

**PATTERNS OF PARASITE INFECTION DYNAMICS DURING A
BIOLOGICAL INVASION**

by

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Abstract

Biological invasions may impact native ecosystems by affecting host-parasite dynamics. Introduced species can harbor parasites transported from their native range and these parasites can spillover to infect native taxa, often with deleterious effects. When parasite-spillover is suspected, it is vital to identify the origin of the parasite accurately, especially if the native taxa are adversely affected. I examined an introduced population of Burmese pythons (*Python bivittatus*) in the Florida Everglades and investigated their potential for altering host-parasite relationships within their invasive range. Native snakes and pythons were collected during road surveys and through opportunistic encounters. All native snakes were salvaged as road-kill to reduce my impact on native populations. Snakes were dissected and lung parasites (*i.e.* pentastomes) were collected. Pentastomes were identified to species using molecular analyses and three pentastome species (*Porocephalus crotali*, *Kiricephalus coarctatus* and *Raillietiella orientalis*) were recovered. Two genera (*Porocephalus* and *Kiricephalus*) are known to infect North American snakes, while *R. orientalis* is native to southeast Asia where it is known to infect Burmese pythons. *Raillietiella orientalis* was collected from pythons and snakes in southern Florida, indicating this parasite has spilled over from pythons to infect native snakes. I examined the impact of *R. orientalis* infection on native snakes through quantifying and comparing parasite infection dynamics within pythons and native snakes. *Raillietiella orientalis* exhibited increased fitness in snake hosts native to the parasite's

introduced range compared with pythons. Native snakes displayed increased prevalence and infection intensity of *R. orientalis*. Additionally, female *R. orientalis* obtained larger sizes in native Florida snakes and adult female parasites consisted of a larger proportion of the total number of *R. orientalis* within a host. The current distribution of *R. orientalis* in Florida extends northward from Miami-Dade and Monroe counties to southern Lake county. The distribution of *R. orientalis* extends beyond the known range of pythons indicating this parasite can spread among native snakes independent of pythons.

Raillietiella orientalis has infected 13 species of native Florida snakes encompassing two families. I investigated the ability of *R. orientalis* to infect a diversity of hosts by examining potential variation of morphological structures associated with feeding that may allow this parasite to successfully infect a wide array of host species. Oral morphology of *R. orientalis* varied significantly depending upon the host species infected, which may allow for *R. orientalis* to adapt to the specific internal conditions of its host. To investigate whether anthropogenic influences, such as landscape disturbance, impact infection dynamics of *R. orientalis* among native snake and pythons, I compared parasite prevalence and intensity in snakes and examined variation of these metrics among disturbed and undisturbed landscapes. Parasite prevalence was significantly higher in undisturbed landscapes for native snakes compared with disturbed habitats, however, prevalence of *R. orientalis* of pythons did not differ across landscapes. Infection intensity of *R. orientalis* did not differ among native snakes and pythons in response to landscape, which indicates that once infected, the ability of the host's

immune system to ameliorate infection is similar in undisturbed and disturbed environments. This study provides the first documentation of a non-native endoparasite that has spilled over from an invasive snake to infect native taxa. As parasites can regulate host populations, and non-native parasites are significantly more virulent in naïve hosts within their invaded range compared to their non-indigenous host, it is likely that native snakes face deleterious impacts at both the level of the individual and population.

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To Mom and Dad (1946 - 2014) for everything.

*"Yours is the light by which my spirit's born. You are my sun,
my moon, and all my stars."*

E.E. Cummings

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Chapter I

Introduction

Biological invasions present unique opportunities to observe natural experiments that aid our understanding of species interactions, as well as evolutionary processes (Mooney and Cleland 2001). As the world is increasingly altered through anthropogenic mechanisms, the introduction of non-native species continues to rise (Kowarik and von de Lippe 2008). Non-native taxa that become established can have direct impacts on native taxa, including increased competition and predation (Human *et al.* 1996; Dorcas *et al.* 2012; McCleery 2015), and indirect effects, including pathogens and parasites introduced along with their non-indigenous host. While direct impacts of biological invasions have been the primary focus of studies examining the effect of introduced species on native taxa, studies of indirect effects, particularly the impact of non-native parasites on novel native hosts, are equally important to furthering our understanding of how organisms are affected during an invasion.

When a parasite is found within a non-indigenous host, it is crucial to identify the origin of the parasite. Careful identification of the parasites of non-indigenous hosts allow us to understand the mechanisms through which the parasite may impact its host. If the parasite is non-native, it may spread from its non-indigenous host to infect native taxa through the process of parasite spillover (Daszak 2000). Alternatively, if the parasite cannot infect taxa within its introduced range, there may be opportunities to use the parasite as a form of biocontrol to manage its non-indigenous host (Duan *et al.* 2017). If parasites found within a non-indigenous host are identified as native to the hosts

introduced range, parasite spillback may occur, a mechanism in which the non-native host acts as a reservoir of infection and functions to increase prevalence of the native parasite among native hosts (Kelly *et al.* 2009; Mastitsky and Veres 2010).

While biological invasions provide an inimitable opportunity to explore parasite infection dynamics, they also provide insight into how a non-native species adapts to a novel environment. In the case of non-native parasites, this adaptation takes place within a novel host, creating a host-parasite relationship that shares no co-evolutionary history (Mastitsky *et al.* 2010). Therefore, invasions create novel challenges to both parasite and host. The parasite must overcome the obstacles of becoming established within its introduced range (MacLeod *et al.* 2010) and, for species with an indirect life cycle, face new challenges at all stages of development. A parasite must adjust to withstand the physiological processes of novel intermediate and definitive hosts and the parasite's eggs must be able to withstand novel environmental conditions. Despite these obstacles, many non-native parasites exhibit increased fitness in native hosts (Knopf 2004) compared with the non-indigenous host responsible for the parasites introduction. While, a non-native parasite face challenges, a novel host is burdened with eliminating or alleviating infection by a non-native parasite, often at the cost of eliciting a strong immune response to counter infection. Studies focused on the mechanism through which a non-native parasite rapidly adapts to novel host environments can elucidate evolutionary processes and reveal phenotypic plasticity that allows a parasite to adapt in ways that increase their success at infecting multiple novel hosts.

While a parasite and host exert influence on each other's physiology and morphology, abiotic environmental factors may also influence infection dynamics

(McKinney 2002). Landscape disturbance has been shown to affect host-parasite relationships and infection dynamics (McKinney 2002). As disturbance increases parasite communities often experience a decrease in species richness, with parasite diversity becoming homogenized (Blair 2001; McKinney 2002). This often results in higher parasite abundances and infection intensities of more generalized parasites capable of successfully completing their life cycle in anthropogenically-altered landscapes (McKenzie 2007). These patterns have been witnessed for native parasites with their co-evolved hosts; however, the effect of landscape disturbance on the prevalence and intensity of non-native parasites is less understood. The effect of disturbance on non-native parasite infection dynamics may be exacerbated when non-native parasites infect naïve hosts. Many non-indigenous species, and therefore their parasites, are restricted to disturbed areas (Coates *et al.* 2017) where food and water may be more plentiful, predation may be reduced, and environmental conditions may be more stable (Bradley and Altizer 2006) than undisturbed environments. When this restriction occurs, taxa less tolerant of altered landscapes (*i.e.* urban avoiders, Blair 2001) find refuge from infection by non-native parasites through reduced contact with invading parasites. However, if non-indigenous species establish in undisturbed landscapes, as seen with the Burmese python (*Python bivittatus*) invasion in the everglades of southern Florida (Snow *et al.* 2007), urban-avoider taxa are provided no reprieve from infection by non-native parasites.

The first Burmese python was documented in southern Florida in 1979, and pythons were reported as established in Everglades National Park in the mid-1990s (Meshaka 2000). Pythons were introduced to southern Florida through the pet trade

(Reed 2005; Reed and Rodda 2009) through which tens of thousands of pythons were imported to the U.S. Pythons are known to be infected by a pentastome parasite (*Raillietiella orientalis*) within their native range in southeast Asia (Christoffersen and De Assis 2013). Invasive pythons in Florida were found to be infected with a pentastome of the genus *Raillietiella*. However, identifying the origin of this parasite was complicated due to a morphologically similar species of *Raillietiella* (*R. bicaudata*) reported to be present in snakes in the southeastern U.S. The everglades are home to a diverse assemblage of native snakes, the majority of which encompass the same families known to host *R. orientalis* within the parasites native range. Therefore, if *Raillietiella* observed in snakes was identified as Asian in origin parasite spillover may be occurring. Alternatively, if *Raillietiella* infecting pythons were native to the U.S., parasite spillback may be occurring. This study aims to identify the origin of *Raillietiella* pentastomes in pythons, its role in parasite spillover and parasite spillback, its plasticity within its hosts, and the impact of landscape patterns on infection dynamics during a biological invasion. invasion biology. To examine factors affecting parasite infection dynamics occurring during the python invasion. First, I identify the origin of *Raillietiella* parasites through analysis of a molecular dataset of *Raillietiella* collected from native snakes compared to *Raillietiella orientalis* collected from its native Asian range. Second, I explored the potential of *R. orientalis* to impact native snakes through parasite spillover by quantifying and comparing infection dynamics of *R. orientalis* between native snakes and pythons. Third, I examine morphological plasticity that may aid the ability of *R. orientalis* to infect diverse host taxa through examining variation in the morphology of structures associated with feeding. Lastly, I investigate the effect of landscape disturbance on the

prevalence and intensity of *R. orientalis* in native snakes and pythons by comparing these metrics among snakes collected in disturbed and undisturbed landscapes.

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Chapter 2

Parasite spillover or spillback? Identifying the origin of pentastome parasites of invasive Burmese pythons in the Florida Everglades

Abstract

Identification of the origin of parasites of non-indigenous species (NIS) can be complex. However, NIS may co-introduce parasites from their native range and acquire parasites from within their invaded range. Determination of whether parasites are non-native or native can be complicated when parasite genera occur within both the NIS' native range and its introduced range. We explored potential for spillover and spillback of lung parasites infecting Burmese pythons (*Python bivittatus*) in their invasive range (Florida). We collected 498 indigenous snakes of 26 species and 805 Burmese pythons during 2004 - 2016 and examined them for lung parasites. We used morphology to identify three genera of pentastome parasites, *Raillietiella*, a cosmopolitan form, and *Porocephalus* and *Kiricephalus*, both New World forms. We sequenced these parasites at one mitochondrial and one nuclear locus and showed that each genus is represented by a single species, *R. orientalis*, *P. crotali*, and *K. coarctatus*. Pythons are host to *R. orientalis* and *P. crotali*, but not *K. coarctatus*; native snakes are host to all three species. Sequence data show that pythons introduced *R. orientalis* to North America, where this parasite now infects native snakes. Additionally, our data suggest that pythons are competent hosts to *P. crotali*, a widespread parasite native to North and South America that was previously hypothesized to infect only viperid snakes. Our results indicate invasive Burmese pythons have

affected parasite-host dynamics of native snakes in ways that are consistent with spillover and spillback, and demonstrate potential for indirect effects during invasions.

Introduction

Direct effects of biological invasions are well documented (*e.g.*, biodiversity loss, predation, and competition; Dorcas *et al.* 2012, Moore *et al.* 2008, Mooney and Cleland 2001, Wilcove *et al.* 1998; Human and Gordon 1996), but indirect effects, including alteration of host-parasite dynamics, may also profoundly affect an invaded ecosystem (Dunn *et al.* 2012; Tompkins and Poulin 2006). Non-indigenous species (NIS) often contain half the parasite species richness of conspecifics in their native range (Torchin *et al.* 2003; MacLeod *et al.* 2010), but still, native hosts are at risk of infection by these non-native parasites (*i.e.*, spillover; Daszak *et al.* 2000; Tompkins and Poulin 2006). Adverse effects of NIS-facilitated spillover may be exacerbated as native hosts do not share a co-evolutionary history with an introduced parasite and may lack adaptations to effectively counter novel pathogens (Dogel *et al.* 1958; Anderson and May 1986; Holdich and Reeve 1991; Kohler and Wiley 1997; Daszak *et al.* 2000).

Non-indigenous species may also acquire parasites from their introduced range (Dobson and May 1986; Cornell and Hawkins 1993; Poulin and Mouillot 2003; Kelly *et al.* 2009a), and often the majority of parasites infecting NIS are native to their invaded range (Torchin and Mitchell 2004). If NIS are competent hosts (*i.e.* parasites are capable of establishment, survival, and reproduction within the host) of native parasites, then NIS may serve as reservoirs of indigenous parasites and increase infection in sympatric native hosts through parasite spillback (Daszak *et al.* 2000; Tompkins and Poulin 2006; Kelly *et al.* 2009a). Aside from direct effects on host mortality, increased transmission can

adversely affect competitive interactions and increase vulnerability to predation, resulting in host population declines and altered community structure (Settle and Wilson 1990; Tompkins *et al.* 2000; Tompkins *et al.* 2001). Conversely, if NIS can be infected by parasites from an invaded range, yet the parasites are not able to reproduce within the NIS host, the invading species may act as a sink for native parasites, and subsequently dilute transmission among sympatric native hosts (Telfer *et al.* 2005; Kopp and Jokela 2007; Kelly *et al.* 2009b).

Accurate identification of origin of parasites infecting NIS is vital to understanding mechanisms through which changes to host-parasite relationships occur during biological invasions. Parasite species identification can be challenging if morphological similarities arise from parallel evolution, convergent evolution, or paedomorphism (*e.g.* Kelehear *et al.* 2011; Falk *et al.* 2011). Ambiguity of distinguishing characteristics can lead to misidentification and can hinder understanding of how host-parasite relationships are altered during invasions. For example, Barton (1998) used morphological characteristics to identify nematodes from lungs of an invasive Australian population of cane toads (*Rhinella marina*) as *Rhabdias* cf. *hylae*, a species native to Australia. Dubey and Shine (2008) reevaluated Barton's diagnosis using molecular techniques and found the nematodes were, in fact, *Rhabdias pseudosphaerocephala*, a species of South American origin that was likely introduced with cane toads. Dubey and Shine (2008) examined native frogs for the presence of the *R. pseudosphaerocephala* and found the introduced parasite was not able to infect native anurans, which presented the opportunity to consider use of *R. pseudosphaerocephala* as a biocontrol tool to manage cane toad populations.

Burmese pythons (*Python bivittatus*) are a large constricting snake species native to Southeast Asia that were introduced to southern Florida through the pet trade; with most pythons imported to the US from Vietnam and Thailand (Franke and Telecky 2001; Reed and Rodda 2009). These pythons became established in southern Florida prior to the early 2000s and are now widely distributed (Snow *et al.* 2007; Meshaka *et al.* 2000; Willson *et al.* 2011). Corn *et al.* (2011) examined ectoparasites of non-native reptiles in southern Florida and documented new host-parasite relationships among native and introduced ectoparasites for NIS reptiles, including pythons. Burmese pythons were infested with Neotropical ticks (*Amblyomma rotundatum* and *Amblyomma dissimile*) and two species of chiggers (*Eutrombicula splendens* and *Eutrombicula cinnabaris*) native to the US. Their results suggest a high degree of host switching among non-native and native ectoparasites and NIS reptiles. However, altered host-parasite relationships among other kinds of parasites infecting the invasive python population have not been examined.

Here, we identified pentastome parasites associated with invasion of Burmese pythons in southern Florida as a preliminary step in understanding effects of this invader on the native snake fauna. Despite infecting numerous taxa and being of zoonotic concern, pentastomes remain understudied (Christoffersen and De Assis 2015). Pentastomes are parasitic arthropods found in the respiratory tract of reptiles, toads, mammals, and birds. However, 90% of definitive hosts are reptiles, with squamates comprising the majority of reptilian hosts (Christoffersen and De Assis 2013). Infective pentastome larvae (nymphs) reside in intermediate hosts (mammals, reptiles, amphibians, fish, and insects), which are consumed by the definitive host. Once ingested, larvae migrate from the definitive host's stomach to its lungs where the larvae feed on a host's

blood (Riley 1986; Paré 2008). Pentastome infection, known as pentastomiasis, can induce pathogenic reactions including sepsis and pneumonia that result from decomposed exuvia as a pentastome develops through a series of molts. Scarring, lesions, and hemorrhaging can occur as a parasite migrates through the body and attaches hooks associated with feeding (Riley 1986). Reduced pulmonary function due to blockage of respiratory passages can occur when many pentastomes infect a host (Riley 1986). Larval pentastomes can cause adverse reactions within intermediate hosts, with severe visceral infections documented in several mammalian hosts, including humans (Self 1972; Brookins *et al.* 2009; Tappe and Büttner 2009). North American pentastomes that infect snake definitive hosts include *Kiricephalus coarctatus*, *Porocephalus crotali*, and *Raillietiella bicaudata*. Colubrid snakes are hosts to *K. coarctatus* and *R. bicaudata* while *P. crotali* is only known to infect pit vipers. Each of these North American pentastome species has potential to infect invasive Burmese pythons and spillback into native snake populations. Burmese pythons are known hosts to several species of pentastomes in their native range, including *Raillietiella orientalis*, *Armillifer agkistrodontis*, *A. yoshidai*, *A. armillatus*, and *A. moniliformis*, with the latter two being species of zoonotic concern (Latif *et al.* 2011; Christoffersen and De Assis 2013). Burmese pythons may have brought these Asian pentastome species to North America, where spillover into native snake species may occur. Of particular interest is a possibility that two species of *Raillietiella* – *R. bicaudata* and *R. orientalis* – currently occur in southern Florida snakes, where the former evolved in the Americas and the latter was transported by Burmese pythons from Asia. Thus, examples of spillover and spillback might be present within this single parasite genus.

Because an absence of morphological autapomorphies may impede identification of these pentastome taxa via visual inspection, we employed molecular methods to distinguish among possible species present in our samples. For example, we expected to recover two clades within each genus if two species are present. We use both GenBank and novel sequence data to identify the geographic origin of the samples (*i.e.*, North America or Old World). The presence of Asian pentastomes in native snakes would be a necessary condition for demonstrating spillover, and the presence of North American pentastomes in pythons would be a necessary condition for demonstrating the potential for parasite spillback. Additionally, evidence of a genetic bottleneck and subsequent range expansion was expected within the genome of parasites of Old World origin that co-invaded with the pythons, due to the population originating from a few founding individuals. Such genetic bottlenecks were not expected in pentastomes native to North America.

Methods

Snake and pentastome collection

Burmese pythons (*Python bivittatus*; n = 805) were collected throughout their introduced range in southern Florida, including Everglades National Park (ENP) and private lands in Collier, Miami-Dade, and Monroe Counties. These snakes were collected alive or salvaged after being hit by automobiles. Pythons captured alive were euthanized per Everglades National Park Python Removal Program protocol. We also collected 498 native snakes of 26 species from regions of allopatry and sympatry with pythons. The allopatric region included Alabama, Georgia, Florida north Hardee, Highlands, Manatee, Okeechobee and St. Lucie counties. The sympatric region included

all remaining counties within Florida. Native snakes from the allopatric region were examined to obtain specimens of *R. bicaudata* from locations that were not confounded by the potential presence of *R. orientalis*. We only collected native snakes that had been previously killed by automobiles to reduce our impact on native snake populations. These snakes were collected during 2012 – 2016 during nocturnal road surveys conducted on consecutive nights to ensure snakes had been killed within the last 24 hours.

Snakes were dissected immediately after euthanasia or frozen for later dissection. During dissection, the lungs, oral cavity, trachea, and body cavity were examined for the presence of pentastomes. Areas outside of the lungs were examined because pentastomes are known to change location within the body, and at times vacate the host entirely when death of the host occurs (Montgomery *et al.* 2006; Paré 2008). Collected pentastomes were stored in 95% ethanol until morphological and molecular analyses were performed. All pentastomes and snakes collected within National Park Service lands were deposited in collections administered by the NPS. All pentastome and snake collections collected outside of NPS lands were deposited at the Auburn University Museum of Natural History.

Molecular methods

We followed the Chelex extraction protocol prescribed by Kelehear *et al.* (2011) to extract whole genomic DNA from pentastome tissue. Two genes were amplified by polymerase chain reaction (PCR): the mitochondrial gene COI and the nuclear ribosomal RNA gene 18S. Amplification procedures for COI followed Kelehear (2011) using primers LCOI490/HCO2189 (Folmer *et al.* 1994). The primers and protocols for 18S amplification followed Brookins *et al.* (2009). Primers used for PCR amplification were

also used for DNA sequencing, which was performed at Beckman Coulter (Danvers, MA). PCR amplification of both COI and 18S were performed on all samples; however, only one gene amplified for some samples. Reactions were performed a second time for failed runs, yet amplification was still not successful for some samples, which were eliminated from the study. Chromatographs from forward and reverse reads were assembled, and contiguous sequences were aligned and edited by eye using the program Geneious version 6.0.6 (<http://www.geneious.com>, Kearse *et al.* 2012).

Pentastome phylogeny

Published sequences for COI and 18S available on GenBank were used in combination with sequence data generated from this study to construct a pentastome phylogeny. Published sequences for *Raillietiella* pentastomes included 11 COI sequences from an introduced population of *R. orientalis* collected from Australian snakes (Kelehear *et al.* 2014), one COI sequence of *R. hebitihamata* (GenBank accession JF975594), one 18S sequence of *R. orientalis* collected in Asia (GenBank accession KC904945), and two 18S sequences labeled as *Raillietiella sp.* (GenBank accession EU370434; AY744887) of Old World origin (Asian and African). Sequence data for *R. bicaudata* were not available from GenBank or from museum collections, and because *R. bicaudata* is reported from snake species that inhabit Florida and Alabama, where we collected our samples, we assumed that any monophyletic lineage that excluded the published *R. orientalis* sequence and exhibited morphology consistent with *R. bicaudata* was that species.

Phylogenetic analyses were performed separately on COI and 18S datasets and then on a concatenated dataset. The concatenated dataset included specimens for which

both markers amplified as well as specimens for which only one marker amplified. Sequences for the pentastome *Linguatula arctica* (Genbank accession KF029445.1) were used as an outgroup for the 18S Bayesian and concatenated phylogenies, and *L. serrate* (GenBank accession KU2400601.1) was used as the outgroup for the COI Bayesian phylogeny. Best-fit models of evolution for each gene and for the concatenated dataset were selected using Akaike information criteria values in PartitionFinder (Lanfear et al. 2012). We inferred a Bayesian phylogeny using MrBayes 3.2.2 on CIPRES Science Gateway (Miller *et al.* 2010; Ronquist *et al.* 2012). Each analysis had two runs with four chains each set at default temperatures. These were allowed to run for 10 million generations and were sampled every 1000 generations. A 25% burnin was calculated using the sump option and a 50% majority rule consensus tree was created using the sumt option in MrBayes. Definitive identity and origin of collected pentastomes were determined based upon their position on the tree relative to the position of reference pentastome species.

Haplotype network and nucleotide diversity

Haplotype networks were generated for COI and 18S datasets using PopART (<http://popart.otago.ac.nz>; Leigh and Bryant, 2015), a program implementing TCS Networks (Clement *et al.* 2000). The TCS Networks were generated using statistical parsimony to estimate the genealogy of the haplotypes present in the dataset using default parameters.

Nucleotide diversity, Tajima's D, and Fu Li's D* & F* statistics were calculated using DnaSP 5.10.1 software (Librado and Rozas 2009). These statistics were calculated to test for founder effects and subsequent expansion that would be expected if an

introduced parasite species is present. For each test, a score that differed significantly (either positively or negatively) from zero supported rejection of the null hypothesis that a population is stable and evolving neutrally (Kimura and Ohta 1969; Tajima 1989). A significant negative score was indicative of a population that originated from a few individuals and then expanded. A positive score indicated a population that was in a state of contraction or had experienced balancing selection.

Nucleotide diversity (π), the mean number of nucleotide differences among sites within a locus (Nei and Li 1979), was examined and compared within pentastome genera based upon location to determine if the average pairwise differences within sequences may reflect the low genetic variation expected of an introduced parasite species. Low nucleotide diversity would be expected of a recently introduced population founded by a small number of individuals.

Results

Pentastome species identification

Bayesian analyses of 18S (Figure 1), COI (Figure 2), and concatenation of both genes (Figure 3), resulted in similar topologies. All recovered distinct genetic lineages associated with three genera, *Raillietiella*, *Porocephalus*, and *Kiricephalus*. *Raillietiella* samples collected from Florida snakes formed a single clade that included the reference specimen from GenBank for *R. orientalis* (18S) and a series of specimens identified as *R. orientalis* from Australian snakes (COI, Kelehear *et al.* 2014). No Florida specimen of *R. orientalis* was collected from outside the area of sympatry with pythons and phylogenetic reconstructions showed no (18S and concatenated) or limited (COI) structure within the lineage, features consistent with identification of Florida pentastomes as *R. orientalis*.

This cluster also included two unidentified *Raillietiella* specimens from GenBank (GenBank accession: EU370434; AY744887) and a larval specimen collected from an African shrew (*Crocidura sp.*) and sent to one of us (JMK) to be identified. In the COI and concatenated trees, we included a specimen of *R. hebitihamata* (synonym for *R. frenata*) collected in Australia (GenBank accession: JF975594), the only other member of the genus available from GenBank. This specimen failed to cluster within our Florida specimens, suggesting that other species of *Raillietiella*, if present, would have been revealed on our trees.

Our phylogenetic trees also supported the hypothesis of a single species within each of the other two genera. We identified these genera on morphological grounds as *K. coarctatus* and *P. crotali*, species known from North American snakes. All three phylogenetic analyses recovered these as sister taxa relative to *R. orientalis* (Figures 1-3). *Porocephalus crotali* was sampled frequently from pythons as well as from native snakes outside the range of pythons. *Kiricephalus coarctatus* specimens from native snakes were recovered from within and outside the range of pythons, but were never collected from Burmese pythons.

Haplotype network and nucleotide diversity

An analysis of the 18S gene revealed that a haplotype of *R. orientalis* obtained in Southeast Asian and African source populations is present in pythons and native snakes from Florida (Fig 4). We documented three additional rare haplotypes, two from Florida and one of Old World origin. We sequenced only a single 18S haplotype from *P. crotali* collected from pythons and native snakes and three haplotypes from *K. coarctatus* collected from native snakes.

Haplotype analysis of the COI gene of *R. orientalis* revealed one haplotype from North American samples of pythons and native snakes and three from Australian snakes (Figure 5). No haplotype was shared between *R. orientalis* collected in Florida and those from Australia. Four COI haplotypes were recovered from *P. crotali* and one from *K. coarctatus*

We observed significant deviations from neutrality in sequences from *R. orientalis* within its introduced Florida range for the 18S but not the COI gene. Values for tests of neutrality observed for *R. orientalis* within its Florida range were significant for Fu Li's D^* (- 3.046, $P < 0.05$, $n = 52$) and Fu Li's F^* (- 3.072, $P < 0.05$, $n = 52$); Tajima's D (- 1.692, $n = 52$) approached significance at $0.05 > P < 0.1$. Old World *R. orientalis* samples (18S gene: Fu Li's $D^* = - 0.780$, $P > 0.1$, $n = 4$; Fu Li's $F^* = - 0.721$, $P > 0.1$, $n = 4$; Tajima's $D = - 0.780$, $P > 0.1$, $n = 4$) and Australian samples (COI gene: Fu Li's $D^* = - 1.231$, $P > 0.1$, $n = 11$; Fu Li's $F^* = - 1.438$, $P > 0.1$, $n = 11$; Tajima's $D = - 1.403$, $P > 0.1$, $n = 11$) did not deviate significantly from neutrality. Overall mean nucleotide diversity among *R. orientalis* sequences collected from Florida pythons and native snakes were 0.00032 for the 18S gene and 0.00093 for the COI gene. Nucleotide variation among *R. orientalis* of Old World origin (18S gene) and Australian origin (COI gene) were 0.00513 and 0.00291, respectively.

Discussion

We provide several lines of evidence that support the hypothesis that the *Raillietiella* samples we collected from both native snakes and invasive Burmese pythons represent a single species, *R. orientalis*, which was introduced to Florida with the establishment of the Burmese python. First, *Raillietiella* collected from pythons and

sympatric native snakes in Florida were contained within a single 18S lineage that included an exemplar of *R. orientalis* collected from an Asian cobra and published to GenBank. Second, the 18S haplotype of this exemplar is shared with specimens from pythons and native snakes of Florida. Third, diversity of the 18S gene within pentastomes collected from snakes in Florida is statistically consistent with a genetic bottleneck followed by population expansion. Finally, specimens of *Raillietiella* are found only in native snakes within the range of invasive pythons, despite extensive sampling of native snakes outside this range. Evidence from the COI gene supports a similar conclusion because of close phylogenetic ties of Florida *Raillietiella* to specimens of *R. orientalis* introduced to Australia. Additionally, when based on the COI gene, values of Tajima's D for both Australian and Floridian samples, while not showing a statistically significant deviation from zero, are more strongly negative than those for samples from the native range of *R. orientalis*, a feature suggestive of a bottleneck and range expansion at both invaded sites.

We suspect Burmese pythons are responsible for the introduction of *R. orientalis* to Florida, as this parasite is known to infect Burmese pythons in their native range of Southeast Asia (Christoffersen and De Assis 2013). *Raillietiella orientalis* has been noted to infect a wide array of Asian snakes as its primary definitive host, including the widespread snake families Colubridae, Elapidae, and Viperidae, which may result in the parasite being pre-adapted to infect snakes within its introduced range in the US and Australia. Florida is home to many introduced reptiles in addition to Burmese pythons (Meshaka *et al.* 2004) including the northern African python, (*Python sebae*). *Python sebae* is established in Florida (Reed *et al.* 2010), originates from a continent that is

known to contain *R. orientalis*, and is host to pentastomes. However, *R. orientalis* is not known from *P. sebae*, and because observations of *P. sebae* in Florida are relatively recent and from a limited geographic area, it is less likely that this host transported *R. orientalis* to Florida or plays a significant role in its range expansion.

Our data are the first to document *R. orientalis* from Nearctic snakes. We show that the distribution of this invasive parasite among native snakes is extensive, suggesting that intermediate hosts required for transmission of *R. orientalis* are present in Florida. Intermediate hosts of *R. orientalis* in its native range are unknown, but it has been suggested that raillietiellids have multiple intermediate hosts (Riley 1986). Kelehear *et al.* (2014) examined Australian snakes for pentastomes and found 38% of native snakes surveyed were infected with the introduced *R. orientalis*. Due to the similarity among diets of infected snake species, Kelehear *et al.* (2014) concluded that ground-dwelling frogs were the likely intermediate host of this pentastome in Australia. Aquatic snakes comprised the majority of native snake species infected with *R. orientalis* in southern Florida, making anurans a plausible intermediate host of this parasite in the North America. However, an anuran intermediate host is not likely to allow transmission of *R. orientalis* to invasive pythons. The diet of Burmese pythons in southern Florida is known to include over 40 species, primarily consisting of mammals and birds, occasionally including reptiles (*e.g.* American alligator, *Alligator mississippiensis*; Dove *et al.* 2011, Rochford *et al.* 2010; Reed and Rodda 2009; Snow *et al.* 2007), but not frogs. Thus, alternate intermediate hosts must be used by *R. orientalis* in Florida. Our samples include a larval pentastome collected from an African shrew and identified as *R. orientalis* by our molecular analysis. This represents the first record of *Raillietiella* from a mammalian

intermediate host. Diet overlap of native terrestrial snake hosts and invasive pythons likely include additional mammalian hosts, especially among rodents. A *Raillietiella* sp. sample, obtained from GenBank, was collected from the common house gecko (*Hemidactylus frenatus*), a lizard of Old World origin that has established in southern Florida. Therefore, *H. frenatus* is likely to play a role as an intermediate host in the life cycle of *R. orientalis* within its introduced range.

We searched extensively for *R. bicaudata* and did not recover a lineage attributable to this species in the 498 native snakes examined. Therefore, we question the validity of this taxon. Originally reported from North American colubrid snakes (Heymons and Vitzthum 1935, Heymons 1935), the type specimens of *R. bicaudata* include two adult males collected from two captive snakes housed at the Berlin Aquarium in Germany (Christoffersen and De Assis 2013). These specimens cannot be distinguished from *R. orientalis* or *R. furcocercum* (a species of Central and South American snakes) based on morphological features (Kinsella, pers. observ; Mahon 1954). Therefore, the type specimens might represent infections of accidental hosts in a zoo environment rather representing a raillietiellid native to North America. The ambiguity surrounding the original description of *R. bicaudata* and its hosts, the lack of morphological traits useful in differentiating pentastomes beyond genus (Riley 1986), and the fact that we did not recover evidence of *R. bicaudata* in our extensive survey of native snakes lead us to conclude that *R. bicaudata* is not a valid pentastome species. Our example expands the use of molecular data in understanding parasite diversity by noting that these data can be used both to reveal hidden diversity (*e.g.* Falk *et al.* 2011) and to reduce apparent diversity.

Our data demonstrate that *P. crotali* is capable of infecting Burmese pythons in Florida. This parasite is native to viperid snakes of North and South America (Riley and Self 1979) and its presence in invasive Burmese pythons introduces the potential that pythons could negatively impact native pit vipers through parasite spillback (Kelly *et al.* 2009a). The intermediate hosts of *P. crotali* are primarily small rodents (Christoffersen and De Assis 2013) including the hispid cotton rat (*Sigmodon hispidus*), Florida mouse (*Peromyscus floridana*), rice rat (*Oryzomys palustris*) and cotton mouse (*Peromyscus gossypinus*) (Kinsella 1974; Layne 1967). The diet overlap between pythons and pit vipers aids the transmission of *P. crotali* within these taxa and may result in pythons acting as a reservoir of *P. crotali* infection, with spillback of *P. crotali* from pythons to native pit vipers facilitating increased prevalence of this parasite among pit vipers.

Our observations allowed us to identify pentastomids in invasive pythons and native snakes. We demonstrate that *R. orientalis* has spilled over from pythons to native snakes, an important first step in understanding challenges to conservation of native snake assemblages in southern Florida. The diverse intermediate hosts apparently used by *R. orientalis* have allowed this invasive parasite to infect many native snake species. Several of these native hosts are abundant and widespread, suggesting a fundamental niche for *R. orientalis* that extends well beyond that of the invasive host responsible for the parasite's introduction to Florida. Similarly, the potential of pythons to affect pit vipers through parasite spillback could alter composition of snake predators in an area of intense restoration efforts (Estenoz and Bush 2015). Our future studies will examine the prevalence, infection intensity, and impact of pentastomes, specifically *R. orientalis* and *P. crotali*, on native snakes and invasive pythons.

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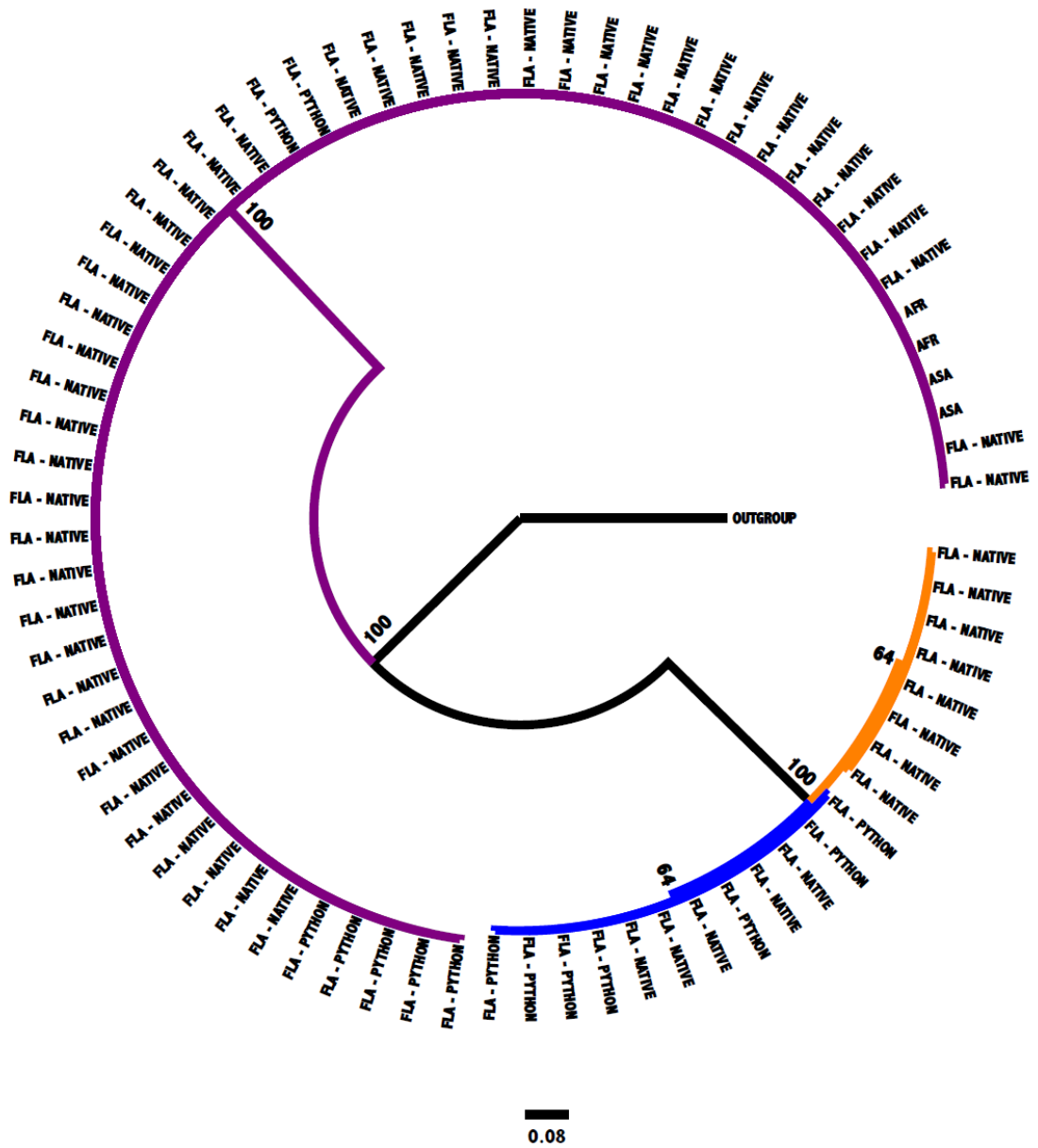


Figure 1 A 50% majority rule Bayesian Inference phylogeny inferred from the 18S gene. Clades for *Raillietiella orientalis* (purple), *Porocephalus crotali* (blue) and *Kiricephalus coarctatus* (orange) are shown with collection location of parasite (FLA = Florida; AFR = Africa; and Asia = ASA) and whether the host was native or non-native (python). Nodes are labeled with the posterior probability as a percent. *Linguatula arctica* was used as an outgroup.

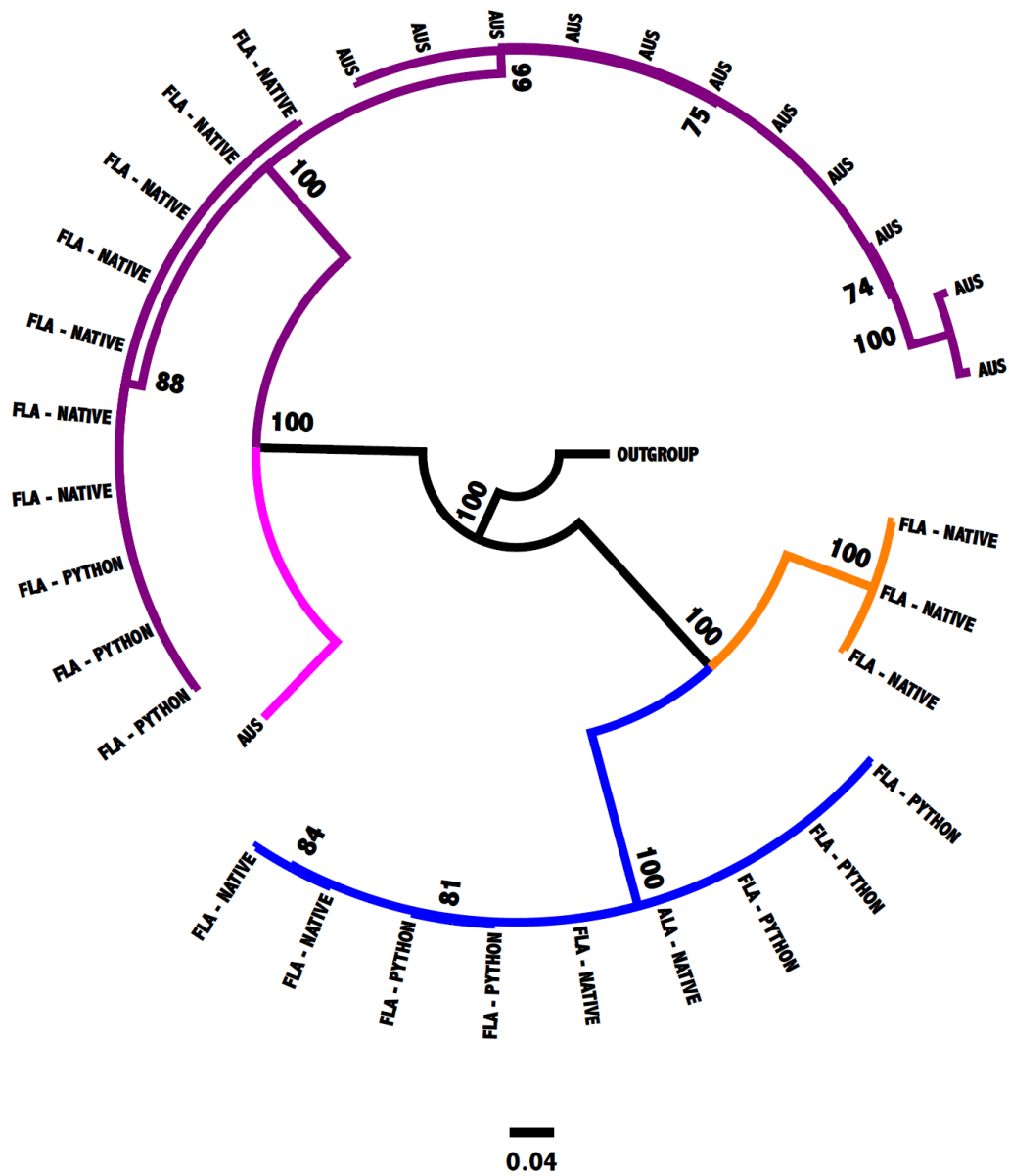


Figure 2 A 50% majority rule Bayesian Inference phylogeny inferred from the COI gene. Clades for *Raillietiella orientalis* (purple), *Porocephalus crotali* (blue) and *Kiricephalus coarctatus* (orange) are shown with collection location of parasite (FLA = Florida; AUS = Australia) and whether the host was native or non-native (python). Nodes are labeled with the posterior probability as a percent. *Linguatula serrata* was used as an outgroup.

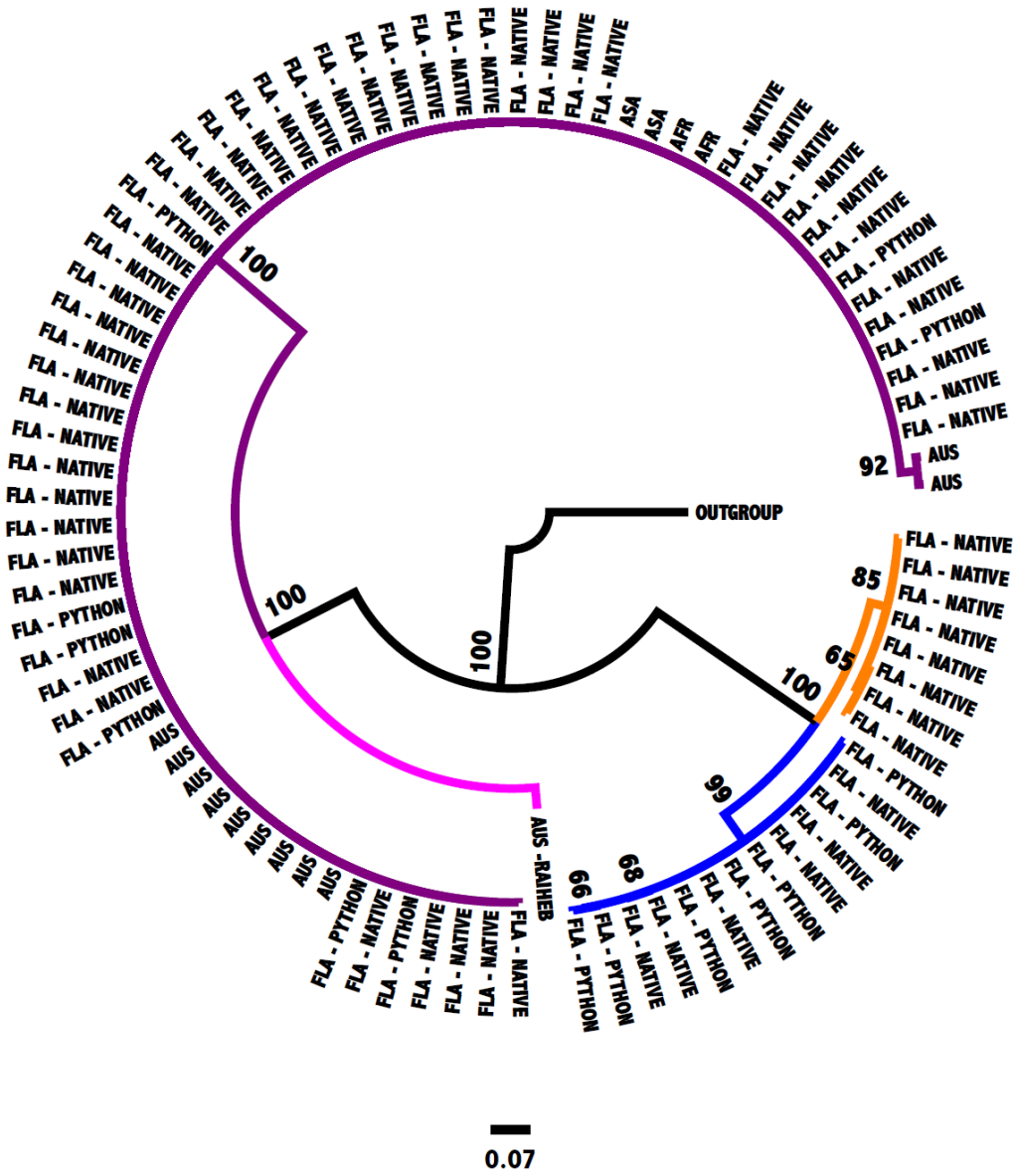


Figure 3 Concatenated tree with a 50% majority rule Bayesian Inference phylogeny inferred from the 18S and COI gene. Clades for *Raillietiella orientalis* (purple), *Porocephalus crotali* (blue) and *Kiricephalus coarctatus* (orange) are shown with collection location of parasite (FLA = Florida; AUS = Australia; AFR = Africa; and Asia = ASA) and whether the host was native or non-native (python). Nodes are labeled with the posterior probability as a percent. *Linguatula arctica* was used as an outgroup.

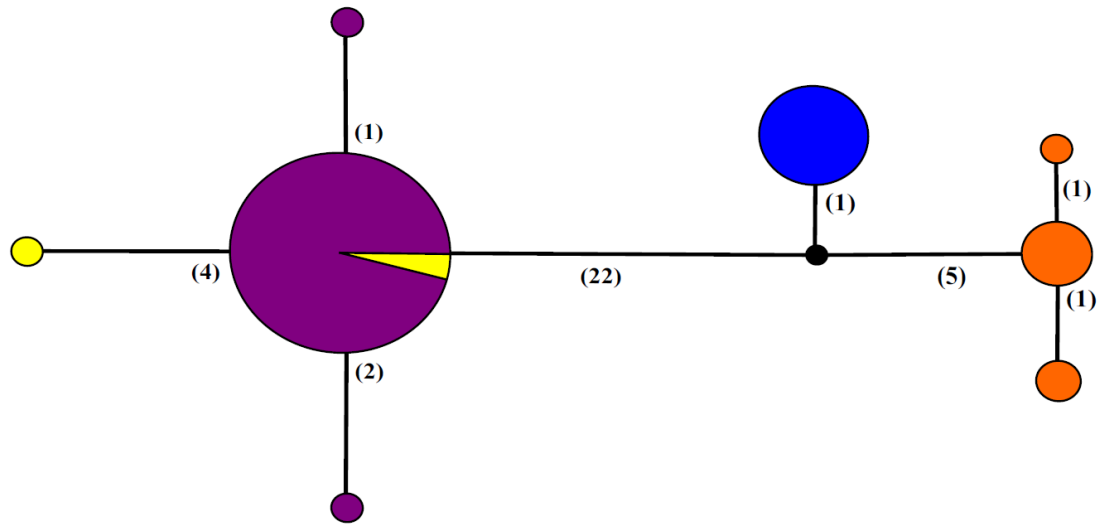


Figure 4 Haplotype network of the 18S gene sequenced from pentastomes collected from Burmese pythons and native snakes (allopatric or sympatric with pythons) is shown. Haplotype variation is shown for *Raillietiella orientalis* (purple = collected from invasive Burmese pythons and sympatric native snakes in Florida; and yellow = Asian/African samples). *Porocephalus crotali* (blue = collected from native snakes sympatric or allopatric with pythons) and *Kiricephalus coarctatus* (orange = collected from native snakes sympatric or allopatric with pythons) are shown for comparison. Each circle represents one haplotype unique to the 18S gene of each group and the size of the circle corresponds to the number with of individuals with that haplotype. Numbers in parentheses represent the number of mutations between haplotypes. Black circles represent hypothetical haplotypes generated by PopART software based upon the 18S gene data provided.

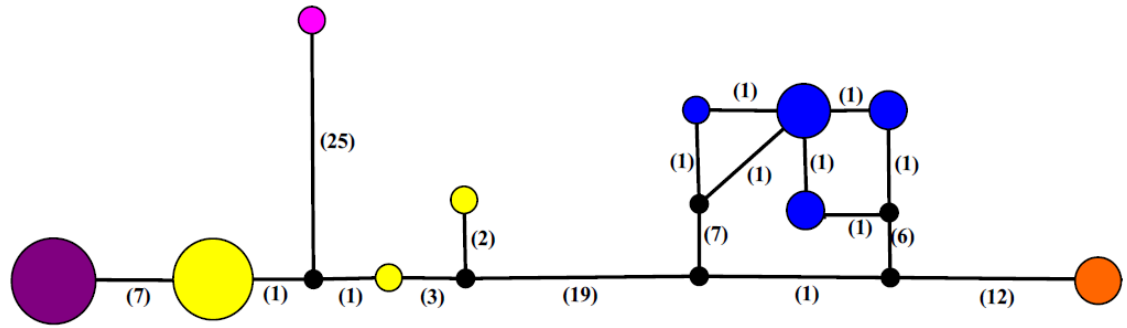


Figure 5 Haplotype network of the COI gene sequenced from pentastomes *Raillietiella*, *Porocephalus*, and *Kiricephalus* collected from Burmese pythons and native snakes. Haplotype variation is shown for *Raillietiella orientalis* collected from invasive Burmese pythons and native snakes in Florida (purple), *R. orientalis* obtained from GenBank from an introduced Australian population (yellow) and *R. hebitihamata* (pink). Haplotypes of *Porocephalus crotali* (blue) and *Kiricephalus coarctatus* (orange) are shown for comparison. Each circle represents one haplotype unique to the COI gene of each group and the size of the circle corresponds to the number with of individuals with that haplotype. Numbers in parentheses represent the number of mutations between haplotypes. Black circles represent hypothetical haplotypes generated by PopART software based upon the COI gene data provided.

Supplemental Data

Table S1 Catalog number, host species, host identification number, and collection locality are shown for pentastomes (*Raillietiella orientalis*, *Porocephalus crotali* and *Kiricephalus coarctatus*) used in the 18S, COI and concatenated phylogenetic analyses and haplotype analyses. GenBank accession number and haplotype number are provided for each sample for the 18S gene and/or the COI gene. Reference *R. orientalis* samples obtained from GenBank and used in this study are listed in the text with their respective accession number. Haplotype number 1 was obtained from the 18S gene of *R. orientalis* (GenBank accession KC904945) collected in Asia from a Chinese cobra (*Naja atra*). Haplotype number 4 was shared among the 18S gene of Old World samples and *R. orientalis* collected from native Florida snakes and pythons. Parasite catalog number and host ID number are for specimen records at the Auburn University Museum (AUM). Specimens collected within Everglades National Park are retained by that entity; all others are housed at AUM. Host genera are: *P* = *Python* (*bivittatus*) and *Pantherophis* (*guttatus*; *obsoletus*); *N* = *Nerodia*; *T* = *Thamnophis*; *A* = *Agkistrodon*; *C* = *Coluber*; *D* = *Drymarchon*; and *L* = *Lampropeltis*). Samples that were consumed in analyses are noted as NS.

Parasite Species	Parasite Catalog No.	Host Species	Host ID No.	Collection Locality	GenBank Accession	Haplotype COI	18S
<i>P. crotali</i>	28681 A	<i>P. bivittatus</i>	1464	Miami-Dade, FL	MG559566		5
<i>P. crotali</i>	28681 A	<i>P. bivittatus</i>	1464	Miami-Dade, FL	MG559647	6	
<i>P. crotali</i>	28681 B	<i>P. bivittatus</i>	1464	Miami-Dade, FL	MG559567		5
<i>P. crotali</i>	28681 B	<i>P. bivittatus</i>	1464	Miami-Dade, FL	MG559652	6	
<i>P. crotali</i>	28722	<i>P. bivittatus</i>	914	Miami-Dade, FL	MG559574		5
<i>P. crotali</i>	28722	<i>P. bivittatus</i>	914	Miami-Dade, FL	MG559648	7	
<i>P. crotali</i>	28724 A	<i>A. piscivorus</i>	23	Covington, AL	MG559578		5
<i>P. crotali</i>	28724 A	<i>A. piscivorus</i>	23	Covington, AL	MG559649		9
<i>P. crotali</i>	28724 E	<i>A. piscivorus</i>	23	Covington, AL	MG559579		5
<i>P. crotali</i>	28724 E	<i>A. piscivorus</i>	23	Covington, AL	MG559650	9	
<i>P. crotali</i>	28688	<i>P. bivittatus</i>	718	Miami-Dade, FL	MG559653	8	
<i>P. crotali</i>	28688	<i>P. bivittatus</i>	718	Miami-Dade, FL	MG559568		5
<i>P. crotali</i>	28748 A	<i>A. piscivorus</i>	53	Alachua, FL	MG559597		5
<i>P. crotali</i>	28748 A	<i>A. piscivorus</i>	53	Alachua, FL		7	
<i>R. orientalis</i>	28743 D	<i>P. guttatus</i>	458	Monroe, FL	MG559593		4
<i>R. orientalis</i>	41548 A	<i>P. bivittatus</i>	1481	Collier, FL	MG559565		4
<i>R. orientalis</i>	41548 B	<i>P. bivittatus</i>	1481	Collier, FL	MG559565		4
<i>R. orientalis</i>	28705 B	<i>P. bivittatus</i>	743	Miami-Dade, FL	MG559570		4
<i>R. orientalis</i>	28717 A	<i>T. sirtalis</i>	578	Miami-Dade, FL	MG559572		4
<i>R. orientalis</i>	28723 A	<i>N. clarkii</i>	301	Martin, FL	MG559575		4
<i>R. orientalis</i>	28723 B	<i>N. clarkii</i>	301	Martin, FL	MG559576		4
<i>R. orientalis</i>	28723 L	<i>N. clarkii</i>	301	Martin, FL	MG559577		4
<i>R. orientalis</i>	28723 F	<i>N. clarkii</i>	301	Martin, FL	MG559628		4
<i>R. orientalis</i>	28739 A	<i>N. fasciata</i>	344	Miami-Dade, FL	MG559585		4
<i>R. orientalis</i>	28739 B	<i>N. fasciata</i>	344	Miami-Dade, FL	MG559586		4
<i>R. orientalis</i>	28739 C	<i>N. fasciata</i>	344	Miami-Dade, FL	MG559587		4
<i>R. orientalis</i>	28759 A	<i>C. constrictor</i>	164	Miami-Dade, FL	MG559600		4
<i>R. orientalis</i>	28763 A	<i>C. constrictor</i>	168	Miami-Dade, FL	MG559601		4
<i>R. orientalis</i>	41551 A	<i>P. bivittatus</i>	1516	Miami-Dade, FL	MG559609		4
<i>R. orientalis</i>	41551 B	<i>P. bivittatus</i>	1516	Miami-Dade, FL	MG559610		4
<i>R. orientalis</i>	41541 B	<i>C. constrictor</i>	518	Highlands, FL	MG559613		4
<i>R. orientalis</i>	30269	<i>C. constrictor</i>	520	Highlands, FL	MG559614		4
<i>R. orientalis</i>	30087	<i>C. constrictor</i>	174	Miami-Dade, FL	MG559615		4
<i>R. orientalis</i>	30055	<i>C. constrictor</i>	163	Monroe, FL	MG559620		4
<i>R. orientalis</i>	28734 A	<i>P. obsoletus</i>	487	Collier, FL	MG559630		4
<i>R. orientalis</i>	28734 B	<i>P. obsoletus</i>	487	Collier, FL	MG559622		2
<i>R. orientalis</i>	28734 C	<i>P. obsoletus</i>	487	Collier, FL	MG559621		4
<i>R. orientalis</i>	30280	<i>N. fasciata</i>	632	Highlands, FL	MG559625		4
<i>R. orientalis</i>	30271	<i>P. guttatus</i>	636	Glades, FL	MG559629		4
<i>R. orientalis</i>	30022 A	<i>T. sirtalis</i>	588	Miami-Dade, FL	MG559635		4
<i>R. orientalis</i>	30022 B	<i>T. sirtalis</i>	588	Miami-Dade, FL	MG559636		4
<i>R. orientalis</i>	30060	<i>C. constrictor</i>	167	Miami-Dade, FL	MG559623		4
<i>R. orientalis</i>	30278 B	<i>N. fasciata</i>	359	Glades, FL	MG559602		4
<i>R. orientalis</i>	30278 C	<i>N. fasciata</i>	359	Glades, FL	MG559603		4

<i>P. crotali</i>	28749	<i>A. piscivorus</i>	55	Alachua, FL	MG559655	7	
<i>P. crotali</i>	28749	<i>A. piscivorus</i>	55	Alachua, FL	MG55958		5
<i>P. crotali</i>	41550	<i>P. bivittatus</i>	1517	Miami-Dade, FL	MG559607		5
<i>P. crotali</i>	28731 J	<i>A. piscivorus</i>	72	Jackson, FL	MG559651	7	
<i>P. crotali</i>	28731 J	<i>A. piscivorus</i>	72	Jackson, FL	MG559581		5
<i>P. crotali</i>	41549	<i>P. bivittatus</i>	1518	Miami-Dade, FL	MG559631		5
<i>P. crotali</i>	28731 A	<i>A. piscivorus</i>	72	Jackson, FL	MG559581		5
<i>P. crotali</i>	28731 B	<i>A. piscivorus</i>	72	Jackson, FL	MG559582		5
<i>R. orientalis</i>	28702 A	<i>P. bivittatus</i>	862	Miami-Dade, FL	MG559637	1	
<i>R. orientalis</i>	28702 A	<i>P. bivittatus</i>	862	Miami-Dade, FL	MG559569		4
<i>R. orientalis</i>	28712 F	<i>P. bivittatus</i>	738	Miami-Dade, FL	MG559638	1	
<i>R. orientalis</i>	28712 F	<i>P. bivittatus</i>	738	Miami-Dade, FL	MG559571		4
<i>R. orientalis</i>	28712 G	<i>P. bivittatus</i>	738	Miami-Dade, FL	MG559639	1	
<i>R. orientalis</i>	28735	<i>N. fasciata</i>	339	Miami-Dade, FL	MG559640	1	
<i>R. orientalis</i>	28735	<i>N. fasciata</i>	339	Miami-Dade, FL	MG559583		4
<i>R. orientalis</i>	28737	<i>N. fasciata</i>	NS	Miami-Dade, FL	MG559584		4
<i>R. orientalis</i>	28746	<i>N. clarkii</i>	306	Monroe, FL	MG559596		4
<i>R. orientalis</i>	28746	<i>N. clarkii</i>	306	Monroe, FL	MG559641	1	
<i>R. orientalis</i>	28720	<i>T. sirtalis</i>	579	Miami-Dade, FL	MG559573		4
<i>R. orientalis</i>	28720	<i>T. sirtalis</i>	579	Miami-Dade, FL	MG559643	1	
<i>R. orientalis</i>	28754 A	<i>A. piscivorus</i>	98	Miami-Dade, FL	MG559599		4
<i>R. orientalis</i>	28754 A	<i>A. piscivorus</i>	98	Miami-Dade, FL	MG559644	1	
<i>R. orientalis</i>	28751 A	<i>A. piscivorus</i>	97	Miami-Dade, FL	MG559645	1	
<i>R. orientalis</i>	28752 A	<i>A. piscivorus</i>	-	Miami-Dade, FL	MG559642	1	
<i>R. orientalis</i>	28743	<i>P. guttatus</i>	458	Monroe, FL	MG559594		4
<i>R. orientalis</i>	28743	<i>P. guttatus</i>	458	Monroe, FL	MG559646	1	
<i>R. orientalis</i>	28743 A	<i>P. guttatus</i>	458	Monroe, FL	MG559590		4
<i>R. orientalis</i>	28743 B	<i>P. guttatus</i>	458	Monroe, FL	MG559591		4
<i>R. orientalis</i>	28743 C	<i>P. guttatus</i>	458	Monroe, FL	MG559592		4

Chapter 3

Highly competent native snake hosts extend the range of an introduced parasite beyond its invasive Burmese python host

Abstract

Invasive Burmese pythons (*Python bivittatus*) have introduced a non-native pentastomid parasite (*Raillietiella orientalis*) to southern Florida that has spilled over to infect native snakes. However, the extent of spillover, regarding prevalence and intensity of *R. orientalis* among native snakes, is unknown. We examined native snakes collected from Florida to determine the degree to which parasite spillover is occurring. We found *R. orientalis* has infected 13 species of native snakes collected from areas of sympatry with pythons. Prevalence and infection intensity of *R. orientalis* was significantly higher among native snakes compared with pythons. Moreover, adult female pentastomes achieved larger sizes and represented a greater proportion of the overall parasite population in native snakes versus pythons, indicating native snakes are more competent hosts of *R. orientalis* than pythons. We also examined native snakes from regions of allopatry with pythons to determine how far *R. orientalis* has spread. We found an infected native snake 348 km north of the northernmost infected python. Our data show that native snakes are highly competent hosts of *R. orientalis* and have facilitated the rapid spread of this non-native pentastome beyond the python's range.

Introduction

Biological invasions can impact native ecosystems by altering host-parasite dynamics (Tompkins and Poulin 2006). Non-indigenous species (NIS) often contain half

the parasite species richness of conspecifics in their native range (Torchin *et al.* 2003) due to the low probability of a non-native parasite successfully establishing in its introduced range (MacLeod *et al.* 2010). Despite obstacles a parasite must overcome during the invasion pathway (*e.g.* low host densities and lack of required intermediate hosts), non-native parasites have become established and spillover of these parasites to native taxa has been documented (Daszak *et al.* 2000; Tompkins and Poulin 2006).

When parasite spillover occurs, adverse effects of introduced parasites and pathogens may be exacerbated as native taxa do not share a co-evolutionary history with the non-native parasite and, therefore, often lack adaptations to effectively mitigate or deter infection (Mastitsky *et al.* 2010; Dogel *et al.* 1958; Anderson *et al.* 1986; Holdich and Reeve 1991; Kohler and Wiley 1997; Daszak *et al.* 2000). In 85% of cases in which an introduced parasite infects native taxa, the parasite is more virulent in the naïve host compared to the NIS host from which the parasite was introduced (Lymberty *et al.* 2014). For example, the swim-bladder nematode (*Anguillicola crassus*), introduced to Europe by the Japanese eel (*Anguilla japonica*), now infects the native European eel (*A. anguilla*) (Kirk 2003). While the effect of the nematode in the Japanese eel is negligible, nematode infection in the European eel yields large worm burdens and causes severe damage to swim-bladder function, which may prevent spawning migrations (Kirk 2003).

Competent hosts are those in which a parasite can undergo all stages of development and reproduction. Non-native parasites within immunologically naïve hosts can occur at higher intensity, increased prevalence, and larger size relative to NIS hosts (Kirk 2003). For female parasites, an increase in body length is strongly and positively correlated with the number of eggs produced, the size of the eggs, and total reproductive

effort (Timi *et al.* 2005). Therefore, when native taxa are highly competent hosts of non-native parasites, they may function to increase parasite transmission among susceptible hosts. With increased transmission, the introduced parasite may spread quickly among novel hosts within its invaded range allowing the parasite to spread beyond the distribution of the NIS host. Spread of an introduced parasite beyond the range of its NIS host has been shown in the nematode (*Camallanus cotti*) that was introduced to Hawaii by non-native swordtail and guppy fishes. Since its introduction, the nematode has infected the native Hawaiian goby (*Awaous stamineus*) and this non-native nematode can now be found in river catchments lacking non-indigenous fish (Gagne *et al.* 2015).

Burmese pythons (*Python bivittatus*), native to Southeast Asia, have been introduced to southern Florida where they have been established for over two decades (Meshaka *et al.* 2000; Snow *et al.* 2007). Miller *et al.* (in review) examined the lung parasites of wild-caught Burmese pythons in southern Florida and found them to be infected with a pentastome parasite, *Raillietiella orientalis*, of Old World origin. Within its native range, *R. orientalis* infects a diverse array of snakes including members of the families Pythonidae, Elapidae, Viperidae, and Colubridae (Christoffersen and De Assis 2013). However, *raillietiellid* pentastomes that infect snakes as their definitive host are not known from North America (Miller *et al.* in review). Since the introduction of *R. orientalis* to southern Florida, spillover of this parasite from pythons to native snakes has occurred (Miller, in review). However, infection dynamics of *R. orientalis* among native snakes and pythons have not been explored.

We examined infection dynamics of *R. orientalis* in novel snake hosts in southern Florida. Specifically, we measured parasite prevalence, infection intensity, and size and

proportion of adult female pentastomes as measures of host competency of *R. orientalis* in infected native Florida snakes and we compared these metrics to those recovered from Burmese pythons in Florida, the host responsible for the introduction of *R. orientalis* (Miller, in review). We also examined a potential for *R. orientalis* to spread through infected native snakes beyond the python's invasive range by comparing prevalence of *R. orientalis* in native snakes collected from locations in sympatry and allopatry with pythons, as well as along the python invasion front. If prevalence of *R. orientalis* does not differ between native snakes from the invasion front and those from areas in sympatry with pythons, yet prevalence of these two populations are significantly higher than prevalence of *R. orientalis* in pythons, this would be consistent with the hypothesis that native snakes, and not pythons, are the primary driver of transmission of *R. orientalis*.

Methods

Burmese pythons were collected from throughout their range in southern Florida (Fig. 1) through road surveys and opportunistic captures. Pythons were also provided through a collaborative python removal effort among the United States Geological Survey, National Park Service, and University of Florida Fort Lauderdale Research and Education Center. Native snakes were salvaged as road-kill during road surveys conducted in the core of the python's distribution, along the python invasion front, and in areas of allopatry with pythons (Alabama, Georgia, and northern Florida; Fig. 1). Pythons and native snakes were dissected, and lungs and respiratory passageways were examined for pentastomes. All members of the genus *Raillietiella* were assumed to be *R. orientalis* based on a molecular analysis of a large series of this genus (Miller, in review).

Prevalence (number of infected hosts and number of uninfected hosts; first grouping variable) of *R. orientalis* was quantified for native snake species which were grouped into taxonomic (crotalines = *Agkistrodon piscivorus* + *Sistrurus miliarius*; colubrines = *Coluber constrictor* + *C. flagellum* + *Drymarchon couperi*; lampropeltines = *Pantherophis guttatus* + *P. obsoletus* + *Lampropeltis getula*) or functional (frog-eaters = *Nerodia clarkii* + *Nerodia fasciata* + *Thamnophis sirtalis*; fish-eaters = *Nerodia floridana* + *Nerodia taxispilota*) groups representing a second grouping variable.. We included regions [python core area (Miami-Dade and Monroe counties, FL) and python invasion front (Broward, Charlotte, Collier, DeSoto, Glades, Hardee, Hendry, Highlands, Lee, Manatee, Martin, Okeechobee, Palm Beach, Sarasota, and Saint Lucie counties, FL)] as a third grouping variable and tested for differences in prevalence among species groups and region with a log-linear model (CATMOD of SAS). Regions of allopatry with pythons (north of Hardee, Highlands, Manatee, Okeechobee, and Saint Lucie counties, FL) represented a third region; however, snakes within this region were not included in this analysis (nor subsequent analyses) due to a low number of infected snakes (n = 3 individuals) recovered from this region.

Infection intensity (mean number of individual parasites per host) was quantified for infected individuals within three groups: native snakes (all taxa pooled together) in the core region (n = 95 infected hosts), native snakes in the invasion front (n = 22 infected hosts), and pythons from the pooled core (n = 119 infected hosts) and invasion front (n = 1 infected host) regions. Data were square-root transformed to improve fit to a normal distribution and a one-way ANOVA was used to test for differences in mean

intensity among groups, followed by a Tukey HSD analysis to determine where any significant differences occurred.

Female pentastome body length was measured (nearest mm) as a proxy for host competency, as female body length in parasites strongly and positively correlates with fecundity (*e.g.* Timi *et al.* 2005). Female *R. orientalis* larger than or equal to 34 mm in body length are capable of producing eggs and males never achieve this body size (Hett 1924); therefore, only individuals 34 mm or greater were included. Body length measurements were square-root transformed to improve normality and pooled into native snakes from the core region, native snakes from the invasion front, and pythons from the core and invasion front combined. ANOVA was used to test for differences in mean body length among these three groups.

Finally, we generated a contingency table enumerating the number of individual pentastomes that were adult females versus a pooled category for juveniles and adult males for native snakes from the core region, native snakes from the invasion front, and pythons from the core region and invasion front. We used a chi-square test to determine whether the proportion of adult female parasites in a population of parasites differed among the three host groups.

Results

We examined 529 native snakes encompassing three families, 16 genera, and 27 species, 13 of which were native to the core python region and were captured frequently enough to include in our statistical analyses. Thirteen native snake species were infected with *R. orientalis*. These species were pooled into taxonomic (crotalines = *Agkistrodon piscivorus* + *Sistrurus miliarius*; colubrids = *Coluber constrictor* + *C. flagellum* +

Drymarchon couperi; lampropeltines = *Pantherophis guttatus* + *P. obsoletus* + *Lampropeltis getula*) or functional (frog-eaters = *Nerodia clarkii* + *Nerodia fasciata* + *Thamnophis sirtalis*; fish-eaters = *Nerodia floridana* + *Nerodia taxispilota*) groups.

A total of 1083 *R. orientalis* were recovered from native snakes, six from allopatry with pythons, 304 from the python invasion front, and 773 from the core python region. The northern-most native snake infected with *R. orientalis* was collected in Lake County, 348 km north of the northern-most infected python (Fig. 1). All native species examined for *R. orientalis* are provided in a supplemental table (S1). We examined 1003 pythons to determine prevalence of *R. orientalis*, 43 of which were from the invasion front. A total of 255 *R. orientalis* was recovered from 120 infected pythons. All but two of these parasites were from the core region of the python invasion.

Prevalence of *R. orientalis* differed among the five native snake taxonomic or functional groups and pythons ($\chi^2 = 37.93$; $df = 5$; $P < 0.0001$; Fig. 2). Prevalence in lampropeltines was significantly greater than for pythons and fish eaters ($\chi^2 = 10.17$; $df = 2$; $P < 0.006$) and significantly lesser than for frog eaters, colubrids and crotalines ($\chi^2 = 9.39$; $df = 3$; $P < 0.02$). None of the three-way interactions (snake group * area * infection) were significant, indicating that prevalence patterns were similar between core and invasion front areas.

Mean intensity of *R. orientalis* differed significantly among groups ($F = 25.56$; $df = 2$; $P < 0.001$; Fig. 3) Mean intensity of *R. orientalis* in pythons differed significantly from native snakes in the core and invasion front. Intensity of parasites within native snakes did not differ between these two regions. Infection intensity ranged from 1 – 77 *R. orientalis* for native snakes and 1- 15 for pythons.

The length of adult female *R. orientalis* differed significantly among groups ($F = 6.47$; $df = 2$; $P = 0.0018$; Fig. 4). Female *R. orientalis* reached significantly longer body lengths in native snakes collected from the core region than in pythons, but did not differ from native snakes from the invasion front. Female body length of *R. orientalis* in native snakes collected from the invasion front also did not differ from pythons.

The proportion of adult female *R. orientalis* differed among native snakes from the core region and pythons ($\chi^2 = 19.10$; $df = 1$; $P < 0.0001$; Fig 5). Native snakes from the invasion front also varied significantly ($\chi^2 = 8.32$; $df = 1$; $P = 0.0039$) from pythons, but not from native snakes from the core region ($\chi^2 = 1.76$; $df = 1$; $P < 0.185$).

Discussion

Burmese pythons have introduced a non-native pentastome, *R. orientalis*, to southern Florida where this parasite has infected native snakes (Miller, in review). The diversity of native snakes infected (13 species) represents 48% of the snake fauna that we sampled and is at least 28% of the 45 species known from Florida (Bartlett and Bartlett 2003). Four families of snakes are infected by *R. orientalis* within its native range (Christoffersen and De Assis 2013), three of which (viperids, elapids, and colubrids) are also native to North America. Within its native range, *R. orientalis* exhibits an exceptionally broad distribution, which likely emerges from use of a diverse assemblage of intermediate and definitive hosts. Therefore, *R. orientalis* appears to have retained these host diversities within its introduced range, features that suggest it may pose a risk to snakes throughout the US.

Aside from this parasite's ability to infect many snake species, our study shows that certain species native to the parasite's introduced range are more competent hosts of

R. orientalis than are pythons. We observed higher prevalence, higher intensity, larger female body size and greater proportion of adult female *R. orientalis* in populations of native snakes in the core region and python invasion front than in pythons. This has enabled native snakes to spread *R. orientalis* approximately 350 km farther north than have pythons. Prevalence of *R. orientalis* among native snakes collected from the python invasion front did not differ from that of native snakes collected from the core of the python's distribution. Yet, prevalence among native snakes from both these regions differed significantly from that of pythons, again suggesting that *R. orientalis* is spread more effectively through native snake hosts, which represents a risk to native species well beyond the physiological/climatic restraints that may limit the northward expansion of pythons (Rodda *et al.* 2008).

Our data suggest that certain native snake groups (*e.g.* crotalines, colubrids, frog-eating aquatic snakes) are more competent hosts than others (fish-eating aquatic snakes). Therefore, highly competent native hosts are not only at increased risk of infection, but also heighten the likelihood of *R. orientalis* transmission compared to less competent native snake hosts (*i.e.* *Nerodia floridana* and *N. taxispilota*). We infer that the reservoir of intermediate hosts used by *R. orientalis* in Florida explains differences in competency among infected native snake species. Dietary patterns of native snakes suggest that fish are unlikely to be an important intermediate host but that frogs are likely to serve this role. Kelehear *et al.* (2014) reached a similar conclusion regarding the role of anurans in transmitting *R. orientalis* to snakes native to Australia. *Raillietiella orientalis* larvae have been documented in lizards (Miller, in review), but the effect of this assemblage of potential intermediate hosts on transmission to native snakes in Florida is unknown.

Miller *et al.* (in review) first documented *R. orientalis* larvae collected from a mammal, an assemblage of intermediate hosts critical to establishment of this parasite within the founding population of pythons. However, pythons have reduced mammal populations in the core range (Dorcas *et al.* 2012). Therefore, the low prevalence of *R. orientalis* in pythons as well as within mammal-eating native snakes (*e.g.* lampropeltines), may result from reduction of mammal populations that serve as intermediate hosts. If this is true, *R. orientalis* eventually should become more prevalent in lampropeltines as the parasite expansion outpaces the effect of pythons on native mammals. Regardless, the pool of intermediate hosts used by *R. orientalis* appears to be diverse and crucial to its rapid range expansion in native snakes.

Our results indicate several native snake species are unlikely to be infected by *R. orientalis*. Many of these will require additional samples to eliminate the possibility that the parasite is present in the species but not detected in current samples. *Raillietiella*. *orientalis* was not detected in our sample of six *Crotalus adamanteus*, a large mammal-consuming viperid. Even if the parasite, eventually is detected in this species, it seems unlikely that prevalence will be as high as it is in other viperids, perhaps conforming to other mammal-eating snakes from which the parasite has been detected, but at low prevalence. An absence of *R. orientalis* in samples of *Liodytes*, *Storeria*, *Tantilla*, and *Virigina*, suggests to us that native invertebrates are unlikely to serve as intermediate hosts. Similarly, our failure to detect the parasite in *Farancia abacura* suggests that aquatic salamanders also are unlikely to serve as intermediate hosts.

When an immunologically-naïve host is infected with a non-native parasite, virulence is predicted to increase, leading to a reduction in host fitness (Combes 2001;

Mastitsky *et al.* 2010). Over time, as the non-native parasite and naïve host co-evolve, the host may adapt to either resist infection or to develop tolerance toward infection, allowing the host to mitigate harmful effects (Best *et al.* 2008; Svensson and Roberg 2010). Pentastomes are parasitic arthropods that infect the lungs of tetrapods and feed on the blood of their host. Effects of pentastome infection on co-evolved hosts may include scarring of lung tissue, haemorrhage, infection, pneumonia, reduced reproductive output, adverse effects on metabolic rate, and blockage of respiratory passageways (Pence and Selcer 1988, Riley 1986; Pare 2008; Caballero *et al.* 2015). These effects may be exacerbated in naïve hosts, leading to lethal effects or sublethal effects that result in reduced host fitness and ultimately host population declines, particularly when parasite prevalence and intensity are high within a naïve host. Increased competency of native snake hosts, witnessed in this study, suggests that native snakes in Florida have not yet adjusted to the presence of *R. orientalis*. The snake hosts representing the highest prevalence, intensity, proportion of adult females, and female body size of *R. orientalis* are among the most ubiquitous and widely dispersed snakes in the US (*e.g.* *A. piscivorus*, *N. fasciata*, and *T. sirtalis*). If *R. orientalis* continues to spread northward independent of pythons, it has the potential to adversely affect snakes throughout North America.

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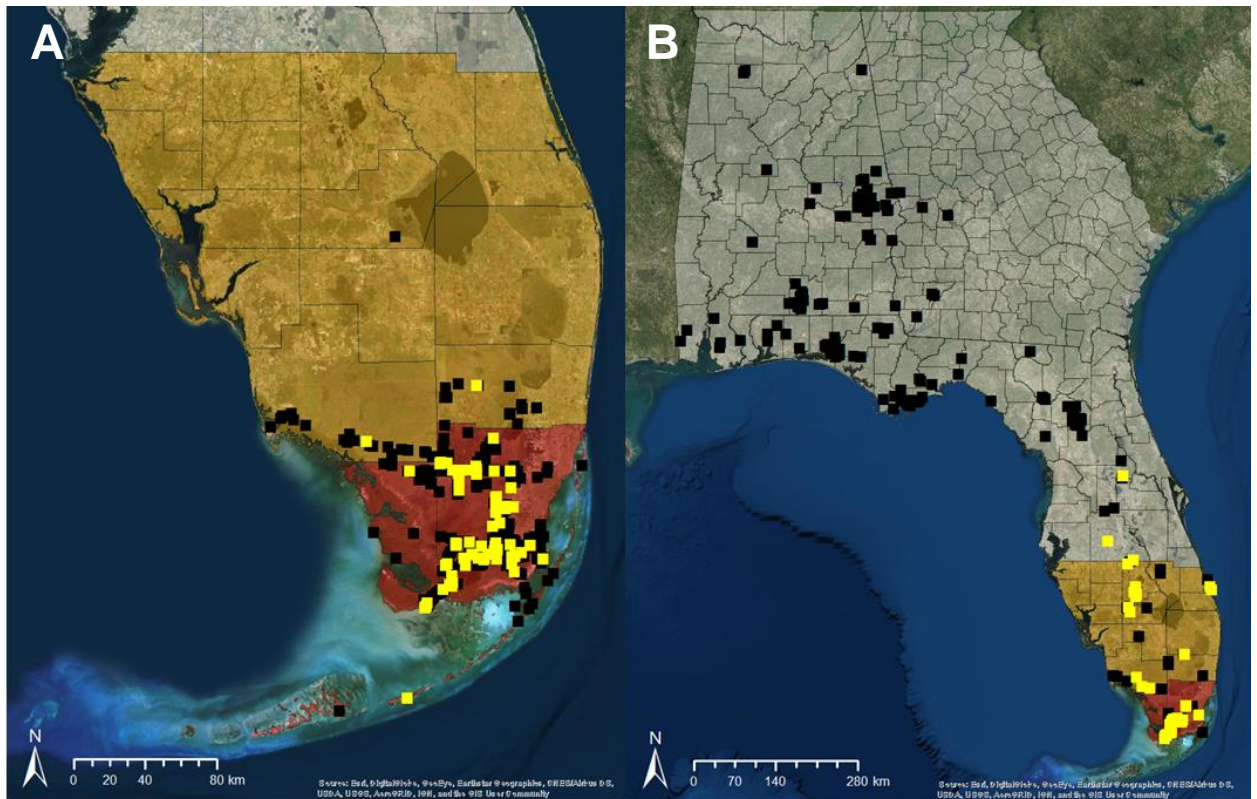


Figure 1 Map of southeastern US showing locations where pythons (A) and native snakes (B) were collected within three regions encompassing Florida, Alabama and Georgia. Regions included the core of the python’s distribution (red shading), the expanding edge of the python’s distribution (orange shading), and a region where pythons are absent (gray shading). Native snakes infected with *R. orientalis* (yellow squares) are shown along with native snakes that were not infected with *R. orientalis* (black squares). Pythons infected with *R. orientalis* are shown (yellow squares) along with pythons that were not infected (black squares) with this parasite. Black lines represent counties within each state.

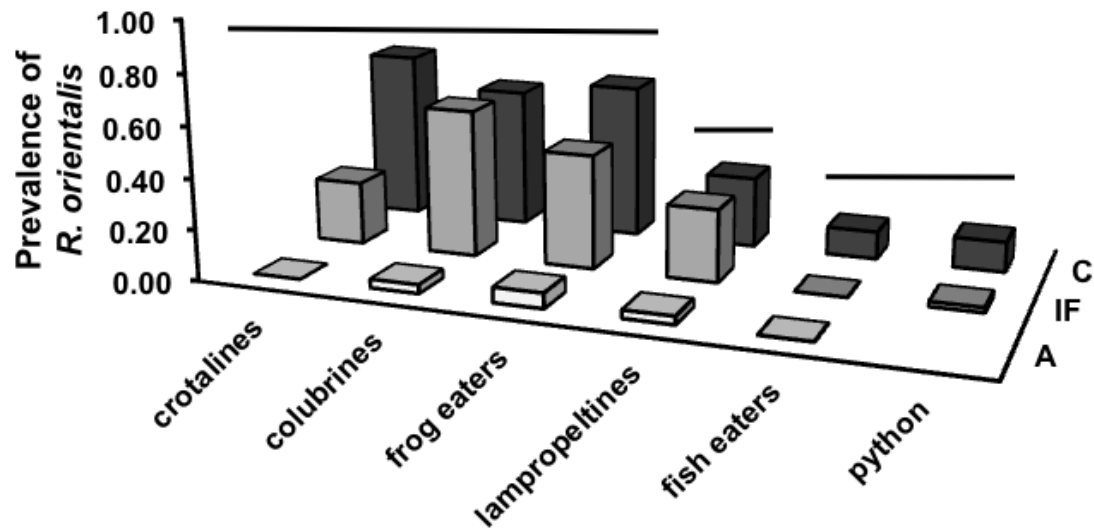


Figure 2 Prevalence of *Raillietiella orientalis* infection for thirteen native snake species and pythons. Native species were combined into taxonomic or functional groups.

Prevalence of each group was quantified for three geographic regions (C = core of python distribution, black bars; IF = python invasion front, gray bars; and A = region of allopatry with pythons, light gray bars). Black lines unite groups that do not differ significantly.

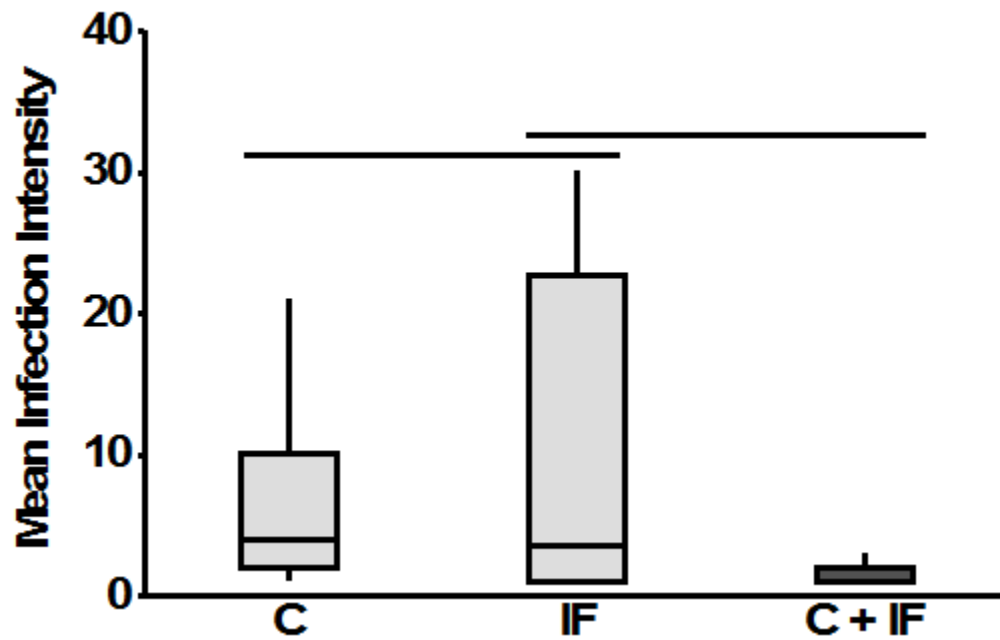


Figure 3 Mean intensity of *Raillietiella orientalis* is shown for pythons (dark gray bar) and native snakes (light gray bars) collected from two regions (C = the core of python distribution and IF = the python invasion front). Black lines unite groups that do not differ significantly.

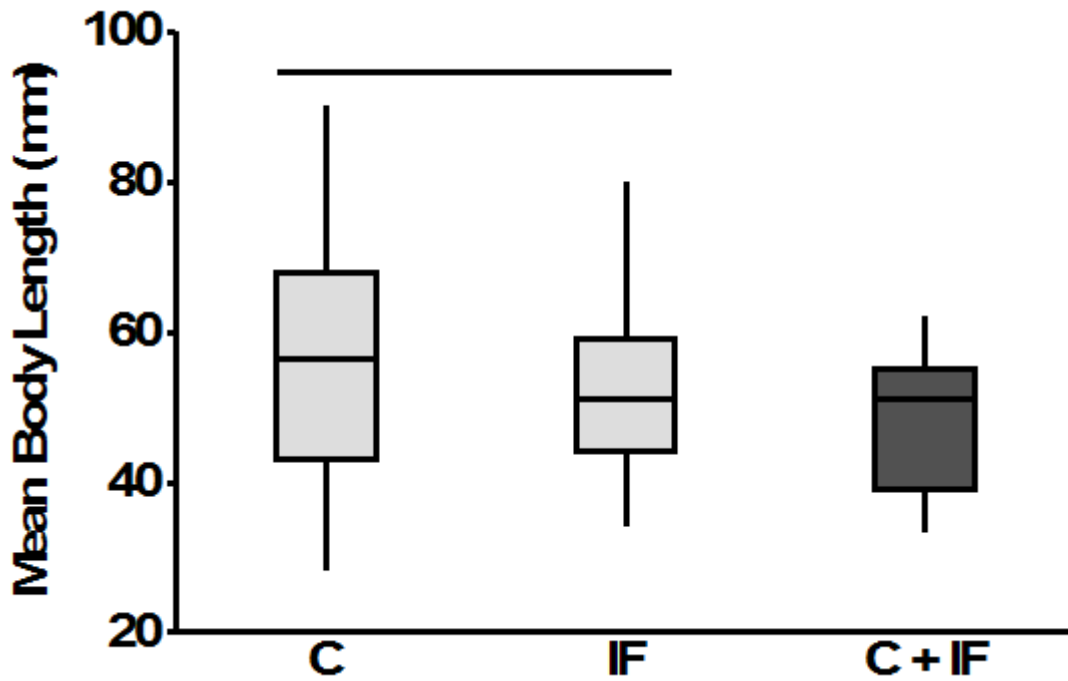


Figure 4 Mean body length of adult female *Raillietiella orientalis* are shown for native snakes (light gray bars) and pythons (dark gray bar) collected from two regions (C = core of python distribution and IF = python invasion front). Black lines unite groups that do not differ significantly.

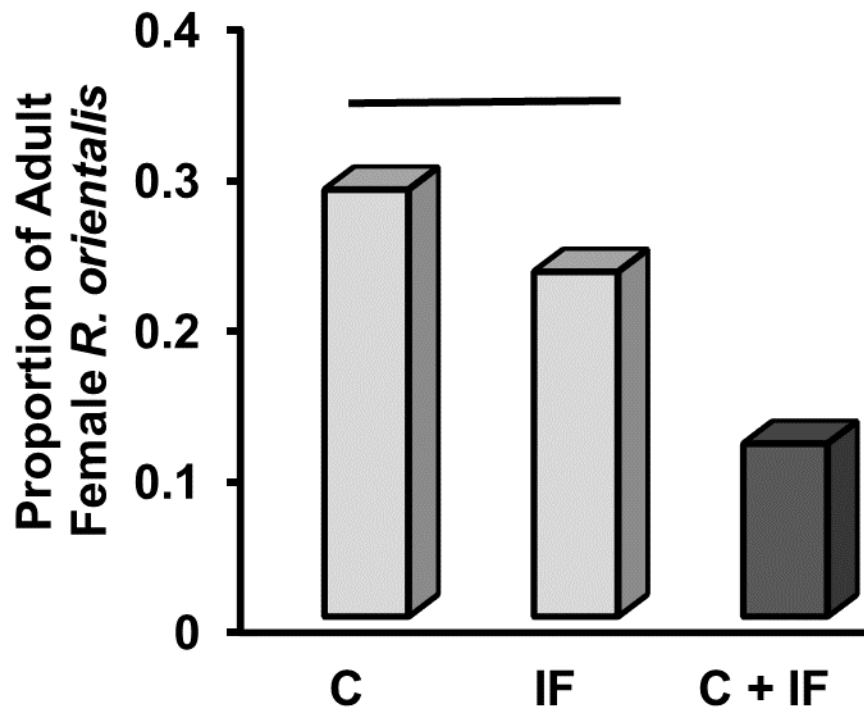


Figure 5 Proportion of adult female *Raillietiella orientalis* are shown for pythons (dark gray bars) and native snakes (light gray bar) collected from two regions (C = core of python's distribution and IF = python invasion front). Black lines indicate groups that do not differ significantly.

S1 Table The sample size for each species examined is presented by region (C = core of python distribution; IF = python invasion front; and A = regions of allopatry with pythons). The total number of individuals examined per species is shown along with the total number of individuals of each species examined per region. Regions outside of a species distribution are noted with a hyphen (-).

Species	Region			Total per Species
	C	IF	A	
<i>Agkistrodon contortrix</i>	-	-	6	6
<i>Agkistrodon piscivorus</i>	43	8	80	131
<i>Cemophora coccinea</i>	3	0	2	5
<i>Coluber constrictor</i>	18	8	19	45
<i>Coluber flagellum</i>	0	2	9	11
<i>Crotalus adamanteus</i>	6	1	31	38
<i>Crotalus horridus</i>	-	-	16	
<i>Drymarchon couperi</i>	0	2	2	4
<i>Farancia abacura</i>	0	1	8	9
<i>Heterodon platirhinos</i>	0	1	8	9
<i>Lampropeltis getula</i>	11	0	3	14
<i>Micrurus fulvius</i>	0	0	2	2
<i>Nerodia clarkii</i>	10	5	1	16
<i>Nerodia erythrogaster</i>	0	0	6	6
<i>Nerodia fasciata</i>	30	7	6	43
<i>Nerodia floridana</i>	19	3	0	22
<i>Nerodia taxispilota</i>	17	1	0	18
<i>Opheodrys aestivus</i>	1	1	3	5
<i>Pantherophis guttatus</i>	9	11	8	28
<i>Pantherophis obsoletus*</i>	5	3	20	28
<i>Pituophis melanoleucus</i>	0	0	11	11

<i>Python bivittatus</i>	960	43	0	1003
<i>Liodytes alleni</i>	4	0	1	5
<i>Sistrurus miliarius</i>	2	0	4	6
<i>Storeria sp.**</i>	1	0	1	2
<i>Thamnophis sauritus</i>	4	2	3	9
<i>Thamnophis sirtalis</i>	31	0	9	40
Total per region	1174	99	259	1532

Chapter 4

Morphological Plasticity of a Non-Native Parasite (*Raillietiella orientalis*)

in Novel Snake Hosts

Abstract

Burmese pythons (*Python bivittatus*) are well established in southern Florida and have introduced a lung parasite (*Raillietiella orientalis*) found within the python's native range in Southeast Asia. Spillover of *R. orientalis* from pythons to native snakes has occurred, with two families and six genera of snakes infected in Florida. The mechanism through which *R. orientalis* infects such diverse taxa has not been explored. We utilized geometric morphometrics to evaluate the morphological plasticity of *R. orientalis* among infected south Florida snakes and pythons. A Procrustes ANOVA revealed significant variation in *R. orientalis* head shape among host taxa. Variation was not attributable to centroid size. *Raillietiella orientalis* from semi-aquatic and aquatic native snakes exhibited the greatest morphological similarity. Morphological analyses of *R. orientalis* recovered from invasive pythons, native pit vipers, and terrestrial snakes each exhibited distinct shapes. Our results suggest *R. orientalis* exhibits significantly different morphology based upon host species infected. Additionally, we provide evidence of host-specific parasite head shape plasticity, an ability that may facilitate infection in a wide array of novel squamate host species.

Introduction

Non-native species can harbor parasites and pathogens capable of infecting native taxa within their introduced range, a process known as parasite spillover (LyMBERG *et al.*, 2014). Potential obstacles to parasite spillover include low host density and lack of an

appropriate intermediate host, for species with indirect life cycles (Paterson *et al.*, 2013). When a parasite species with an indirect life cycle successfully establishes, it may remain host-specific, infecting only the non-native host with which it was introduced, so long as an appropriate intermediate host is present (Dubey & Shine, 2008). This may result from low host susceptibility among potential host taxa within the parasite's invaded range (Vandame *et al.*, 2000), a lack of appropriate intermediate hosts to allow transfer to potential hosts, or from founder effects resulting in reduced plasticity preventing transfer pathways present in the indigenous range of the parasite. Despite the complexity of indirect parasite life cycles, some parasites co-introduced with non-native have demonstrated an ability to infect novel hosts native to the introduced range (Hanselmann *et al.*, 2004; Dobson & Foufopoulos, 2001), often due to a combination of a parasite's phenotypic plasticity among hosts (Kelehear *et al.*, 2011; Huber & Rajakulendran, 1988) and the immunological naivety of a new host, rendering the host unable to deter infection by the novel parasite (Prenter *et al.*, 2004).

Burmese pythons (*Python bivittatus*), native to Southeast Asia, have become established in southern Florida (Snow *et al.*, 2007), where they have co-introduced a lung parasite (*Raillietiella orientalis*) previously unknown from North America (Miller *et al.*, in review). *Raillietiella orientalis* has spilled over into the assemblage of native Floridian snakes, where the parasites experience higher prevalence and intensity, achieve larger body sizes as females, and have populations dominated by reproductive females (Miller *et al.*, in review). In its native range (Asia and Africa), *R. orientalis* infects snakes of at least four families, likely because a variety of intermediate hosts can be used to complete the indirect life cycle of this parasite (Christoffersen & De Assis, 2013). Thus, a diverse

network allowing transmission in the parasite's native range appears to be replicated in Florida. Additionally, *R. orientalis* has been documented to infect non-native lizards in Florida (Nikolakis, unpublished data), and introduced cane toads (*Rhinella marina*) in Australia (Kelehear *et al.*, 2011).

The diversity of definitive hosts known for *R. orientalis* presents challenges, given that lungs of hosts differ greatly in form and function (Perry, 2013). This suggests morphological plasticity as a mechanism to allow attachment to such a wide range of hosts. Geometric morphometric analyses have been utilized to demonstrate phenotypic variability among populations of parasite species (Hugot & Baylac, 1996; Hugot & Baylac 2007; Vignon & Sasal, 2010). Here, we used this analytical technique to investigate plastic responses of *R. orientalis* to phylogenetically and ecologically divergent native host snakes in southern Florida. We used these data to aid in understanding the mechanism that allows this parasite to successfully infect a wide range of native host taxa and to spread rapidly (Miller *et al.*, in review).

Methods

Burmese pythons were collected from their introduced range in southern Florida (Miami-Dade and Monroe Counties) during 2009-2015. Pythons were collected by road surveys, opportunistic encounters, and through a collaborative removal effort between the United States Geological Survey and the National Park Service. Native snakes were salvaged as road-kill during road surveys from locations sympatric with pythons (Table 1). Snakes were dissected and pentastomes were collected and stored in 95% ethanol prior to analyses.

Pentastomes were cleared using an 80% phenol solution for 12 - 24 hours prior to being photographed. Each pentastome was placed on its dorsum on a microscope slide with a cover slip affixed on top of the specimen. Only adult female pentastomes were used, with sex determined based upon the presence (male) or absence (female) of copulatory spicules. Photographs of the ventral hooks and mouthparts of each parasite were taken at 2x magnification using a Nikon Eclipse Ni-E microscope and a Nikon DS-Fi2 camera; a 1000 μm scale was added to each image using Nikon NIS-Elements AR imaging software.

A tps file was prepared with digitized landmarks using tpsDig2 (Rohlf, 2004). Ten homologous landmarks were designated on each photograph to examine placement of the mouth and hooks used to grasp the host's lung tissue during feeding (Fig 1); points 1, 3, 7, and 9 were at the insertion of each hook; points 2, 4, 8, and 10 were at the anterior-most point of the curve of each hook; point 5 was at the esophageal valve at the end of the pharynx; and point 6 was at the anterior-most point of the buccal opening. We used host species as a classifier variable and a Procrustes ANOVA was used to test for differences in parasite head shape among host taxa. Canonical variates analyses (CVA) was used to visualize separation among parasite species in multivariate space. Confidence ellipses (95%) were assigned to all ordinations. Centroid size variation among parasites was analyzed to assess the effect of variation in pentastome length on hook arrangement. Morphological variation of hook structure in *R. orientalis* was compared to a consensus specimen and was visualized in Cartesian space via transformation grids. All analyses were performed using MORPHOJ software (Klingenberg 2011).

Results

Parasite hooks and mouth parts showed significant shape variation (Procrustes $F = 2.27$; $df = 64$; $P = 0.0007$). Centroid size variation was not significant ($F = 2.16$; $df = 4$; $P = 0.09$). Canonical variance analysis (CVA) separated terrestrial (*C. constrictor*) from aquatic (all other species) native snakes along axis 1 and separated pythonoids (*P. bivittatus*) from colubroids (all others) along axis 2 (Fig 2).

Significant variation in pentastome head morphology occurred in length of the buccal cavity and pharynx, hook length, and rotation of the hooks (Fig. 3). *Raillietiella orientalis* samples collected from pythons had the shortest mouthparts, parallel anterior hooks, and posterior hooks that rotated away from the midline of the body compared to *R. orientalis* collected from other snake hosts. Those from *A. piscivorus* exhibited wider placement of the posterior hooks as well as compression along the anteroposterior axis, causing the mouthparts to align with the anterior hooks laterally. The anterior point of each hook also turned medially, a feature unique to *R. orientalis* within this host.

Raillietiella orientalis recovered from *N. clarkii* and *T. sirtalis* did not from each other in morphology, exhibiting comparable anteroposterior compression to *A. piscivorus*, but with hooks that were parallel along the body axis or turned slightly in a lateral direction (Fig. 3). However, increased lateral rotation of the hooks was observed in *T. sirtalis* compared to *N. clarkii*. *Raillietiella orientalis* infecting *Coluber constrictor* exhibited the most compression along the long axis of the body, the longest oral disc, parallel anterior hooks and laterally-rotated posterior hooks (Fig 3).

Discussion

All pentastomes used in this study were identified as *R. orientalis* based on phylogenetic analyses of the COI and 18S genes (Miller *et al.* in review). However, geometric morphometric analyses revealed morphologically distinct parasites according to their host taxa. The greatest similarities observed were exhibited by *R. orientalis* collected from *N. clarkii* and *T. sirtalis*, hosts that are morphologically, ecologically, and phylogenetically similar. *Raillietiella orientalis* recovered from *P. bivittatus*, *A. piscivorus*, and *C. constrictor* each occupied distinct regions of morphological space, with *C. constrictor* parasites displaying the greatest distinction from other host taxa in hook and mouth morphology.

The relationship of parasite morphology and host taxa observed on the CVA ordination plot is best explained by the functional group of the host taxa, with aquatic and semi-aquatic snakes (*A. piscivorus*, *N. clarkii*, *P. bivittatus*, and *T. sirtalis*) separated from a terrestrial snake host (*C. constrictor*) along axis 1. Aquatic native snakes may share similar lung morphology due to the physiological demands of respiration in an aquatic environment. Similar lung function likely requires development of similar mouth and hook arrangements in *R. orientalis* adults. As snakes dive and resurface, pressure changes will cause host lungs to contract and expand beyond that required for respiration in a terrestrial environment.

Significant morphological groupings such as those we observed are likely to have one of two explanations: (1) host immune response affects pentastome development, or (2) pentastomes exhibit morphological plasticity to take advantage of diverse hosts. Host-induced morphology has been documented by numerous studies, but little in the way of

an explanation has materialized (Ponce de León, 1995; Downes, 1990; Kelehear *et al.*, 2011; Blankespoor, 1974; Michel *et al.*, 1972a, 1972b, 1976; Huber & Rajakulendran, 1988). A series of experimental infections performed on calves with the nematode *Ostertagia ostertagi* demonstrated that hosts that had been previously infected, or were older, with a better-developed immune system, contained parasites with significantly underdeveloped vulval flaps (Michel *et al.* 1972a, 1972b, 1976). Further study revealed that parasites were more likely to be underdeveloped if their predecessors had arrested development. However, the strongest factor affecting parasite development was the hosts' immune response.

Variation of *R. orientalis* morphology among host species is not likely a result of host immune response, as *R. orientalis* is competent (*i.e.* able to reproduce) in hosts from both aquatic and terrestrial functional groups. Moreover, if a host immune response elicited a change in mouth and hook morphology, it would be assumed that altering morphology would mitigate infection and limit the success of the parasite within the host. To the contrary, *R. orientalis* exhibits higher prevalence and infection intensity and greater size in snakes native to Florida versus the pythons from which they were introduced (Miller, in review). Therefore, in addition to an ability of *R. orientalis* to exploit immunologically naïve hosts, this parasite is also able to alter its morphology in ways that suggest optimization of an ability to attach to novel taxa within its invaded range.

Other pentastome species have demonstrated significant variation according to their developmental stage. *Raillietiella indica* was considered a distinct species based entirely on morphology, but genetic work elucidated that it is an early instar of

Raillietiella frenatus (Kelehear *et al.*, 2011). Because females included in our study were adults and centroid size variation was found to be insignificant (*i.e.* variation in the length of *R. orientalis* among hosts did not account for the observed taxon-specific parasite hook morphology), ontogenetic variation among pentastomes does not explain the morphological groupings of *R. orientalis* recovered.

Parasites use cues from their microenvironment to alter traits and behaviors that maximize fitness (Mideo & Reece, 2012). Morphological modification in a parasite is a mechanism known to decrease host specificity and, as a result, increases rates of infection (Downes, 1990). Classical morphology-based taxonomies across diverse parasite taxa are beginning to reduce richness to significantly fewer species under molecular analyses, and much of the phenotypic variation has been linked to host taxa (Blankespoor, 1974; Downes, 1990; Huber & Rajakulendran, 1988). Many species complexes have been resolved into a single species, showing that parasites exhibit less co-evolution with a specific host than previously believed and instead optimize the ability to utilize a variety of hosts. *Raillietiella orientalis* demonstrates this phenomenon. Within their native distribution in Asia and Africa, known definitive hosts include snakes from diverse families, including Pythonidae, Colubridae, Elapidae, and Viperidae (Christoffersen & De Assis, 2013). Within its introduced Florida range, *R. orientalis* has been documented to infect snakes from two families and eight genera (Miller in review), along with two genera of non-indigenous lizards (Nikolakis, unpublished data). Within its introduced Australian range, in addition to native snakes, introduced cane toads (*R. marina*) have been infected by this parasite (Kelehear *et al.*, 2011; Kelehear *et al.* 2014). Remarkable phenotypic plasticity may allow *R. orientalis* to infect such a variety of hosts where it has

been introduced. Because these hosts are not co-evolved with *R. orientalis*, their efficacy to resist or ameliorate infection may be reduced, allowing this non-native pentastome to maximize resource use from its novel host. In addition to the capability of *R. orientalis* to infect diverse taxa, the species' demonstrated ability to alter its morphology to successfully infect hosts increases its potential for negatively impacting a multitude of hosts.

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Figure 1 A whole mount of *Raillietiella orientalis* showing placement of ten homologous landmarks used in geometric morphometric analyses to examine variation in hook and mouth morphology among host taxa.

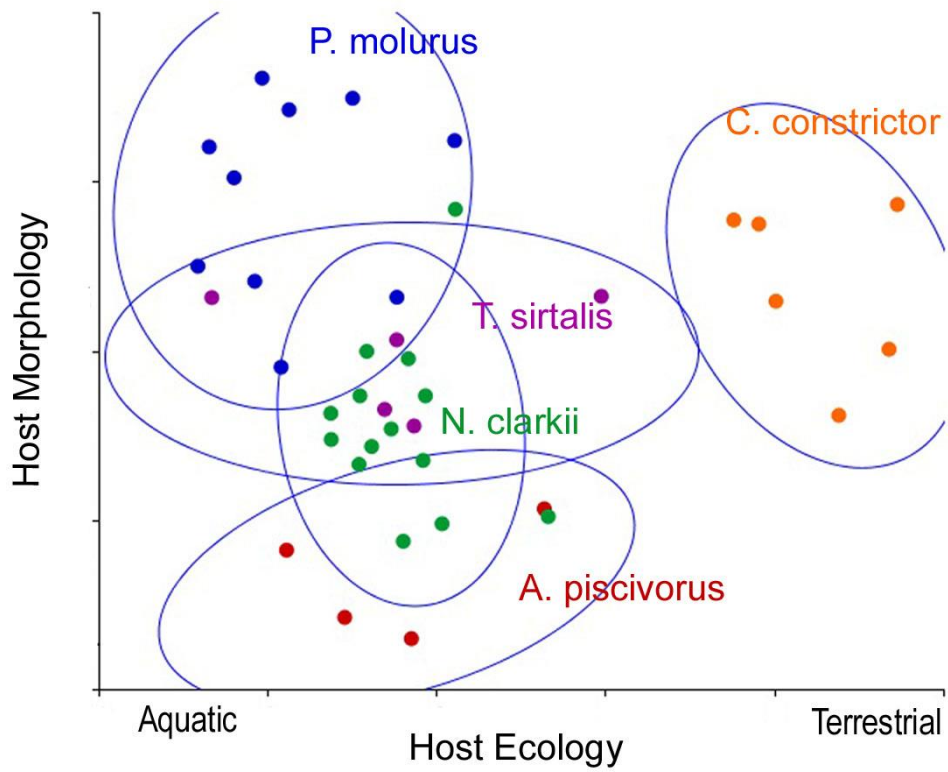


Figure 2 A canonical variance analysis plot depicting the relative morphospace separation of *R. orientalis* among snake hosts. Axes [CV1(x) and CV2 (y)] are replaced by perceived biological axes based on the analysis. 95% confidence ellipses are shown for each centroid.

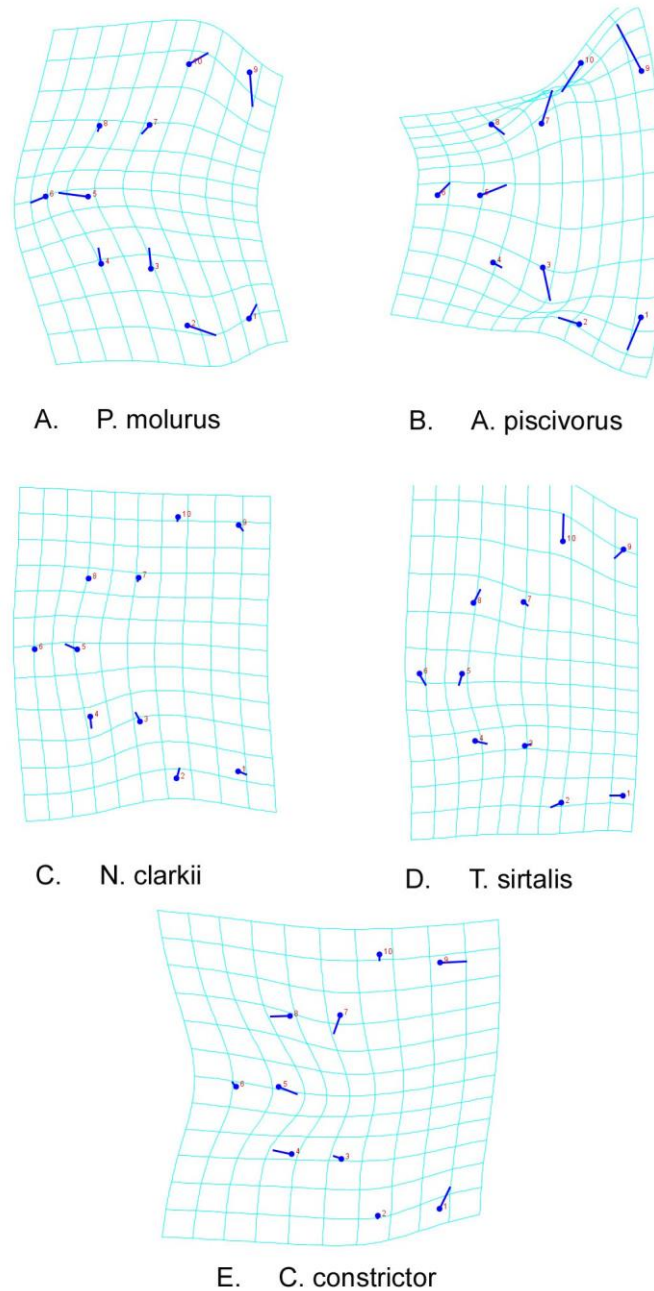


Figure 3 Transformation grids showing host-specific changes in hook and mouth morphology of *R. orientalis*. Variation in morphology among species is shown by lines originating from a circle. Circles indicate the position of the average specimen. The direction of the line relative to a circle indicates specific variation in morphology among species relative to a consensus specimen.

Table 1 Sample sizes of snake hosts and pentastomes (*R. orientalis*) collected from Southern Florida.

Snake host species	Number of host individuals	Total number of parasites
<i>Python bivittatus</i>	4	9
<i>Agkistrodon piscivorus</i>	2	8
<i>Coluber constrictor</i>	2	6
<i>Nerodia clarkii</i>	3	15
<i>Thamnophis sirtalis</i>	3	6
Total	14	44

Chapter 5

Landscape Disturbance Decreases Prevalence of a Non-Native Parasite in Novel Hosts

Abstract

Landscape disturbance often decreases biodiversity and increases the presence of urban-adapted species, including non-native species. How parasites respond to disturbance often mirrors free-living (*i.e.* non-parasitic) taxa, resulting in a homogenized subset of parasite diversity in disturbed environments consisting of urban-adapted (or urban-tolerant) parasites occurring at a higher prevalence and infection intensity. The response of non-native parasites to anthropogenically-altered landscapes is less understood. If non-native parasites follow trends observed for free-living non-indigenous species, we would expect a larger proportion of hosts to be infected with the non-native parasite as well as hosts that exhibit high infection intensity of this parasite in disturbed landscapes. We examined the effect of landscape disturbance on the prevalence and intensity of a non-native pentastomid parasite (*Raillietiella orientalis*). This species, introduced to southern Florida by an invasive population of Burmese pythons (*Python bivittatus*), has spilled-over from pythons to infect snakes native to Florida. We examine prevalence and infection intensity of *R. orientalis* to address whether this non-native parasite follows infection patterns of increased abundance and incidence of many free-living taxa and native parasitic species. To the contrary, we found that prevalence of *R. orientalis* of native snakes was significantly lower in disturbed landscapes. Prevalence of *R. orientalis* of pythons did not vary with disturbance. Infection intensity of *R. orientalis* was not affected by landscape disturbance for native snakes or pythons. Our results support that prevalence of *R. orientalis* is higher among native snakes occupying undisturbed

landscapes and elucidates the complexity of host-parasite relationships in variable environments.

Introduction

Landscape disturbance is a leading cause of biodiversity loss, with urban-sensitive species replaced by urban-adapted and urban-tolerant species as disturbance increases along an urban-rural gradient for many taxa (McKinney 2002; Bradley and Altizer 2006; McKinney 2006; McKinney 2008; Olden *et al.* 2006). Anthropogenic alteration of landscapes exerts stressors on native taxa including habitat loss and fragmentation, pollutants, and altered climatic regimes. These stressors can facilitate the invasion of non-native species (McDonnell and Pickett 1990; McKinney 2002, McKinney 2006).

While many native species decrease with increasing disturbance, non-native species tend to increase with habitat disturbance (Marzluff 2001; McKinney 2002). Biological invasions occurring in disturbed landscapes are an additional stressor on native taxa, and can result in negative synergistic effects such as altered parasite-host relationships (Lafferty and Kuris 1999). Parasites of non-native species may spillover to infect native species within the non-native species' introduced range (Daszak *et al.* 2000; Hatcher *et al.* 2012). The impact of introduced parasites on hosts native to the parasite's invaded range may be augmented in disturbed landscapes where stressors associated with disturbance may result in increased parasite prevalence and infection intensity (Bradley *et al.* 2008; Giraudeau *et al.* 2014).

Parasite communities in anthropogenically-altered landscapes often consist of a homogenized subset of parasite diversity found within hosts in undisturbed landscapes. Often associated with this reduction in parasite species richness, is an increase in the

prevalence and infection intensity of those parasite species capable of completing their life cycle in disturbed environments (King *et al.* 2007; McKinney 2006, McKenzie 2007; Lehrer *et al.* 2010; Schotthoefer *et al.* 2011). Keas and Blankespoor (1997) examined trematode communities in anthropogenically-altered sites and found that, while overall parasite species richness declined, several trematode species associated with urban-adapted hosts had increased in abundance. This effect was observed for a parasite that had co-evolved with its natural host; few studies have examined parasite infection dynamics in disturbed landscapes when a non-native parasite has been introduced to immunologically naïve hosts. The additional stressor of an introduced parasite in disturbed systems may further complicate host-parasite dynamics, as hosts within the non-native parasite's range are likely immunologically naïve to novel parasites and may suffer consequences of infection (Mastitsky *et al.* 2010, but see Lymbery *et al.* 2014).

Many regions of Florida are heavily disturbed by human activities, which threaten native diversity primarily through habitat loss and introduction of non-native species. Burmese pythons (*Python bivittatus*) have been introduced to southern Florida and have been well established since the early 2000s. Miller *et al.* (in review) found that pythons have introduced a pentastome (*Raillietiella orientalis*) parasite, native to the python's range in Southeast Asia, to Florida that is a hematophagous, lung parasite with an indirect life cycle. This pentastome has spilled over from pythons to infect snakes native to Florida. Native snake hosts experience higher prevalence and infection intensity of *R. orientalis* compared with pythons (Miller *et al.*, in review). However, the influence of landscape disturbance on parasite infection dynamics of this non-native pentastome in Florida's native snakes is unknown. Inquiry into how non-native parasites respond to

landscape disturbance can aid in identification of areas of increased infection risk to taxa within the parasites' introduced range.

We examine the effect of landscape disturbance on infection patterns of a non-native parasite. Specifically, we measure parasite prevalence and intensity of *R. orientalis* of native Florida snakes and Burmese pythons to determine if *R. orientalis* fits the pattern of increased prevalence and infection intensity observed for more generalist parasites in disturbed landscapes (King *et al.* 2007; Bradley *et al.* 2008; Machut and Limberg 2008; Schotthoefer *et al.* 2011; Giraudeau *et al.* 2014).

Methods

Specimen collection & study location

Native snakes were collected as road kill during organized road surveys and opportunistically throughout Florida (Monroe, Miami-Dade, Broward, Glades, Hendry, Highlands, Lake, Martin, Okeechobee, Orange, Polk, St. Lucie, and Collier counties) during 2012 – 2016. Additionally, live Burmese pythons were collected throughout their range in Monroe, Miami-Dade, Collier, and Broward counties as part of an invasive species removal program. Coordinates of the capture location for each snake were recorded. Live snakes were euthanized, and all were necropsied and pentastomes collected and stored in 95% ethanol. Pentastomes were identified to genus based on morphological features and to species by molecular analyses (Miller *et al.*, in review).

GIS analysis

Coordinates of each capture location for each snake were plotted using ArcGIS 10.3 software. Buffer zones, encompassing three sizes (500 m, 1000 m, and 1500 m radius), were created for each snake surrounding its capture location. Buffer zone sizes

were selected to provide a snapshot of the habitat occupied by a snake at the time of capture (*e.g.* 500 m buffer size), while larger buffer sizes (1000 m and 1500 m) provided a broader spatial view of landscapes utilized by each snake. In addition, we chose to examine several buffer sizes to account for variation in home-range size due to the body size disparity among native Florida snakes and pythons. Land cover data available from the U.S. Geological Survey Land Cover Database (Homer *et al.* 2015), were used to identify land cover categories (*i.e.* wetland, forest, grassland, cultivated crops, pasture/hay, developed: open space; low intensity; medium; and high intensity) within each buffer. Land cover categories are defined by Anderson (1976). These categories were then grouped into two classes representing un-altered landscapes (wetland + forest + grassland; hereafter referred to as “undisturbed”) or altered landscapes (cultivated crops + pasture/hay + developed: open space, low intensity; medium; and high intensity; hereafter referred to as “disturbed”). Geospatial Modelling Environment Version 0.7.4.0 software (Beyer 2015) was used to calculate the proportion of each land-use class (*i.e.* disturbed or undisturbed) within its respective buffer for each snake.

Statistical analyses

Parasite prevalence (the proportion of infected hosts within a sample) and intensity (the number of parasites per host) of *R. orientalis* were quantified independently for native Florida snakes (all native snake species were grouped, and hereafter referred to collectively as “native snakes”) and invasive pythons within each of the three buffer zone sizes. Comparisons of prevalence of *R. orientalis* were examined for native snakes and then for pythons using binomial logistic regression with a binary response and the disturbed landscape class included as the predictor. Infection intensities of *R. orientalis*

for native snakes and pythons were quantified using negative binomial regression to account for overdispersion of the dependent variable and were regressed against the disturbed landscape class. All analyses were performed using R statistical software, Version 3.4.0 (R Core Team 2017).

Results

We examined 140 native snakes and 894 pythons for the presence of *R. orientalis*. We documented 69 native snakes and 98 pythons infected with *R. orientalis* (see S1 Appendix for data). Prevalence of *R. orientalis* of native snakes decreased significantly with increasing landscape disturbance at all three buffer sizes (500 m buffer, $P = 0.033$, $df = 139$, $SE \pm 0.787$, Fig.1; 1000 m buffer, $P = 0.030$, $df = 139$, $SE \pm 0.757$, Fig. 2; 1500 m buffer, $P = 0.036$, $df = 139$; $SE \pm 0.7431$, Fig. 3). There was no significant effect of disturbance on prevalence of *R. orientalis* of pythons at any of the buffer sizes examined. Infection intensity of *R. orientalis* did not vary by landscape disturbance for native snakes or for pythons at any buffer size.

Discussion

Disturbed landscapes often result in a homogenized subset of biodiversity, with an increase in the intensity and prevalence of urban-adapted species, including non-native species, observed for many taxa (McKinney 2002; Bradley and Altizer 2006; McKinney 2006; Olden *et al.* 2006). Parasites, while studied in this context to a lesser extent, have been shown to follow these trends observed for free-living taxa (McKenzie 2007; Machut and Limberg 2008). However, how non-native parasites respond to differing landscapes is less understood. Our results do not support an increase in the proportion of infected individuals and parasite load of *R. orientalis* with disturbance. To the contrary, we

observed the prevalence of *R. orientalis* in native snakes to be lower in disturbed versus undisturbed landscapes, and no variation in the infection intensity of this parasite among landscapes. In addition, prevalence and intensity of *R. orientalis* in pythons did not vary among disturbed or undisturbed landscapes. This result suggests that while undisturbed landscapes may provide a refuge from introduced parasites for many taxa, certain taxa, such as snakes observed in this study, may face infection risk in both disturbed and undisturbed landscapes.

The increased probability of *R. orientalis* infection for native snakes associated with undisturbed landscapes likely reflects the ecology of pythons and the invasion process of *R. orientalis* in southern Florida. Willson *et al.* (2011) examined plausible models for the establishment of Burmese pythons in Florida and identified the most biologically supported model for their establishment which supports the release of a small number of pythons in in Flamingo, the southernmost tip of the Florida peninsula in Everglades National Park. Since their introduction, pythons are now widely distributed in southern Florida, primarily inhabiting the Greater Everglades Ecosystem, which consists of large expanses of undisturbed wetland habitat. Pythons are responsible for the introduction of *R. orientalis* to Florida (Miller, in review), therefore, *R. orientalis* has co-existed with pythons and native snakes for the longest duration in undisturbed landscapes. The increased prevalence of this parasite in undisturbed versus disturbed landscapes for native snakes may result from longer exposure between parasite and host compared with more disturbed landscapes. The python population in southern Florida has been growing exponentially since the early 2000s (Snow *et al.* 2007), and therefore *R. orientalis* may be early in the invasion process. This species' efficacy of using native

snakes as hosts may not yet be maximized, resulting in a higher prevalence in undisturbed landscapes where pythons and native snakes have co-existed for the longest duration of time.

Miller *et al.* (in review) found that prevalence and infection intensity of *R. orientalis* did not differ among native snakes that were collected in the python invasion front or the core of the python's distribution. As python density is higher in the core region compared with the invasion front, this implies that python density is not the primary factor driving the transmission of this parasite among native snakes. In addition, Miller *et al.* (in review) documented native snakes infected with *R. orientalis* collected just northward of the python invasion front where python density among counties is low to non-existent, further supporting that a high density of pythons is not necessary for *R. orientalis* to spread among native snakes. However, it is still likely that python density does contribute to the spread of *R. orientalis*, particularly in the initial stages of the parasites invasion. Landscape characteristics may be a more important factor in determining the transmission of *R. orientalis*, as prevalence of this parasite in native snakes observed in this study varied significantly with land-use. While Miller *et al.* (in review) examined variation in prevalence and intensity of *R. orientalis* of native snakes in response to geographic variation in the distribution and abundance of pythons, our results demonstrate that the type of landscape, undisturbed or disturbed, may play an additional role in predicting the probability and severity of *R. orientalis* infection in snake hosts.

Variation in prevalence among landscapes may reflect disparity in the presence and abundance of intermediate hosts in undisturbed and disturbed landscapes. Disturbed habitats are likely to have fewer snakes and fewer intermediate hosts necessary for the

life cycle of *R. orientalis*, leading to reduced prevalence in this landscape and/or a spotty distribution of *R. orientalis* infection due to a patchy distribution of parasite and host. The life cycle of *R. orientalis* is unknown, however there is support that intermediate hosts may include frogs, lizards, and small mammals (Kelehear *et al.* 2014; Miller *et al.*, in review). A better understanding of the life cycle of this parasite is needed to advance our understanding of the interplay of intermediate hosts and landscape disturbance on infection dynamics of *R. orientalis*.

Miller *et al.* (in review) found that intensity of *R. orientalis* in native snakes was higher compared with pythons. Nonetheless, intensity among native snakes did not vary among snakes collected from the python invasion front and those collected from the core of the python distribution. Regarding landscape disturbance, infection intensity of *R. orientalis* did not differ for native snakes or pythons among undisturbed and disturbed landscapes indicating that once infected, the parasite does equally well among native snakes and among pythons irrespective of landscape type. As native snakes are immunologically naïve to infection by *R. orientalis*, their immune response may be similarly inadequate at keeping the number of parasites in check in disturbed and undisturbed landscapes. Pythons did not vary in infection intensity in disturbed and undisturbed landscapes, which may reflect the co-evolutionary history shared among *R. orientalis* and pythons and the ability of pythons to mitigate infection intensity despite stressors associated with disturbed landscapes.

While native snakes and pythons infected with *R. orientalis* may not follow trends of parasite infection dynamics in disturbed landscapes, this is not the case for some other reptilian taxa. For example, the presence of the pentastome (*R. frenata*), which was co-

introduced to Australia with the invasive Asian house gecko, (*Hemidactylus frenatus*), was found to decrease along an urban-rural gradient (Coates *et al.* 2017) with the non-native *R. frenata* replaced by pentastome species native to Australia as landscapes became increasingly rural. Non-indigenous hosts such as *H. frenatus*, which are found almost exclusively in disturbed landscapes, may keep non-native parasites restrained to these environments through habitat restrictions of the non-indigenous hosts. Conversely, when a non-native host is capable of invading both disturbed and undisturbed landscapes, such as Burmese pythons, native taxa may not be afforded reprieve from infection by non-native parasites in undisturbed environments. Thus, these non-native parasites present a risk to host species that are urban-adapted or urban-tolerant, as well as those that are urban-avoiders.

The complexities of land-use effects on parasite infection patterns are relatively unknown (Murray and Daszak 2013). Our results present a study of host-parasite dynamics that does not conform to trends observed for other taxa in disturbed environments and stresses the need for further study of how landscape variation effects parasite transmission in variable environments.

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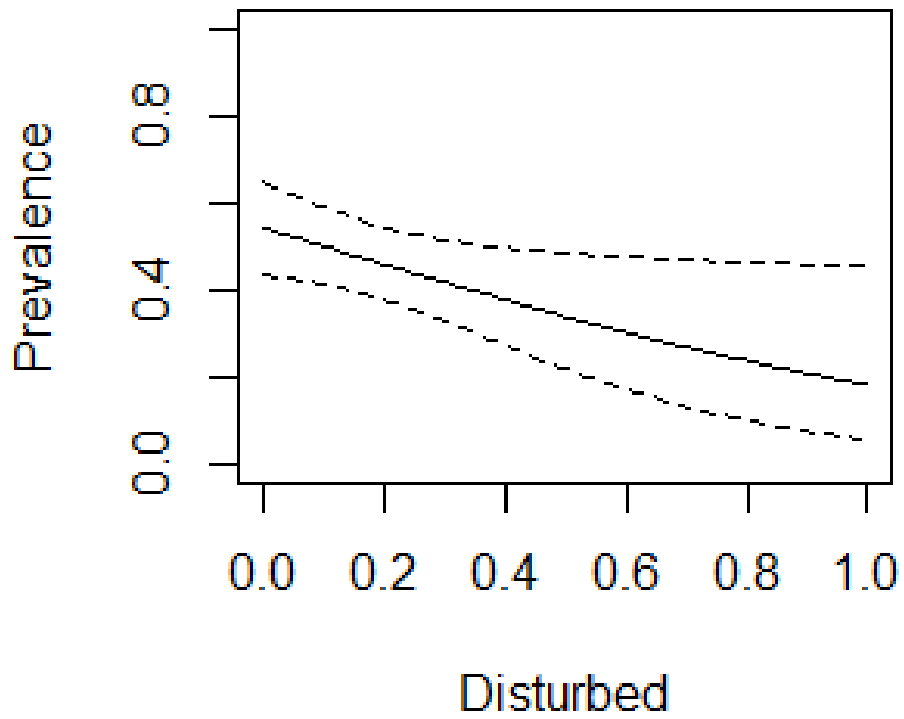


Figure 1 Prevalence of *R. orientalis* is shown for native snakes at a 500 m buffer created around the capture location of each snake. As the proportion of disturbed landscapes increased the prevalence of *R. orientalis* decreased among snakes.

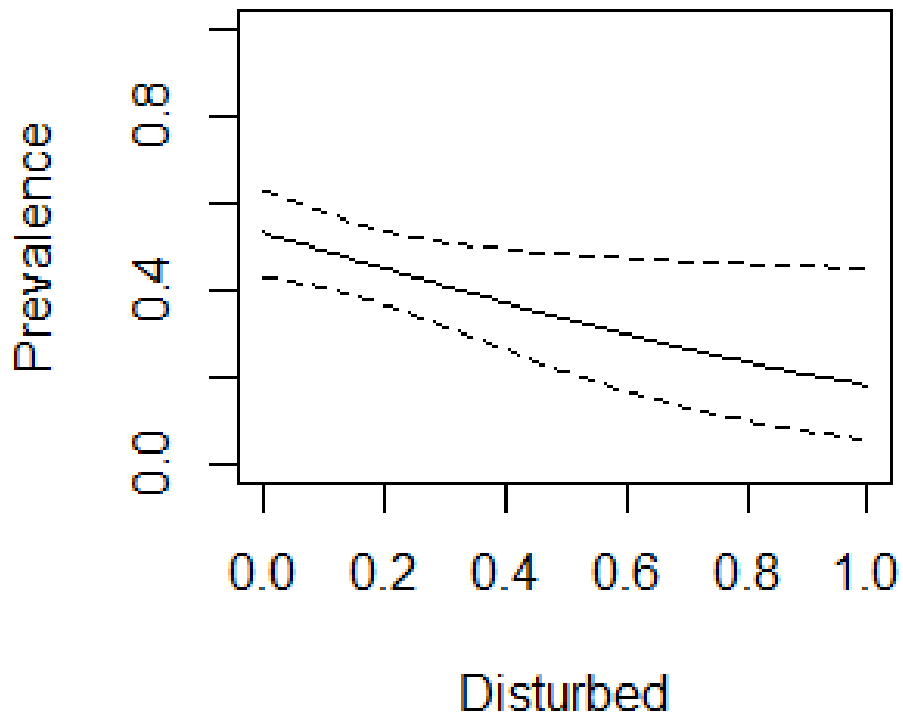


Figure 2 Prevalence of *R. orientalis* is shown for native snakes at a 1000 m buffer created around the capture location of each snake. As the proportion of disturbed landscapes increased the prevalence of *R. orientalis* decreased among snakes.

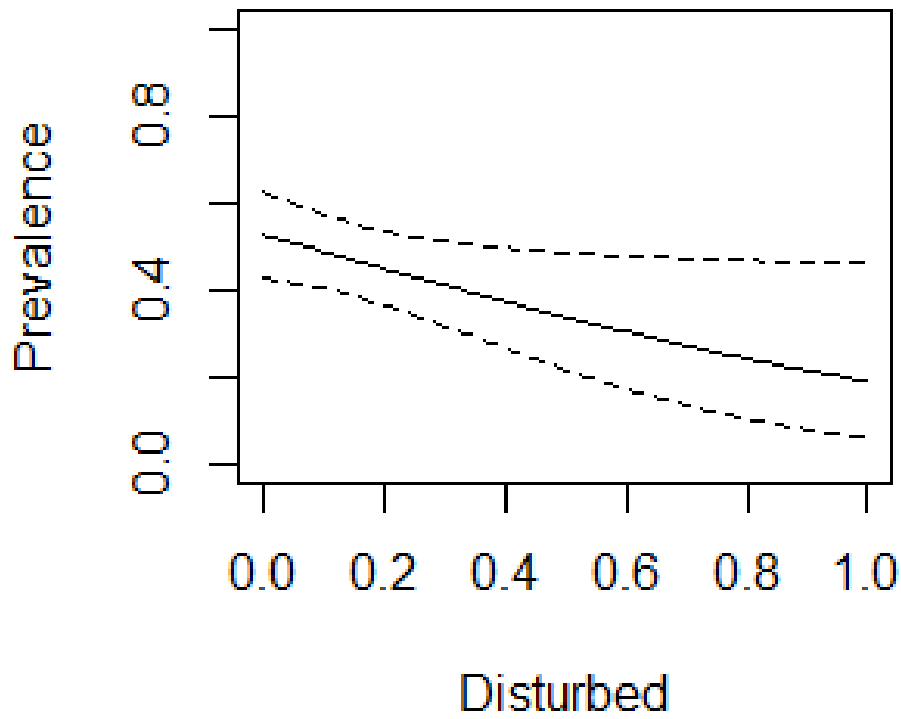


Figure 3 Prevalence of *R. orientalis* is shown for native snakes at a 1500 m buffer created around the capture location of each snake. As the proportion of disturbed landscapes increased the prevalence of *R. orientalis* decreased among snakes.

Chapter 6

Conclusion

Burmese pythons (*Python bivittatus*) have introduced a pentastomid lung parasite, *Raillietiella orientalis*, to southern Florida that is infecting native snakes. This parasite is native to the python's range in southeast Asia, and is documented for the first time in North America in this study. Typically, in co-evolved parasite-host relationships, parasite virulence is kept in check by the immune response of the host. However, native snakes are immunologically naïve to this parasite, as these hosts lack prior exposure to *R. orientalis*, which may increase any negative impact imparted on native snakes from infection (Mastitsky *et al.* 2010). In a study comparing the virulence of a non-native parasite in naïve hosts and the host responsible for the parasite's introduction, 85% of the cases examined documented increased virulence in the naïve host (Lymbery *et al.* 2014). However, Lymbery (*et al.* 2014) note that increased virulence may arise due to the low likelihood of a non-indigenous host becoming established if that host harbors a highly virulent parasite. Therefore, in comparison, a naïve host within the non-native parasite's introduced range is likely to exhibit higher virulence. Nonetheless, naïve hosts infected by parasites with which they do not share a co-evolutionary history often suffer deleterious effects of infection. *Raillietiella orientalis* was highly prevalent among native snakes observed in this study and snakes often suffered high parasite loads indicating that native snakes may incur high energetic costs at the expense of eliciting an immune response to combat infection.

An increase in the severity of pentastome infection is positively correlated with increased negative impacts to the host (Caballero *et al.* 2015). Approximately half of the

total number of snake species in southern Florida were infected with *R. orientalis*. Compared with pythons, native snakes exhibited higher prevalence, higher infection intensity, larger females, and adult female *R. orientalis* comprised a significant majority of the total number of pentastomes within a host. *Raillietiella orientalis* appears capable of exploiting immunologically naïve native snakes, increasing the risk of population declines of infected native species (Anderson and May 1978). The risks from *R. orientalis* infection to native snakes is not limited to those that occur in sympatry with pythons. *Raillietiella orientalis* has been documented just northward of the python invasion front, indicating *R. orientalis* can expand its range independent of pythons through infected native snakes and well beyond the python's current distribution. While the geographic expansion of pythons may be limited due to physiological/climactic constraints on the host (Rodda *et al.* 2009), *R. orientalis* appears to have few obstacles restricting its ability to spread within native snakes throughout North America if intermediate hosts suitable for its life cycle are available.

As a diversity of snake species in Florida are infected with *R. orientalis*, some of which share very little, if any, dietary overlap, multiple hosts are implicated for the transmission of this parasite in Florida. *Raillietiella orientalis* has been introduced to Australia where it infects predominantly frog-eating snakes leading to the supposition that frogs are key to the life cycle of this parasite (Kelehear *et al.* 2014). Snakes that exhibited the highest prevalence and intensity in this study were also frog-eating snakes, which corroborates Kelehear *et al.* (2014) that frogs are likely involved in the life cycle of *R. orientalis*. However, I have documented a shrew (*Crocidura sp.*) infected with larval *R. orientalis*, representing the first time this parasite has been recorded in a

mammalian intermediate host. Shrews native to Florida, including the Southern Short-tailed Shrew (*Blarina carolinensis*), are known to consume vertebrates, including frogs, and/or feed on insects that are attracted to vertebrate carcasses. The diet of some rodents, such as the invasive Black Rat (*Rattus rattus*), which was introduced to Florida in the 1800s, also include frogs. As intermediate hosts can be exposed to pentastome eggs through contact with contaminated food and water, this accidental exposure along with predatory encounters among frogs, shrews, and rats may prove to be important links in the life cycle of *R. orientalis* in southern Florida.

While *R. orientalis* has shown efficacy in its transmission to multiple hosts, one mechanism allowing this success appears to involve the morphology of the hooks and oral disc associated with feeding on the blood of its host. *Raillietiella orientalis* attaches to the lungs of its host using a pair of hooks located atop appendages on either side of the oral disc (*i.e.* mouth) of the parasite (Riley 1986). A geometric morphometric analysis of the position of these structures among native snakes and pythons yielded significant morphological variation dependent upon host species, with water snakes sharing the most morphometric similarity. Morphological plasticity of hook and oral structures may be advantages to *R. orientalis* when infecting hosts with varying life history strategies. For example, aquatic snakes would experience different lung pressure than a terrestrial snake due to occupying an aquatic environment. Furthermore, the micro-structure of lung tissue may vary among snake species and hook and oral disc morphology may change to adapt to differences in lung morphology. The ability of *R. orientalis* to adjust to different environments within a host may increase its capacity to infect a diverse array of taxa.

While morphology aids the success of *R. orientalis* infection at the level of the individual, other factors, such as land-use, appear to influence the prevalence and intensity of *R. orientalis*. Parasite communities in disturbed landscapes are often a homogenized subset of the diversity found in undisturbed environments, with more generalist parasites able to successfully adapt to anthropogenically-altered landscapes occurring at higher prevalence and intensity (Blair 2001; McKinney 2002; McKenzie 2007). Contrary to these findings, I documented decreased prevalence of *R. orientalis* among native snakes in disturbed landscapes. Therefore, *R. orientalis* does not conform to trends in parasitism in altered landscapes. This result stresses the complexity of host-parasite dynamics in variable environments and identifies an area of research where further study is required. Urban-adapted or urban-tolerant taxa often include non-native species, capable of adjusting to altered landscapes (Blair 2001). The partitioning of species that occupy disturbed and undisturbed landscapes may shield taxa that are urban-avoiders from exposure to the non-native parasites of non-indigenous hosts (Coates *et al.* 2017). Native snakes infected with *R. orientalis* are not afforded this reprieve from introduced parasites as pythons are adept at occupying undisturbed and disturbed environments making host life history and land-use an important consideration when examining spatial trends in the infection dynamics of non-native parasites.

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