress.

Please be notified that the UTRGV Institutional Animal Care and Use Committee (IACUC) approved your above-referenced protocol. The approval of this protocol is limited to the animal model(s) and procedures described in the application. You must obtain prior approval from IACUC if any modifications or additions to this protocol are initiated. The approval of your project includes:

ANIMAL SUBJECT(S): The grand total number of animal by species approved for this protocol is:

Total Animal Approved: 5 Heterodon; 5 Lampropeltis; 5 Leptodeira; 5 Nerodia; 5 Pituopnis; 5 Sonora; 5 Tantilla

ANIMAL WELFARE ASSURANCE AND ACCREDITATION

The Executive Vice President for Research, Graduate Studies & New Program Development at The University of Texas Rio Grande Valley received confirmation from the NIH Office of Laboratory Animal Welfare (OLAW) that our Animal Welfare Assurance (AWA) became effective September 25, 2019 and will expire on August 31, 2023. The UTRGV Animal Welfare Assurance identification number with the Department of Health and Human Services is: A4730-01. UTRGV's USDA Certification Number is: 74-R-0216

Christopher Vitek, Ph.D.
IACUC Chair and Animal Assurance Officer
Associate Professor

APPENDIX B

SCIENTIFIC PERMIT NUMBER SPR-0919-144
IS HEREBY ISSUED TO:

Manuel Zavala
University of Texas – Rio Grande Valley

UNDER THE AUTHORITY OF CHAPTER 43, SUBCHAPTER C OF THE
TEXAS PARKS AND WILDLIFE CODE

The activities permitted by this document are to be carried out in accordance with the Texas Parks and Wildlife Code, the Rules and Regulations of the Texas Parks and Wildlife Commission, and all of the following provisions:

1. This permit may not be transferred, assigned or conveyed by the holder.

2. The issuance of this permit is not a guarantee that a subsequent permit or renewal of this permit will be granted.

3. Required information and data shall be maintained at the address of the permit holder and shall be available for inspection at the request of personnel of the Texas Parks and Wildlife Department during the active life of the permit.

4. Acceptance of this permit constitutes an acknowledgment and agreement that the holder will comply with all Rules, Regulations, Orders and Proclamations of the Texas Parks and Wildlife Commission issued in accordance with the law and the conditions precedent to the granting of this permit. Failure to comply with any and all provisions of this permit may result in enforcement action, including any criminal penalties authorized by the Parks and Wildlife Code.

5. This permit does not relieve the holder of the responsibility to obey all other local, county, state and federal laws while carrying out the authorized activities.

- ADDITIONAL PROVISIONS FOLLOW ON ATTACHED PAGES. -

Issued by:

September 5, 2019
Effective date

Chris Maldonado
Wildlife Permits Specialist

6. This permit will expire at midnight, **September 5, 2022**

7. The following individuals may conduct the activities authorized by this permit under the guidance of the permittee:

SUBPERMITTEES: NONE

UNPERMITTED ASSISTANTS: A permittee engaging unpermitted assistants shall maintain on file at their office and possess on their person in the field a signed and dated list of all unpermitted persons assisting in permitted activities. **(Individuals under the direct on-site supervision of permit holder).**

8. The following wildlife species in the specified quantities are authorized by this permit to be:

a. DETECTING SNAKE FUNGAL DISEASE (*OPHIDIOMYCES OPHIODIICOLA*) IN THE LOWER RIO GRANDE VALLEY - This permit gives authority to live-capture (snake hook and tong) native snakes in Cameron, Hidalgo, Starr, and Willacy counties, swab each snake for culturing, and their immediate release unharmed at capture site for scientific purposes. In addition, this permit authorizes the salvage (specimens found dead) of native snakes. GPS coordinates will be taken at all sites where any *Species of Greatest Conservation Need* were captured and submitted with Annual Report, preferably using the respective Texas Natural Diversity Database (TXNDD) Form. All non-target species will be immediately released at site of capture. This authority will end on 12/31/2021.*

Common name	Scientific Name		Quantity
Snakes	Serpentes	(capture and release)	Unrestricted
		(salvage)	Unrestricted

Including species listed by the Department as threatened or endangered

Federal and State Listed Species and other Species of Greatest Conservation Need - http://tpwd.texas.gov/huntwild/wild/wildlife_diversity/nongame/listed-species/

* *In accordance with specifications listed on a valid federal permit, if applicable.*

9. The following means for taking or capture are authorized by this permit:

a. Snake hook, snake tong.

10. The following locations for taking or capture are authorized by this permit:

a. Counties of Texas (Cameron, Hidalgo, Starr, Willacy) - Permit holder is prohibited from collecting on private or public premises without prior written consent of the owner or manager of the premises. Please be sure to review paragraph 14 b-d of this permit.

11. If authorized above, all specimens taken or salvaged shall be deposited with an appropriate collection, or otherwise disposed of in accordance with paragraph 13d of this permit.

12. All collection gear left unattended shall be clearly marked with permittee's name and permit number.

13. **PERMIT HOLDER IS REQUIRED TO**:

a. File a completed report form annually (provided on issuance of this permit), and any reports or publications based on data collected under authority of this permit, with the Texas Parks and Wildlife Department, Wildlife Permits Section, 4200 Smith School Rd., Austin, TX, 78744**, no later than fourteen days following the anniversary date of the permit** (or the expiration date if the permit is due for renewal).
YOUR PERMIT WILL NOT BE VALID UNLESS YOUR REPORT HAS BEEN RECEIVED.

b. Carry a copy of this permit at all times when exercising the provisions of this permit, which shall be subject to inspection by any authorized enforcement officer of the Department upon request.

c. Notify the Texas Parks and Wildlife Department Law Enforcement Office(s) in the region(s) of your field activities by telephone not less than 24 hours nor more than 72 hours prior to collection if collection techniques or devices being used are ordinarily classified as illegal (i.e. using gill nets or electro-shocking devices to collect fish, hunting/collecting along public roads and rights-of-way). **A confirmed response from the local game warden is required prior to collection if the sampling activities being conducted involve methods of capture ordinarily classified as illegal.** To determine appropriate regional office location and/or telephone number, please see http://www.tpwd.state.tx.us/warden/connect/offices. If the regional office(s) or telephone number(s) is

unknown, the number(s) may be obtained at any time by calling a Parks and Wildlife Communication Center: Austin - (512) 389-4848.

d. Dispose of protected wildlife taken under the authority of this permit in only one of the following ways:

(1). Kill and utilize by examination, experimentation, necropsy or dispose of as waste in accordance with state law and city or county regulations (burning is suggested if not in conflict with city, county or state regulations).

(2). Hold permanently for scientific or educational purposes, or donate to another educational display, scientific, or zoological permit holder authorized to receive such specimens, **with required specimen donation form provided by the Department. A copy of the completed form must be submitted with the annual report.**

(3). Release unharmed at collection site.

(4). Donate edible portions of game species to charitable organizations, public hospitals, orphanages or indigent persons. Arrangements for donations are the responsibility of the permit holder.

14. **PERMIT HOLDER IS PROHIBITED FROM**:

a. Selling or bartering specimens collected under the authority of this permit. Specimens may be donated to other permit holders by completing the receipt form enclosed with the permit.

b. Collecting on private premises without prior written consent of the owner or operator of the premises.

c. Collecting in a state park without a separate permit from the Texas Parks and Wildlife Department Natural Resource Program: email: sppermits@tpwd.texas.gov.

d. Wildlife Management Areas (WMA) – This permit cannot give authority to enter a wildlife management area (WMA) to conduct your proposed activity. You will be required to coordinate with Mr. Dennis Gissell (dennis.gissell@tpwd.texas.gov) before any activity in a WMA can occur.

e. Taking species listed by the department as threatened or endangered without express authority in paragraph 8 of this permit.

15. **ADDITIONAL PROVISIONS:**

a. No hunting or fishing license is required for permit holders or individuals listed in paragraph 7 while conducting the activities expressly authorized by this permit. Each listed individual should carry a copy of this permit during collection activities, and a letter of permission from the permittee if working independently.

b. This permit is subject to any applicable federal permit requirements. Where a federal permit is required, the permit holder is cautioned to carry a copy of the federal as well as the state permit during collecting activity. For information on the federal permit contact: U.S. Fish and Wildlife Service, PO Box 709, Albuquerque, NM, 87103-0709; (505)248-7882.

16. PERMIT HOLDER'S ADDRESS FOR RECORDKEEPING PURPOSES:
Manuel Zavala
University of Texas – Rio Grande Valley
1201 W. University Dr.
Edinburg, TX 78539

APPENDIX C

APPENDIX C

TEXAS PARKS AND WILDLIFE DEPARTMENT
LAS PALOMAS WILDLIFE MANAGEMENT AREA
154 B Lakeview Drive
Weslaco, Texas 78596
956-565-1223

This permit serves as an agreement between Texas Parks and Wildlife Department (TPWD) and Manuel Zavala for the purpose of detecting snake fungal disease (*Ophidiomyces ophiodiicola*) on units of Las Palomas WMA (LPWMA).

CONDITIONS

1. Wet weather and soil conditions may prohibit access to the Land. In the event of adverse weather conditions, the area manager may temporarily halt all research activities.
2. Cutting, clearing, or other vegetation disturbances shall be prohibited.
3. Entrance to and travel within TPWD lands shall be over established routes or as authorized by the area manager. Fences must not be cut for any reason. Existing gates must be used. Gates shall remain closed, unless otherwise authorized by the area manager. Only authorized researchers shall be allowed access to the Land. All vehicular and research activities shall be directly related to the project.
4. Operations shall not be conducted at a time that will interfere with public hunts or other scheduled activities on the Land. Manuel Zavala shall contact the area manager for information about other scheduled activities.
5. Manuel Zavala must not travel on the Land or its roads during wet weather, as determined by the area manager. Hunting and/or fishing by researchers or agents associated with Manuel Zavala during the course of operations is prohibited in all circumstances.
6. Fires are prohibited in all circumstances.
7. Manuel Zavala shall be directly responsible to TPWD for any damages caused by operations directly related to his research hereunder to wildlife, livestock, houses, fences, gates, roads, tanks, or other improvements or pasturage or growing crops on units of LPWMA and this permit is issued subject to the prior rights of any lessee of the land.
8. Nothing in this permit is intended or should be construed as absolving Manuel Zavala of any legal claim for damages TPWD may be entitled to assert resulting from negligence or excessive or wrongful conduct of any agent, researcher, contractor, or subcontractor of Manuel Zavala, and no expressed or implied waiver of any claim is intended.
9. All reasonable precautions, including consultation with the area manager, shall be taken to avoid undo disturbance of critical fish, wildlife, or plant resources during operations.
10. Speed limit for vehicular traffic on the land shall be 15 miles per hour or less as conditions warrant.
11. Manuel Zavala must advise TPWD at least 24 hours in advance of collecting activity by telephoning the area

manager or biologist.

12. Manuel Zavala must notify TPWD by telephoning the area manager upon completion of all activities associated with the research activities.
13. Manuel Zavala hereby releases and hold harmless the Texas Parks and Wildlife Department, the Texas Parks and Wildlife Commission, and their officers, employees, agents and commissioners from all legal responsibility and liability for any injuries or death, and any loss, damage, or theft of personal property.
14. Manuel Zavala must take all reasonable precautions to prevent capture, mortality or injury to non-target species.
15. Manuel Zavala must treat all trapped species as humanely and to insure target species are dispatched in the most humane manner possible, consistent with the objectives of the study.
16. Manuel Zavala must mention TPWD as a participating/contributing partner in this research project and any publications associated.
17. The area manager is Jimmy Stout, office telephone (956) 498-4791. The biologist is Tony Henehan, phone number (956) 532-3009.
18. This permit is valid from February 15, 2020 through December 31, 2020, and is subject to renewal at that date.

TEXAS PARKS AND WILDLIFE DEPARTMENT

Approved by: _____ Date: 2/10/20
Jimmy Stout, Area Manager,
Las Palomas Wildlife Management Area

Manuel Zavala

by: _____ Date: 2/6/2020

APPENDIX D

Alignment Report - Fungal (D2) Analysis

Customer: Zavala , Manuel
Company: University of Texas Rio Grande Valley
Address: 2431 Sentry Palm Drive, Rio Grande City, TX 78582 USA

125 SANDY DRIVE•NEWARK, DE 19713•PH 302-737-4297•FX 302-737-7781•WWW.MIDILABS.COM

FY28M2 Library Revision: 2.23

Created: 9/28/2020 11:13:16 AM
Sample ID: C2009247602-1-1

28S DNA: 320 base pairs

FY28M2 DNA Match Report

Match	%Diff	Length	Library Entry Name
1	0.00	320	Fusarium-chlamydosporum-M9774
2	0.00	320	Fusarium-incarnatum
3	0.63	320	Fusarium-chlamydosporum-M9766
4	0.94	320	Fusarium-nygamai-M10106
5	0.94	320	Fusarium-proliferatum/verticillioides
6	1.25	320	Fusarium-subglutinans-M10143
7	1.56	320	Fusarium-anthophilum/napiforme
8	1.56	320	Fusarium-nygamai-M9528
9	1.56	320	Fusarium-poae/robustum
10	1.56	320	Fusarium-subglutinans-M10119

Concise Alignment (maximum difference 0.63):

```
                  13|  205|
Sample:             A     T
Lib Match 1:        A     T
Lib Match 2:        A     T
Lib Match 3:        C     C
```

Closest Match: **Fusarium-chlamydosporum/ Fusarium-incarnatum**

GenBank Match: 100%, Fusarium sp. AY234909*

**GenBank is a public database supported by The National Center for Biotechnology Information. GenBank is used for reference purposes only and is not a validated database.*

Reviewer's Signature 09-28-20

Shurnevia Strickland

Shurnevia Strickland, Data Analyst

RAW SEQUENCE DATA>C2009247602-1-1
AGACCGATAGCGAACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAA
AAGAGAGTTAAAAAGTACGTGAAATTGTTGAAAGGGAAGCGTTTATGACC
AGACTTGGGCTTGGTTAATCATCTGGGGTTCTCCCCAGTGCACTTTTCCA
GTCCAGGCCAGCATCAGTTTTCGCCGGGGGATAAAGGCTTCGGGAATGTG
GCTCTCTCCGGGGAGTGTTATAGCCCGTTGCGTAATACCCTGGCGGGGAC
TGAGGTTCGCGCATCTGCAAGGATGCTGGCGTAATGGTCATCAACGACCC
GTCTTGAAACACGGACCAAG

Alignment Report - Fungal (D2) Analysis

Customer: Zavala , Manuel
Company: University of Texas Rio Grande Valley
Address: 2431 Sentry Palm Drive, Rio Grande City, TX 78582 USA

125 SANDY DRIVE•NEWARK, DE 19713•PH 302-737-4297•FX 302-737-7781•WWW.MIDILABS.COM

FY28M2 Library Revision: 2.23

Created: 9/28/2020 11:13:16 AM
Sample ID: C2009247603-2

28S DNA: 321 base pairs

FY28M2 DNA Match Report

Match	%Diff	Length	Library Entry Name
1	0.93	321	Aspergillus-versicolor-M9480
2	0.93	321	Aspergillus-versicolor-M9835
3	2.18	321	Aspergillus-aureolatus
4	2.34	321	Aspergillus-asperescens
5	2.80	322	Aspergillus-fruticulosus
6	2.80	322	Aspergillus-nidulans-M10113
7	2.80	322	Eupenicillium-cinnamopurpureum-M8844
8	2.80	321	Aspergillus-egyptiacus-M8835
9	2.95	322	Aspergillus-quadrilineatus
10	3.11	322	Aspergillus-unguis

Concise Alignment (maximum difference 1.25):

```
                  97|  170|  290|
Sample:             A     T     CG
Lib Match 1:        G     C     TG
Lib Match 2:        G     C     CA
```

Closest Match: **Aspergillus-versicolor**

GenBank Match: **100%, Aspergillus sp. GU265713***

**GenBank is a public database supported by The National Center for Biotechnology Information. GenBank is used for reference purposes only and is not a validated database.*

Reviewer's Signature 09-28-20

Shurnevia Strickland

Shurnevia Strickland, Data Analyst

RAW SEQUENCE DATA >C2009247603-2
AGACCGATAGCGAACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAA
AAGAGAGTTAAACAGCACGTGAAATTGTTGAAAGGGAAGCGCTTGCAACC
AGACTCGGCCTCGGGGTTCAGCCAGCATTCGTGCTGGTGTACTTCCCCGG
GGCCGGGCCAGCGTCGGTTTGGGCGGCCGGTCAAAGGCCCCAGGAATGTA
TCGTCCTCCGGGACGTCTTATAGCCTGGGGTGCAATGCGGCCAGCCTGGA
CCGAGGAACGCGCTTCGGCACGGACGCTGGCGTAATGGTCGCAAACGACC
CGTCTTGAAACACGGACCAAG

MIDI LABS

Incomplete Analysis Report

Customer: Zavala , Manuel
Company: University of Texas Rio Grande Valley
Address: 2431 Sentry Palm Drive, Rio Grande City, TX 78582 USA
125 SANDY DRIVE•NEWARK, DE 19713•PH 302-737-4297•FX 302-737-7781•WWW.MIDILABS.COM

Date: 10/05/2020

Sample(s): C202009247604- 3-1
C202009247607- 5-1
C202009247622- 14
C202009247626- 17-1
C202009247627- 17-A

We are unable to provide a report for the above sample(s) due to the following reason:

- [ ] Insufficient cell growth
- [ ] Mixed culture
- [ ] Failed Bacteria PCR*
- [ ] Failed Yeast/Fungi PCR*
- [ ] Insufficient sequence data
- [X] Other (See comments.)

*Several attempts were made to obtain an acceptable PCR product. The following items were checked and found to be in compliance with our laboratory's standard operating procedures:

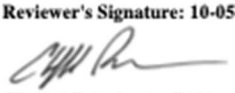
- [ ] Reviewed Sample Submission Plate
- [ ] Reviewed PCR Positive and Negative Controls
- [ ] Reviewed Sample Preparation Process Documentation

PCR failure may result from the presence of PCR inhibitors, mixed cultures and/or the use of universal PCR primers which may not be optimal for all organisms.

Comments: During sample processing it was determined that these sample plates only had bacteria growth. The customer was notified and asked that no further testing be conducted on these samples at this time.

If you have any questions or comments regarding this report, please feel free to contact MIDI Labs.

Reviewer's Signature: 10-05-20



Clifford Poindexter, Laboratory Manager

Alignment Report - Fungal (D2) Analysis

Customer: Zavala , Manuel
Company: University of Texas Rio Grande Valley
Address: 2431 Sentry Palm Drive, Rio Grande City, TX 78582 USA

125 SANDY DRIVE•NEWARK, DE 19713•PH 302-737-4297•FX 302-737-7781•WWW.MIDILABS.COM

FY28M2 Library Revision: 2.23

Created: 9/28/2020 11:13:17 AM
Sample ID: C2009247605-4-1

28S DNA: 321 base pairs

FY28M2 DNA Match Report

Match	%Diff	Length	Library Entry Name
1	0.00	321	Aspergillus-flavus
2	1.25	321	Petromyces-alliaceus
3	3.12	321	Aspergillus-awamori/foetidus/niger
4	3.12	321	Aspergillus-terricola
5	3.12	321	Fennellia-flavipes
6	3.43	321	Aspergillus-ochraceus
7	3.72	323	Aspergillus-sclerotiorum
8	3.74	321	Aspergillus-petrakii
9	4.35	321	Aspergillus-candidus
10	4.36	321	Aspergillus-carbonarius

Exact match with Aspergillus-flavus

Closest Match: **Aspergillus-flavus**

GenBank Match: **100%, Aspergillus flavus AY235013***

**GenBank is a public database supported by The National Center for Biotechnology Information. GenBank is used for reference purposes only and is not a validated database.*

Reviewer's Signature 09-28-20

Shurnevia Strickland

Shurnevia Strickland, Data Analyst

RAW SEQUENCE DATA >C2009247605-4-1
AGACCGATAGCGAACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAA
AAGAGAGTTAAAAAGCACGTGAAATTGTTGAAAGGGAAGCGCTTGCGACC
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GGGCGGGCCAGCGTCGGTTTGGGCGGCCGGTCAAAGGCTCCCGGAATGTA
GTGCCCTCCGGGGCACCTTATAGCCGGGAGTGCAATGCGGCCAGCCTGGA
CCGAGGAACGCGCTTCGGCACGGACGCTGGCATAATGGTCGTAAACGACC
CGTCTTGAAACACGGACCAAG

MIDI LABS

Incomplete Analysis Report

Customer: Zavala , Manuel
Company: University of Texas Rio Grande Valley
Address: 2431 Sentry Palm Drive, Rio Grande City, TX 78582 USA
125 SANDY DRIVE•NEWARK, DE 19713•PH 302-737-4297•FX 302-737-7781•WWW.MIDILABS.COM

Date: 10/05/2020

Sample(s): 202009247606- 5
202009247608- 6
202009247609- 6-1
202009247610- 7

We are unable to provide a report for the above sample(s) due to the following reason:

- [ ] Insufficient cell growth
- [ ] Mixed culture
- [ ] Failed Bacteria PCR*
- [x] Failed Yeast/Fungi PCR*
- [ ] Insufficient sequence data
- [ ] Other (See comments.)

*Several attempts were made to obtain an acceptable PCR product. The following items were checked and found to be in compliance with our laboratory's standard operating procedures:

- [x] Reviewed Sample Submission Plate
- [x] Reviewed PCR Positive and Negative Controls
- [x] Reviewed Sample Preparation Process Documentation

PCR failure may result from the presence of PCR inhibitors, mixed cultures and/or the use of universal PCR primers which may not be optimal for all organisms.

Comments: N/A

If you have any questions or comments regarding this report, please feel free to contact MIDI Labs.

Reviewer's Signature: 10-05-20

Clifford Poindexter, Laboratory Manager

Alignment Report - Fungal (D2) Analysis

Customer: Zavala , Manuel
Company: University of Texas Rio Grande Valley
Address: 2431 Sentry Palm Drive, Rio Grande City, TX 78582 USA

125 SANDY DRIVE•NEWARK, DE 19713•PH 302-737-4297•FX 302-737-7781•WWW.MIDILABS.COM

FY28M2 Library Revision: 2.23

Created: 9/28/2020 11:13:17 AM
Sample ID: C2009247611-7-1

28S DNA: 322 base pairs

FY28M2 DNA Match Report

Match	%Diff	Length	Library Entry Name
1	0.62	322	Paecilomyces-variotii-M8709
2	1.86	322	Paecilomyces-puntonii
3	2.48	322	Byssochlamys-fulva
4	2.48	322	Byssochlamys-nivea-M10126
5	2.48	322	Byssochlamys-nivea-M8726
6	3.11	322	Paecilomyces-variotii-M9846
7	4.04	321	Hamigera-striata
8	4.04	322	Penicillium-argillaceum
9	4.35	321	Hamigera-avellanea
10	4.66	321	Penicillium-dierckxii

Concise Alignment with Paecilomyces-variotii-M8709

```
Sample:     (151) T (248) C
            .....   .....
LibEnt 1:   (151) G (248) T
```

Closest Match: **Paecilomyces-variotii**

GenBank Match: **99%, Byssochlamys fulva MG877730***

**GenBank is a public database supported by The National Center for Biotechnology Information. GenBank is used for reference purposes only and is not a validated database.*

Reviewer's Signature 09-28-20

Shurnevia Strickland

Shurnevia Strickland, Data Analyst

RAW SEQUENCE DATA >C2009247611-7-1
AGACCGATAGCGAACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAA
AAGAGAGTTAAACAGCACGTGAAATTGTTGAAAGGGAAGCGCTTGCGACC
AGACTCGCCCGCGGGGGTTCAGCCGGTACTCGTACCGGTGTACTCCCCCG
TGGGCGGGCCAGCGTCGGTTTGGGCGGTCGGTCAAAGGCCTCCGGAATGT
GTCGCCCCCCGGGGCGTCTTATAGCCGGAGGTGCAATGCGGCCAGCCCGG
ACCGAGGAACGCGCTTCGGCTCGGACGCTGGCGTAATGGTCGTAAGCGGC
CCGTCTTGAAACACGGACCAAG

Alignment Report - Fungal (D2) Analysis

Customer: Zavala , Manuel
Company: University of Texas Rio Grande Valley
Address: 2431 Sentry Palm Drive, Rio Grande City, TX 78582 USA

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125 SANDY DRIVE•NEWARK, DE 19713•PH 302-737-4297•FX 302-737-7781•WWW.MIDILABS.COM

FY28M2 Library Revision: 2.23

Created: 9/28/2020 11:13:18 AM
Sample ID: C2009247612-7-2

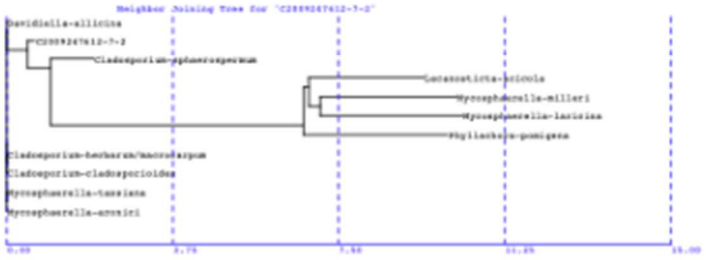
28S DNA: 316 base pairs

FY28M2 DNA Match Report

Match	%Diff	Length	Library Entry Name
1	0.63	316	Cladosporium-cladosporioides
2	0.63	316	Cladosporium-herbarum/macrocarpum
3	0.63	316	Davidiella-allicina
4	0.63	316	Mycosphaerella-aronici
5	0.63	316	Mycosphaerella-tassiana
6	1.90	316	Cladosporium-sphaerospermum
7	8.78	317	Lecanosticta-acicola
8	9.40	317	Mycosphaerella-milleri
9	9.46	315	Phyllachora-pomigena
10	9.72	317	Mycosphaerella-laricina

Concise Alignment (maximum difference 0.63):

```
                  80|  285|
Sample:             G    T
Lib Match 1:        A    C
Lib Match 2:        A    C
Lib Match 3:        A    C
```



Closest Match: **Cladosporium-spp./ Davidiella-allicina/ Mycosphaerella-spp.**

GenBank Match: **100%, Naganishia albidosimilis MT267486***
**GenBank is a public database supported by The National Center for Biotechnology Information. GenBank is used for reference purposes only and is not a validated database.*

Reviewer's Signature 09-28-20

Shurnevia Strickland

Shurnevia Strickland, Data Analyst

RAW SEQUENCE DATA >C2009247612-7-2
AGACCGATAGCGAACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGGA
AAGAGAGTTAAAAAGCACGTGAAATTGTTGAAAGGGAAGGGATTGCAACC
AGACTTGCTCGCGGTGTTCCGCCGGTCTTCTGACCGGTCTACTCGCCGCG
TTGCAGGCCAGCATCGTCTGGTGCCGCTGGATAAGACTTGAGGAATGTAG
CTCCCTCGGGAGTGTTATAGCCTCTTGTGATGCAGCGAGCGCCGGGCGAG
GTCCGCGCTTCGGCTAGGATGCTGGCGTAATGGTTGTAATCCGCCCGTCT
TGAAACACGGACCAAG

MIDI LABS

Incomplete Analysis Report

Customer: **Zavala , Manuel**
Company: **University of Texas Rio Grande Valley**
Address: **2431 Sentry Palm Drive, Rio Grande City, TX 78582 USA**

125 SANDY DRIVE•NEWARK, DE 19713•PH 302-737-4297•FX 302-737-7781•WWW.MIDILABS.COM

Date: 10/05/2020

Sample(s): 202009247613- 8
202009247615- 8-2
202009247616- 9
202009247618- 9-2

We are unable to provide a report for the above sample(s) due to the following reason:

- [ ] Insufficient cell growth
- [ ] Mixed culture
- [ ] Failed Bacteria PCR*
- [x] Failed Yeast/Fungi PCR*
- [ ] Insufficient sequence data
- [ ] Other (See comments.)

*Several attempts were made to obtain an acceptable PCR product. The following items were checked and found to be in compliance with our laboratory's standard operating procedures:

- [x] Reviewed Sample Submission Plate
- [x] Reviewed PCR Positive and Negative Controls
- [x] Reviewed Sample Preparation Process Documentation

PCR failure may result from the presence of PCR inhibitors, mixed cultures and/or the use of universal PCR primers which may not be optimal for all organisms.

Comments: N/A

If you have any questions or comments regarding this report, please feel free to contact MIDI Labs.

Reviewer's Signature: 10-05-20

Clifford Poindexter, Laboratory Manager

Alignment Report - Fungal (D2) Analysis

Customer: Zavala , Manuel
Company: University of Texas Rio Grande Valley
Address: 2431 Sentry Palm Drive, Rio Grande City, TX 78582 USA

125 SANDY DRIVE•NEWARK, DE 19713•PH 302-737-4297•FX 302-737-7781•WWW.MIDILABS.COM

FY28M2 Library Revision: 2.23

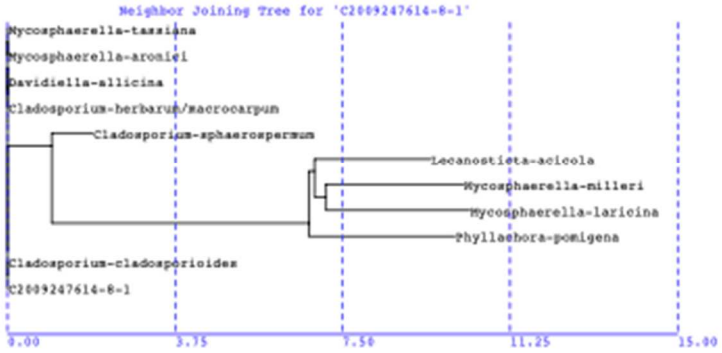
Created: 9/28/2020 11:13:18 AM
Sample ID: C2009247614-8-1

28S DNA: 316 base pairs

FY28M2 DNA Match Report

Match	%Diff	Length	Library Entry Name
1	0.00	316	Cladosporium-cladosporioides
2	0.00	316	Cladosporium-herbarum/macrocarpum
3	0.00	316	Davidiella-allicina
4	0.00	316	Mycosphaerella-aronici
5	0.00	316	Mycosphaerella-tassiana
6	1.90	316	Cladosporium-sphaerospermum
7	9.40	317	Lecanosticta-acicola
8	10.03	317	Mycosphaerella-milleri
9	10.09	315	Phyllachora-pomigena
10	10.34	317	Mycosphaerella-laricina

Exact match with top 3 entries including Cladosporium-cladosporioides



Closest Match: **Cladosporium-spp./ Davidiella-allicina/ Mycosphaerella-spp.**

GenBank Match: **100%, Naganishia albidosimilis MT267486***

**GenBank is a public database supported by The National Center for Biotechnology Information. GenBank is used for reference purposes only and is not a validated database.*

Reviewer's Signature 09-28-20

Shurnevia Strickland

Shurnevia Strickland, Data Analyst

RAW SEQUENCE DATA >C2009247614-8-1
AGACCGATAGCGAACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGGA
AAGAGAGTTAAAAAGCACGTGAAATTGTTAAAAGGGAAGGGATTGCAACC
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TTGCAGGCCAGCATCGTCTGGTGCCGCTGGATAAGACTTGAGGAATGTAG
CTCCCTCGGGAGTGTTATAGCCTCTTGTGATGCAGCGAGCGCCGGGCGAG
GTCCGCGCTTCGGCTAGGATGCTGGCGTAATGGTCGTAATCCGCCCGTCT
TGAAACACGGACCAAG

Alignment Report - Fungal (D2) Analysis

Customer: Zavala , Manuel
Company: University of Texas Rio Grande Valley
Address: 2431 Sentry Palm Drive, Rio Grande City, TX 78582 USA

125 SANDY DRIVE•NEWARK, DE 19713•PH 302-737-4297•FX 302-737-7781•WWW.MIDILABS.COM

FY28M2 Library Revision: 2.23

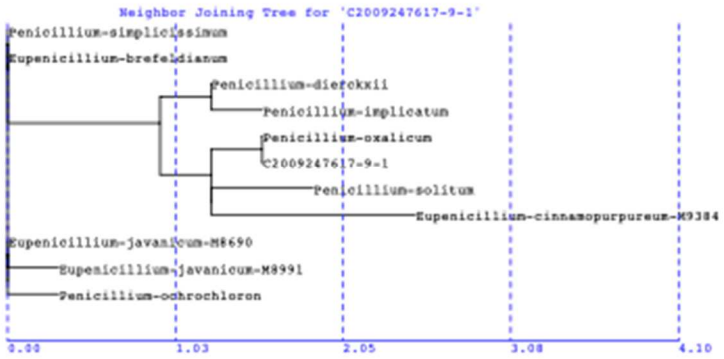
Created: 9/28/2020 11:13:19 AM
Sample ID: C2009247617-9-1

28S DNA: 321 base pairs

FY28M2 DNA Match Report

Match	%Diff	Length	Library Entry Name
1	0.00	321	Penicillium-oxalicum
2	0.93	321	Penicillium-dierckxii
3	0.93	321	Penicillium-solitum
4	1.25	321	Penicillium-implicatum
5	1.56	321	Eupenicillium-brefeldianum
6	1.56	321	Eupenicillium-cinnamopurpureum-M9384
7	1.56	321	Eupenicillium-javanicum-M8690
8	1.56	321	Penicillium-simplicissimum
9	1.87	321	Eupenicillium-javanicum-M8991
10	1.87	321	Penicillium-ochrochloron

Exact match with Penicillium-oxalicum



Closest Match: **Penicillium-oxalicum**

GenBank Match: **100%, Penicillium sp. MH443374***

**GenBank is a public database supported by The National Center for Biotechnology Information. GenBank is used for reference purposes only and is not a validated database.*

Reviewer's Signature 09-28-20

Shurnevia Strickland

Shurnevia Strickland, Data Analyst

RAW SEQUENCE DATA >C2009247617-9-1
AGACCGATAGCGAACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAA
AAGAGAGTTAAACAGCACGTGAAATTGTTGAAAGGGAAGCGCTTGCGACC
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GGGCGGGCCAGCGTCGGTTTGGGCGGCCGGTCAAAGGCCCTCGGAATGTA
ACGCCCCCCGGGGCGTCTTATAGCCGAGGGTGCCATGCGGCCAGCCCAGA
CCGAGGAACGCGCTTCGGCTCGGACGCTGGCATAATGGTCGTAAGCGACC
CGTCTTGAAACACGGACCAAG

Alignment Report - Fungal (D2) Analysis

Customer: Zavala , Manuel
Company: University of Texas Rio Grande Valley
Address: 2431 Sentry Palm Drive, Rio Grande City, TX 78582 USA

125 SANDY DRIVE•NEWARK, DE 19713•PH 302-737-4297•FX 302-737-7781•WWW.MIDILABS.COM

FY28M2 Library Revision: 2.23

Created: 9/28/2020 8:52:09 PM
Sample ID: C2009247619-10-1

28S DNA: 319 base pairs

FY28M2 DNA Match Report

Match	%Diff	Length	Library Entry Name
1	1.25	319	Pseudallescheria-ellipsoidea
2	1.57	319	Pseudallescheria-angusta
3	4.39	319	Melanospora-asymmetrica
4	4.70	319	Petriella-boulangeri
5	5.02	318	Petriella-setifera
6	5.31	317	Microascus-manginii-M9403
7	5.33	319	Parascedosporium-putredinis
8	5.92	317	Microascus-manginii-M8870
9	5.92	317	Microascus-chartarum-M8780
10	5.94	317	Microascus-manginii-M8749

Concise Alignment with Pseudallescheria-ellipsoidea

```
Sample:     (063) T (133) C (193) GC
            .....   .....   .....
LibEnt 1:   (063) C (133) T (193) AT
```

Closest Match: **Pseudallescheria-ellipsoidea**

GenBank Match: **100%, Pseudallescheria ellipsoidea KX639347***

**GenBank is a public database supported by The National Center for Biotechnology Information. GenBank is used for reference purposes only and is not a validated database.*

Reviewer's Signature 09-28-20

Shurnevia Strickland

Shurnevia Strickland, Data Analyst

RAW SEQUENCE DATA >C2009247619-10-1
AGACCGATAGCGAACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAA
AAGAGAGTTAAATAGCACGTGAAATTGTTGAAAGGGAAGCGCTTGCGACC
AGACTTGTGCCCGTCGAATCAGCCGCCGCTCGCCGGCGGCGCACTTCGGC
GGGCTCAGGCCAGCATCAGTTCGCTGCAGGGGGAGAAAGGCGGCGGGAAT
GTGGCTCTTCGGAGTGTTATAGCCCGCCGCGCAATACCCCTCGGCGGACT
GAGGACCGCGCATCTGCAAGGATGCTGGCGTAATGGTCGTCAGCGACCCG
TCTTGAAACACGGACCAAG

Alignment Report - Fungal (D2) Analysis

Customer: Zavala , Manuel
Company: University of Texas Rio Grande Valley
Address: 2431 Sentry Palm Drive, Rio Grande City, TX 78582 USA
125 SANDY DRIVE•NEWARK, DE 19713•PH 302-737-4297•FX 302-737-7781•WWW.MIDILABS.COM

FY28M2 Library Revision: 2.23

Created: 9/28/2020 11:13:20 AM
Sample ID: C2009247620-11-1

28S DNA: 316 base pairs

FY28M2 DNA Match Report

Match	%Diff	Length	Library Entry Name
1	0.63	316	Cladosporium-cladosporioides
2	0.63	316	Cladosporium-herbarum/macrocarpum
3	0.63	316	Davidiella-allicina
4	0.63	316	Mycosphaerella-aronici
5	0.63	316	Mycosphaerella-tassiana
6	1.90	316	Cladosporium-sphaerospermum
7	8.78	317	Lecanosticta-acicola
8	9.40	317	Mycosphaerella-milleri
9	9.46	315	Phyllachora-pomigena
10	9.72	317	Mycosphaerella-laricina

Concise Alignment (maximum difference 0.63):

```
                80|  285|
Sample:           G     T
Lib Match 1:      A     C
Lib Match 2:      A     C
Lib Match 3:      A     C
```

Closest Match: **Cladosporium-spp./ Davidiella-allicina/ Mycosphaerella-spp.**

GenBank Match: **100%, Naganishia albidosimilis MT267486***
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Reviewer's Signature 09-28-20

Shurnevia Strickland

Shurnevia Strickland, Data Analyst

RAW SEQUENCE DATA >C2009247620-11-1
AGACCGATAGCGAACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGGA
AAGAGAGTTAAAAAGCACGTGAAATTGTTGAAAGGGAAGGGATTGCAACC
AGACTTGCTCGCGGTGTTCCGCCGGTCTTCTGACCGGTCTACTCGCCGCG
TTGCAGGCCAGCATCGTCTGGTGCCGCTGGATAAGACTTGAGGAATGTAG
CTCCCTCGGGAGTGTTATAGCCTCTTGTGATGCAGCGAGCGCCGGGCGAG
GTCCGCGCTTCGGCTAGGATGCTGGCGTAATGGTTGTAATCCGCCCGTCT
TGAAACACGGACCAAG

Alignment Report - Fungal (D2) Analysis

Customer: Zavala , Manuel
Company: University of Texas Rio Grande Valley
Address: 2431 Sentry Palm Drive, Rio Grande City, TX 78582 USA

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FY28M2 Library Revision: 2.23

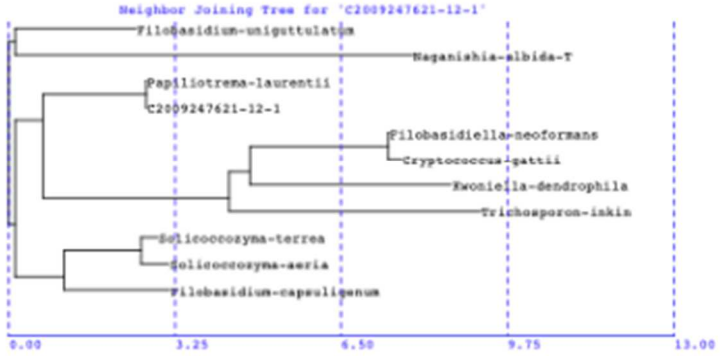
Created: 9/28/2020 11:13:20 AM
Sample ID: C2009247621-12-1

28S DNA: 347 base pairs

FY28M2 DNA Match Report

Match	%Diff	Length	Library Entry Name
1	0.00	347	Papiliotrema-laurentii
2	4.61	345	Filobasidium-uniguttulatum
3	5.73	347	Filobasidium-capsuligenum
4	5.73	347	Solicoccozyma-aeria
5	5.73	347	Solicoccozyma-terrea
6	9.46	347	Filobasidiella-neoformans
7	9.74	347	Cryptococcus-gattii
8	10.06	345	Trichosporon-inkin
9	10.34	347	Kwoniella-dendrophila
10	10.95	328	Naganishia-albida-T

Exact match with Papiliotrema-laurentii



Closest Match: **Papiliotrema-laurentii**

GenBank Match: **100%, Filobasidium magnum NG_069409***

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Shurnevia Strickland

Shurnevia Strickland, Data Analyst

RAW SEQUENCE DATA >C2009247621-12-1
AGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGCACTTTGGA
AAGAGAGTTAAACAGTATGTGAAATTGTTGAAAGGGAAACGATTGAAGTC
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GGGGTCAACATCAGTTTTGATCGCTGGATAAAGGCTGGAGGAACGTAGTA
CCCTCGGGTAAACTTATAGCCTCCTGTCACATACAGTGGTTGGGACTGAG
GAACGCAGCACGCCTTTATGGCCGGGATTCGTCCACGTACGTGCTTAGGA
TGTTGACATAATGGCTTTAAACGACCCGTCTTGAAACACGGACCAAG

Alignment Report - Fungal (D2) Analysis

Customer: Zavala , Manuel
Company: University of Texas Rio Grande Valley
Address: 2431 Sentry Palm Drive, Rio Grande City, TX 78582 USA

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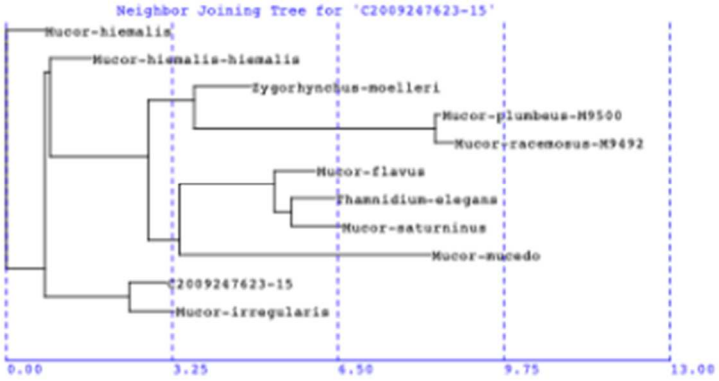
FY28M2 Library Revision: 2.23

Created: 9/28/2020 11:13:21 AM
Sample ID: C2009247623-15

28S DNA: 428 base pairs

FY28M2 DNA Match Report

Match	%Diff	Length	Library Entry Name
1	1.64	428	Mucor-irregularis
2	3.50	428	Mucor-hiemalis-hiemalis
3	3.86	428	Mucor-hiemalis
4	6.78	428	Zygorhynchus-moelleri
5	7.83	434	Mucor-flavus
6	8.05	435	Mucor-saturninus
7	8.29	434	Thamnidium-elegans
8	9.61	435	Mucor-mucedo
9	10.26	426	Mucor-plumbeus-M9500
10	10.72	426	Mucor-racemosus-M9492



Closest Match: **Mucor-irregularis**

GenBank Match: **99%, Mucor hiemalis MH872064***

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Shurnevia Strickland

Shurnevia Strickland, Data Analyst

RAW SEQUENCE DATA >C2009247623-15
AGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGAACTTTGAA
AAGAGAGTTAAACAGTATGTGAAATTGTTAAAAGGGAACCGTTTGGAGCC
AGACTGGCTTAATCGTAATCACTCTAGGCTTCGGCCTGGATGCACTTGCG
GTTTATGCCGGCCAACGACAGTTTTGTTTGAGGGAAAAAATTACATTGAA
TGTGGCCCCTCGGGGTGTTATAGCTTTGTAAAAAATACCTTGGATGGGAC
TGAGGAACGCAGTGAATGCCTTTAGGCGAGATTGCTGGGTGCTTGCGCTG
ATACATGCTAGAATTTCTGCTTCGGGTGGTGCTAGTGTGTAAAGGAGTAA
CCCGCTTAGTATATTTTTTATTCACTTAGGTTGTTGGCTTAATGACTCTA
AATGACCCGTCTTGAAACACGGACCAAG

Alignment Report - Fungal (D2) Analysis

Customer: Zavala , Manuel
Company: University of Texas Rio Grande Valley
Address: 2431 Sentry Palm Drive, Rio Grande City, TX 78582 USA

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FY28M2 Library Revision: 2.23

Created: 9/28/2020 11:13:21 AM
Sample ID: C2009247624-16-1

28S DNA: 320 base pairs

FY28M2 DNA Match Report

Match	%Diff	Length	Library Entry Name
1	0.31	320	Fusarium-chlamydosporum-M9766
2	0.31	320	Fusarium-chlamydosporum-M9774
3	0.31	320	Fusarium-incarnatum
4	1.25	320	Fusarium-nygamai-M10106
5	1.25	320	Fusarium-poae/robustum
6	1.25	320	Fusarium-proliferatum/verticillioides
7	1.56	320	Fusarium-chlamydosporum-M8851
8	1.56	320	Fusarium-subglutinans-M10143
9	1.88	320	Fusarium-anthophilum/napiforme
10	1.88	320	Fusarium-graminearum

Concise Alignment (maximum difference 0.63):

```
                 13|  205|
Sample:           A    C
Lib Match 1:      C    C
Lib Match 2:      A    T
Lib Match 3:      A    T
```

Closest Match: **Fusarium-spp.**

GenBank Match: **99%, Fusarium chlamydosporum MH870726***

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Shurnevia Strickland

Shurnevia Strickland, Data Analyst

RAW SEQUENCE DATA >C2009247624-16-1
AGACCGATAGCGAACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAA
AAGAGAGTTAAAAAGTACGTGAAATTGTTGAAAGGGAAGCGTTTATGACC
AGACTTGGGCTTGGTTAATCATCTGGGGTTCTCCCCAGTGCACTTTTCCA
GTCCAGGCCAGCATCAGTTTTCGCCGGGGGATAAAGGCTTCGGGAATGTG
GCTCCCTCCGGGGAGTGTTATAGCCCGTTGCGTAATACCCTGGCGGGGAC
TGAGGTTCGCGCATCTGCAAGGATGCTGGCGTAATGGTCATCAACGACCC
GTCTTGAAACACGGACCAAG

Alignment Report - Fungal (D2) Analysis

Customer: Zavala , Manuel
Company: University of Texas Rio Grande Valley
Address: 2431 Sentry Palm Drive, Rio Grande City, TX 78582 USA

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FY28M2 Library Revision: 2.23

Created: 9/28/2020 11:13:22 AM
Sample ID: C2009247625-16-2

28S DNA: 322 base pairs

FY28M2 DNA Match Report

Match	%Diff	Length	Library Entry Name
1	0.00	322	Alternaria-alternata
2	0.00	322	Cochliobolus-carbonum
3	0.00	322	Stemphylium-vesicarium-M9954
4	0.31	322	Alternaria-gaisen/geophila
5	0.62	322	Alternaria-longipes
6	1.24	322	Ulocladium-atrum/botrytis
7	1.24	322	Ulocladium-chartarum/consortiale
8	1.55	323	Alternaria-cichorii/cucumerina/dauci
9	1.55	322	Embellisia-chlamydospora
10	1.85	324	Alternaria-porri/solani

Exact match with top 3 entries including Alternaria-alternata

Closest Match: **Alternaria-alternata/ Cochliobolus-carbonum/ Stemphylium-vesicarium**

GenBank Match: **100%, Alternaria sp. MT154534***

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Shurnevia Strickland

Shurnevia Strickland, Data Analyst

RAW SEQUENCE DATA >C2009247625-16-2
AGACCGATAGCGAACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGGA
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TAGGCAGGCCAGCATCAGTTTGGGCGGTAGGATAAAGGTCTCTGTCACGT
ACCTCCTTTCGGGGAGGCCTTATAGGGGAGACGACATACTACCAGCCTGG
ACTGAGGTCCGCGCATCTGCTAGGATGCTGGCGTAATGGCTGTAAGCGGC
CCGTCTTGAAACACGGACCAAG

Alignment Report - Fungal (D2) Analysis

Customer: Zavala , Manuel
Company: University of Texas Rio Grande Valley
Address: 2431 Sentry Palm Drive, Rio Grande City, TX 78582 USA

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FY28M2 Library Revision: 2.23

Created: 9/28/2020 11:13:22 AM
Sample ID: C2009247628-18

28S DNA: 320 base pairs

FY28M2 DNA Match Report

Match	%Diff	Length	Library Entry Name
1	0.00	320	Fusarium-chlamydosporum-M9774
2	0.00	320	Fusarium-incarnatum
3	0.63	320	Fusarium-chlamydosporum-M9766
4	0.94	320	Fusarium-nygamai-M10106
5	0.94	320	Fusarium-proliferatum/verticillioides
6	1.25	320	Fusarium-subglutinans-M10143
7	1.56	320	Fusarium-anthophilum/napiforme
8	1.56	320	Fusarium-nygamai-M9528
9	1.56	320	Fusarium-poae/robustum
10	1.56	320	Fusarium-subglutinans-M10119

Concise Alignment (maximum difference 0.63):

```
                 13|  205|
Sample:            A     T
Lib Match 1:       A     T
Lib Match 2:       A     T
Lib Match 3:       C     C
```

Closest Match: **Fusarium-chlamydosporum/ Fusarium-incarnatum**

GenBank Match: **100%, Fusarium sp. AY234909***

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Reviewer's Signature 09-28-20

Shurnevia Strickland

Shurnevia Strickland, Data Analyst

RAW SEQUENCE DATA >C2009247628-18
AGACCGATAGCGAACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAA
AAGAGAGTTAAAAAGTACGTGAAATTGTTGAAAGGGAAGCGTTTATGACC
AGACTTGGGCTTGGTTAATCATCTGGGGTTCTCCCCAGTGCACTTTTCCA
GTCCAGGCCAGCATCAGTTTTCGCCGGGGGATAAAGGCTTCGGGAATGTG
GCTCTCTCCGGGGAGTGTTATAGCCCGTTGCGTAATACCCTGGCGGGGAC
TGAGGTTCGCGCATCTGCAAGGATGCTGGCGTAATGGTCATCAACGACCC
GTCTTGAAACACGGACCAAG

Alignment Report - Fungal (D2) Analysis

Customer: Zavala , Manuel
Company: University of Texas Rio Grande Valley
Address: 2431 Sentry Palm Drive, Rio Grande City, TX 78582 USA

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FY28M2 Library Revision: 2.23

Created: 9/30/2020 11:59:06 AM
Sample ID: C2009247629-18-1

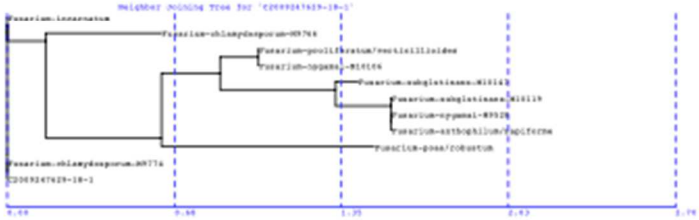
28S DNA: 320 base pairs

FY28M2 DNA Match Report

Match	%Diff	Length	Library Entry Name
1	0.00	320	Fusarium-chlamydosporum-M9774
2	0.00	320	Fusarium-incarnatum
3	0.63	320	Fusarium-chlamydosporum-M9766
4	0.94	320	Fusarium-nygamai-M10106
5	0.94	320	Fusarium-proliferatum/verticillioides
6	1.25	320	Fusarium-subglutinans-M10143
7	1.56	320	Fusarium-anthophilum/napiforme
8	1.56	320	Fusarium-nygamai-M9528
9	1.56	320	Fusarium-poae/robustum
10	1.56	320	Fusarium-subglutinans-M10119

Concise Alignment (maximum difference 0.63):

```
                 13|  205|
Sample:            A     T
Lib Match 1:       A     T
Lib Match 2:       A     T
Lib Match 3:       C     C
                   *     *
```



Closest Match: **Fusarium-chlamydosporum/ Fusarium-incarnatum**

GenBank Match: **100%, Fusarium sp. AY234909***

**GenBank is a public database supported by The National Center for Biotechnology Information. GenBank is used for reference purposes only and is not a validated database.*

Reviewer's Signature 09-30-20

Shurnevia Strickland

Shurnevia Strickland, Data Analyst

RAW SEQUENCE DATA >C2009247629-18-1
AGACCGATAGCGAACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAA
AAGAGAGTTAAAAAGTACGTGAAATTGTTGAAAGGGAAGCGTTTATGACC
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GCTCTCTCCGGGGAGTGTTATAGCCCGTTGCGTAATACCCTGGCGGGGAC
TGAGGTTCGCGCATCTGCAAGGATGCTGGCGTAATGGTCATCAACGACCC
GTCTTGAAACACGGACCAAG

BIOGRAPHICAL SKETCH

Manuel Zavala (manuelzavala365@gmail.com) is the researcher behind this study. Prior to earning his Master of Science in Biology from The University of Texas Rio Grande Valley in December 2020, he earned his Bachelor of Science in Biology from the very same university. In the summers of 2016 and 2017 during his undergraduate career he completed an internship with the US Army Corps of Engineers in North Dakota monitoring two threatened and endangered bird species known as the Piping Plover and Interior Least Tern. This only strengthened his passion for working with all kinds of wildlife and specifically endangered species. Once returning from his second round of the internship in North Dakota he returned to complete his bachelors and obtained a part time job working at the McAllen Nature Center in McAllen, Texas. There he worked with like-minded people who had a deep love for the environment and everything it had to offer. He learned valuable skills in being able to identify various bird and flora species and honed his public speaking skills with the visitors. Once he was accepted into the Biology Graduate program at UTRGV he had recently read articles discussing Snake Fungal Disease and the negative impact it had on snake populations which was his favorite group of animals and decided to focus his research on that topic in the Lower Rio Grande Valley. He also had the privilege of working as a Graduate Teaching Assistant where he picked up valuable skills in preparing chemicals, maintaining laboratory equipment, sanitization and teaching undergraduate students how to perform the weekly experiments.