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Documenting Range Expansion of the Invasive Pentastome Parasite, *Raillietiella orientalis*, Using Southern Black Racer and Eastern Coachwhip Road Mortality

Jenna N. Palmisano^{1,*}, Terence M. Farrell², Corinna M. Hazelrigg³, and Michael N. Brennan⁴

Abstract - The spillover of an invasive lung parasite, *Raillietiella orientalis*, is an important conservation concern for snake species in the southeastern US, as native snake hosts exhibit severe infections that can be fatal. In this report, we used opportunistic captures of road-killed snakes to document the presence of *R. orientalis* in 4 central Florida counties where it had not previously been documented. These new localities expand the known range of *R. orientalis* on the eastern and western coasts of Florida as well as fill in gaps of its known range. In 2 of 4 cases, *R. orientalis* individuals moved into the snake's mouth and emerged after host death. This evidence indicates that the use of road-killed specimens of frequently infected snake species may be a useful method for detecting *R. orientalis*. Additional surveys for *R. orientalis* are needed throughout the southeastern US, as this parasite is a major conservation concern for many snake species, its use of synanthropic intermediate hosts make its geographic spread unpredictable, and it is rapidly infiltrating additional populations of snakes.

Introduction. Spillover of the Asian pentastome parasite *Raillietiella orientalis* (Sambon) to snake species in Florida was likely initiated with the establishment of *Python bivittatus* Kuhl (Burmese Python) (Miller et al. 2018). *Raillietiella orientalis* is an endoparasitic crustacean that lives in the lungs of its definitive hosts, which are most often snake species (Kelehear et al. 2014, Miller et al. 2020). In the definitive host, adult pentastomes feed on host blood and reproduce. The eggs pass through the definitive host's digestive tract and enter the environment with the host's feces (Pare 2008). *Raillietiella orientalis* has an indirect life cycle that appears to utilize multiple intermediate hosts in Florida (Palmisano et al. 2022). Coprophagous insects, such as roaches, ingest pentastome eggs that develop into larvae that then infect a variety of insectivores, including lizards and frogs after infected insects are consumed (Palmisano et al. 2022). Native snakes can be infected when they consume the second intermediate host (Miller et al. 2020). The impacts of *R. orientalis* on snakes are largely unknown, but pentastome infections may be associated with mortality of *Sistrurus miliarius* (L.) (Pygmy Rattlesnake) and *Drymarchon couperi* (Holbrook) (Eastern Indigo Snake) (Bogan et al. 2022, Farrell et al. 2019).

In addition to invading both Australia (Kelehear et al. 2014) and southern Florida (Miller et al. 2018), *R. orientalis* now appears to be rapidly expanding its geographic range to central and north Florida, with high potential to cross into neighboring southeastern states. Miller et al. (2018) initially found that *R. orientalis* infections were restricted to the Burmese Python's range in southern Florida from a large sample of snakes collected from 2004–2016. However, soon thereafter, infections were documented in Volusia County, FL, at least 160 km north of the Burmese Python's range (Farrell et al. 2019) as well as in Lake County (Miller et al. 2020). Walden et al. (2020) documented *R. orientalis* in Alachua County, FL, a site that was 340 km north of the Burmese Python's established range in

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Florida. Given the apparent rapid spread and potentially fatal host impacts of *R. orientalis*, there is a need for continued field surveys of *R. orientalis* to determine the pace and extent of its geographic spread.

It can be difficult to monitor the spread of endoparasites, including *R. orientalis*, as they reside within their hosts. The only method to document the presence of *R. orientalis* in intermediate hosts involves dissecting the host and examining the viscera under magnification (Palmisano et al. 2022). In definitive hosts, pentastome infection can be determined either by dissection or examination of feces for eggs (Pare 2008). Typically, studies have performed necropsies of snakes that were road-killed (Miller et al. 2020) or found dead in the field (Farrell et al. 2019, Walden et al. 2020). In this note, we opportunistically sampled road-killed snakes and documented the presence of *R. orientalis* in 4 counties in central Florida where it was previously unknown. These cases expand the known geographic distribution of this parasite and illustrate the utility of examining road-killed snakes to detect *R. orientalis*.

New localities. Seminole County – We found a dead *Coluber constrictor priapus* Dunn and Wood (Southern Black Racer) lying beside a road in a residential neighborhood (28°38'60.36"N, 81°16'39.07"W) on 27 April 2022. This Southern Black Racer had a snout–vent length of 102 cm and was a reproductive female with developing follicles. The snake had a live pentastome emerging from its mouth extending onto the pavement (Fig. 1A). Inspection of its oral cavity revealed at least 6 more pentastomes (Fig. 1B). In



Figure 1. (A) An adult female *Raillietiella orientalis* crawling out of a dead female Southern Black Racer in Seminole County. (B) Six adult female *R. orientalis* exiting the glottis of the snake from Seminole County. (C) An adult *R. orientalis* on the road after exiting the glottis of a dead Southern Black Racer in Hernando County. (D) Multiple *R. orientalis* and trematode parasites exiting the glottis of a dead Pygmy Rattlesnake in Volusia County.

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a necropsy, we removed 61 *R. orientalis* from the mouth, trachea, lungs, and body cavity of the snake. There were 28 smaller pentastomes, presumably males given the findings of Miller et al. (2020), with an average length of 12 mm and 33 larger pentastomes, presumably females, with an average length of 55 mm. We tentatively identified the pentastomes as *R. orientalis* based on morphological features (head shape and the arrangement of hooks). We confirmed our identification with conventional PCR using 18S rRNA (von Reumont et al. 2009). We sequenced the amplified DNA using Sanger sequencing and compared them to sequences available in GenBank confirming the identification of *R. orientalis* (NCBI accession OQ361694). This new record in Seminole County extends the range of *R. orientalis* 30 km to the southwest of the nearest prior record in Lake County (Fig. 2; Miller et al. 2020).

Flagler County – We found a Southern Black Racer dead on state road 11 (29°24'32.65"N, 81°18'19.58"W) on 6 May 2021. In this area, the road transects a large expanse of pine flatwoods habitat. No pentastomes were emerging from the mouth, but 1 pentastome was found

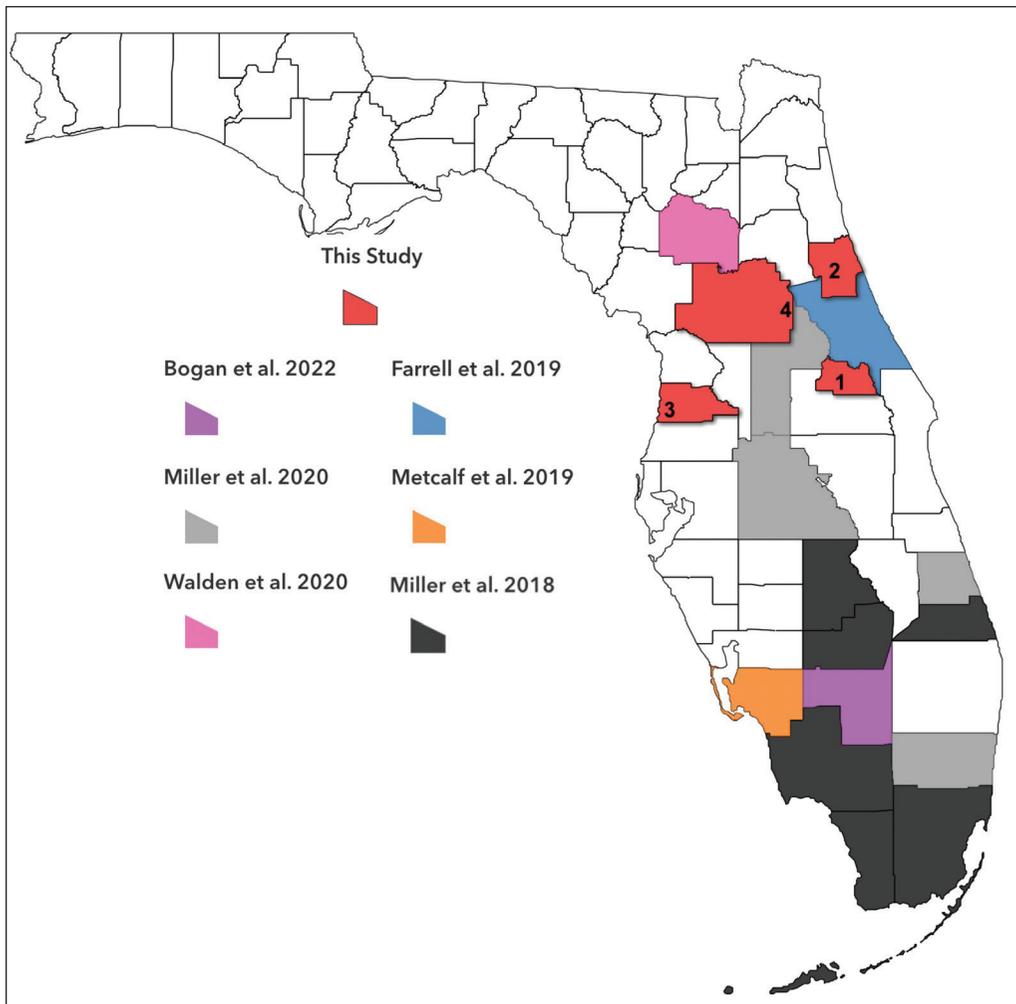


Figure 2. A map of Florida counties showing previous and new localities for the invasive pentastome *Raillietiella orientalis*. The numbers (1–4) show the position of each new locality within the county: 1 = Seminole County, 2 = Flagler County, 3 = Hernando County, and 4 = Marion County.

in the lung during a necropsy. We examined its morphology with a dissecting microscope to confirm its identity as *R. orientalis* based on the highly identifiable hook morphology, head-shape, and annulation (Kelehear et al. 2011). This observation extends the known range of *R. orientalis* 36 km to the northeast from the nearest known location in Volusia County (Fig. 2; Farrell et al. 2019).

Hernando County – We received a message on our Facebook account about a snake infected with parasites. An amateur natural historian found a Southern Black Racer on 10 July 2019 in a residential area (28°28'25.86"N, 82°36'04.18"W) of Spring Hill, FL. She observed parasitic worms crawling out of the snake's mouth and photographed the specimen (Fig. 1C). No specimen was preserved, but from the photograph, we identified the parasite as *R. orientalis* given its flattened head shape and lack of highly apparent annulation on the body. This location is ~120 km southwest of the nearest previously known specimen (Fig. 2; Miller et al. 2020).

Marion County – We found a dead *Masticophis flagellum flagellum* (Shaw) (Eastern Coachwhip) lying on the side of State Road 40 in the Ocala National Forest (29°10'36.30"N, 81°40'36.23"W) on 23 June 2022. The road passes through *Pinus clausa* (Chapm. ex Engelm.) Vasey ex Sarg. (Sand Pine) scrub habitat in this area. The snake had a snout-vent length of 124 cm and was a female of unknown reproductive status due to damage to the posterior of the body. We conducted a partial necropsy on site and collected 13 pentastomes of varying sizes. We did not measure the full intensity of infection due to time constraints in the field, though an additional 15 pentastomes were observed around the heart, lungs, liver, and stomach that were not collected. No pentastomes were observed in the mouth or trachea. We identified the pentastomes as *R. orientalis* based on morphological features and molecular evidence. We conducted conventional PCR using 18S rRNA (Brookins et al. 2009). We sequenced the amplified DNA using Sanger sequencing, and the sequences matched those available in Genbank for *R. orientalis* with high homology (NCBI accession OQ360656). This locality is 30.3 km WNW of the closest previously published known locality for *R. orientalis* (Fig. 2; Farrell et al. 2019) and fills a large distributional gap between the Alachua County record (Walden et al 2020) and records in Lake (Miller et al. 2020) and Volusia (Farrell et al. 2019) counties.

Discussion. *Raillietiella orientalis* detections in the 4 infected snakes described above greatly expand the known geographic range of this parasite in central Florida. The new localities extend the known geographic range of this parasite much closer to the east (18 km from the Atlantic Ocean) and west (6.5 km from the Gulf of Mexico) coasts in this region. We suspect the apparent range restriction to interior central Florida was a result of search efforts being limited to that part of central Florida, as in Miller et al. (2020), rather than a result of the actual distribution of *R. orientalis*. Therefore, the continued opportunistic inspection of road-killed specimens throughout the southeastern US could be an effective technique for documenting the presence of this parasite and assessing the threat of infection to native snake populations.

Our findings and the records of high prevalence of *R. orientalis* infection in South Florida for the Southern Black Racer and Eastern Coachwhip (Miller et al. 2020) indicate efforts of future inspection of road-killed specimens could target such species. We suggest collection of the parasites for careful morphological and molecular analysis to ensure correct species identification as we have done for the specimens collected in Seminole and Marion counties. In addition to the accounts of pentastome movements after death in this note, we have observed *R. orientalis* emerging from the mouth of Pygmy Rattlesnakes (Fig. 1D), and

Kelehear et al. (2014) noted the presence of *R. orientalis* in the oral cavity of road-killed Australian snakes. Hopefully, researchers and amateur natural historians in the southeastern US can use parasite movements after host death, combined with the fairly large body size of adult female *R. orientalis* (40–70 mm), to enable quick road-side inspection of snakes to reveal pentastome infection and indicate dead snakes that should be collected for further examination. If feasible, we recommend the collection of the host for necropsy, as pentastomes may fail to move into oral cavity after host death. Specimens should be frozen as soon as possible after collection to preserve morphological and molecular integrity.

Raillietiella orientalis is now an important conservation concern in the southeastern United States. It has high infection prevalence in many snake species in south Florida and is known to infect many genera of native snake species including *Agkistrodon*, *Coluber*, *Crotalus*, *Drymarchon*, *Lampropeltis*, *Masticophis*, *Nerodia*, *Thamnophis*, *Pantherophis*, and *Sistrurus* (Metcalf et al. 2019, Miller et al. 2020). *Raillietiella orientalis* can also have high parasite intensity as shown by the Southern Black Racer from Seminole county with more than 60 pentastomes and the Eastern Coachwhip with at least 28 pentastomes. There is growing evidence that infection with *R. orientalis* may have major negative health consequences for native species including Pygmy Rattlesnakes (Farrell et al. 2019), *Nerodia fasciata pictiventris* (Cope) (Florida Watersnake) (Walden et al. 2020), and Eastern Indigo Snakes (Bogan et al. 2022). Unfortunately, this study and several other recent investigations (Farrell et al. 2019, Miller et al. 2020, Walden et al. 2020) indicate *R. orientalis* is spreading rapidly. The ability of *R. orientalis* to use abundant, synanthropic intermediate hosts (Palmisano et al. 2022) may facilitate range expansion. While Miller et al. (2018) surveyed roadkill snakes in Georgia and Alabama and did not find *R. orientalis*, additional surveys for *R. orientalis* north of Alachua County, FL, are warranted to determine how rapidly this parasite is spreading.

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Literature Cited

- Bogan, J.E., Jr., D.A. Steen, B. O'Hanlon, M.M. Garner, H.D.S. Walden, and J.F.X. Wellehan Jr. 2022. *Drymarchon couperi* (Eastern Indigo Snake). Death associated with *Raillietiella orientalis*. Herpetological Review 53:147.
- Brookings, M.D., J.F.X. Wellehan, J.F. Roberts, K. Allison, S.S. Curran, A.K. Childress, and E.C. Greiner. 2009. Massive visceral pentastomiasis caused by *Porocephalus crotali* in a dog. Veterinary Pathology 46:460–463.
- Farrell, T.M., J. Agugliaro, H. Walden, J.F.X. Wellehan, A.L. Childress, and C. Lind. 2019. Spillover of pentastome parasites from invasive Burmese Pythons (*Python bivittatus*) to Pygmy Rattlesnakes (*Sistrurus miliarius*), Extending Parasite Range in Florida, USA. Herpetological Review 50:75–78.
- Kelehear, C., D.M. Spratt, S. Dubey, G.P. Brown, and R. Shine. 2011. Using combined morphological, allometric, and molecular approaches to identify species of the genus *Raillietiella* (pentastomida). PLoS ONE 6(9):e24936.
- Kelehear, C., D.M. Spratt, D. O'Meally, and R. Shine. 2014. Pentastomids of wild snakes in the Australian tropics. International Journal for Parasitology: Parasites and Wildlife 3:20–31.
- Metcalf, M.F., A. Marsh, W. Brosse, and J.E. Herman. 2019. *Crotalus adamanteus*: Endoparasite. Herpetological Review 50:389.
- Miller, M.A., J.M. Kinsella, R.W. Snow, M.M. Hayes, B.G. Falk, R.N. Reed, F.J. Mazzotti, C. Guyer, and C.M. Romagosa. 2018. Parasite spillover: Indirect effects of invasive Burmese Pythons. Ecology and Evolution 8:830–840.

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- Miller, M.A., J.M. Kinsella, R.W. Snow, B.G. Falk, R.N. Reed, S.M. Goetz, F.J. Mazzotti, C. Guyer, and C.M. Romagosa. 2020. Highly competent native snake hosts extend the range of an introduced parasite beyond its invasive Burmese Python host. *Ecosphere* 11:03153.
- Palmisano, J.N., C.M. Bockoven, S.M. McPherson, R.J. Ossiboff, H.D.S. Walden, and T.M. Farrell. 2022. Infection experiments indicate common Florida anurans and lizards serve as intermediate hosts for the invasive pentastome parasite *Raillietiella orientalis*. *Journal of Herpetology* 56(3):355–361.
- Pare, J.A. 2008. An overview of pentastomiasis in reptiles and other vertebrates. *Journal of Exotic Pet Medicine* 17(4):285–294.
- von Reumont, B.M., K. Meusemann, N.U. Szucsich, E. Dell’Ampio, V. Gowri-Shankar, D. Bartel, S. Simon, H.O. Letsch, et al. 2009. Can comprehensive background knowledge be incorporated into substitution models to improve phylogenetic analyses? A case study on major arthropod relationships. *BMC Evolutionary Biology* 9:119.
- Walden, H.D.S., M.E. Iredale, A. Childress, J.F. Wellehan, and R.J. Ossiboff. 2020. Case report: Invasive pentastomes, *Raillietiella orientalis* (Sambon, 1922), in a free-ranging Banded Water Snake (*Nerodia fasciata*) in north central Florida, USA. *Frontiers in Veterinary Science* 7:467.