TRANSMISSION, HOST SPECIFICITY, AND SEASONAL OCCURRENCE OF *CYRTOSOMUM PENNERI* (NEMATODA: ATRACTIDAE) IN LIZARDS FROM FLORIDA

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ABSTRACT: Experimental infections and field-collected lizards were used to investigate issues of transmission, host specificity, and seasonal occurrence in the nematode *Cyrtosomum penneri* (Cosmocercoidea: Atractidae). *Anolis sagrei* (87 males, 42 females) were captured from the Florida Southern College campus, Polk County, Florida, from October 2010 to September 2011, and 8,803 *C. penneri* were collected from their intestines. During the breeding season all sexually mature (SVL ≥34 mm) *A. sagrei* were infected, whereas juvenile lizards (SVL <34 mm) were never infected. Experimental infections, using *A. sagrei*, found that worms were transferred to new hosts venereally, but not during oral exposures. Mating trials confirmed that worms were consistently transferred between hosts during copulation under natural conditions. Experimental exposures found that land snails and crickets do not serve as transport or intermediate hosts, which supports the idea that *C. penneri* is transferred only during host copulation. Experimental infections to test host specificity in *C. penneri* successfully infected *A. sagrei*, *Hemidactylus turcicus*, and *Sceloporus undulatus*, but not *Anolis carolinensis* or *Plestiodon inexpectatus*. Overall, this is the first study to fully elucidate the life cycle of any atractid nematode, and we suggest a venereal route of transmission for all atractid worms that infect reptilian hosts. Our findings also have implications for the host's reproductive and behavioral biology, e.g., support for covert or satellite males in the *A. sagrei* mating system.

Nematodes of the cosmopolitan family Atractidae Railliet, 1917 (Cosmocercoidea), produce juveniles that develop to the third stage in utero. These autoinfective worms can establish high-intensity infections in the lower gastrointestinal tract or lungs of several vertebrate groups, including fishes, amphibians, reptiles, and mammals (Baker, 1982; Anderson, 2000; Bursey et al., 2009). The autoinfective section of the atractid life cycle is well understood (Chabaud, 1978; Baker, 1982; Anderson, 2000); however, the means of transmission from host to host is unknown for all species in the family (Anderson, 2000). Despite a lack of experimental infections, several authors have suggested that transmission of atractids occurs by venereal and oral exposure. Jerke (1902) reported that *Probstymayria viviparia* (Probstmayr, 1965) from equine hosts could survive in manure for 4–5 days, which suggested an oral-fecal transmission route. Likewise, Da Costa (1963) believed that *Rondonia rondoni* Travassos, 1917, passed in feces, following matricidal endotoky, i.e., maternal retention of juvenile worms that results in female death, and was directly infective to fishes feeding on the worms. Alternatively, Petter (1966) postulated a venereal transmission route for *Atractis chabaudi* Petter, 1966, because tortoises became infected only after reaching sexual maturity. In support of venereal transmission, Pfaffenberger et al. (1986) and Norval et al. (2011) found that infection rates of *Cyrtosomum penneri* Gambino, 1957, were significantly higher in sexually mature lizards when compared to juveniles. Norval et al.'s (2011) seasonal prevalence and abundance data provide additional support for copulation as a route of transmission; specifically they found that cohorts of recently mature anoles were infected during the subsequent seasonal mating period. Alternatively, Goldberg et al. (1995) suggested that atractids infecting anoles were transferred between hosts via coprophagy.

According to Bursey and Flanagan (2002), members of the atractid genus *Cyrtosomum* infect the large and small intestine of lizards and chelonians, although all 4 known species from the New World have been collected only from lizards. Worms in the genus *Cyrtosomum* were previously thought to possess strict

physiologically host specificity based on lizards collected in nature (Gambino and Heyneman, 1960). This view was changed when Bowie and Franz (1974) synonymized *Cyrtosomum readi* Gambino, 1958, and *Cyrtosomum heynemani* Gambino, 1958, with *C. penneri*. Currently, it appears that *C. penneri*, *Cyrtosomum longicaudatum* Brenes and Bravo Hollis, 1960, and *Cyrtosomum scelopori* Gedoelst, 1919, display little host specificity because they infect multiple genera of New World lizards in nature (Gambino and Heyneman, 1960; Goldberg et al., 2003; Bursey et al., 2005; Bursey and Brooks, 2010; Norval et al., 2011), whereas *Cyrtosomum mega* Bowie and Franz, 1974, is known only from *Cyclura carinata*.

Cyrtosomum penneri is known to infect several lizard genera in North and Central America, such as *Anolis*, *Callisaurus*, *Holbrookia*, *Sceloporus,* and *Uma* (for a species list see Bursey et al., 2012). In addition to infections found in lizards within their native ranges, the nematode is known to infect the lizard *Anolis sagrei* in the lizard's introduced range in Taiwan (Norval et al., 2011) and Florida (Goldberg et al., 1994). Kolbe et al. (2004) suggested that *A. sagrei* from Taiwan originated in Florida, and Norval et al. (2011) concluded that *C. penneri* also originated in Florida. However, it is not clear if *A. sagrei* in Florida arrived from its native Cuba already infected with *C. penneri*, or if *A. sagrei* acquired the infection from native Florida lizards (or both). To our knowledge, *C. penneri* has not been reported from Cuban lizards (reviewed by Norval et al., 2011), but Cuba's helminth fauna is relatively undersampled compared to much of North America. The worm has been reported from Florida lizards, e.g., collected from *Sceloporus woodi* (Gambino and Heyneman, 1960; Bowie and Franz, 1974), but these collections occurred after the introduction of *A. sagrei* (Oliver, 1950). It is also of interest to note that the worm infects *A. sagrei* but has never been collected from the closely related *Anolis carolinensis* (Gambino and Heyneman, 1960; Sellers, 1971). Taken together, the lack of a fully elucidated life cycle and reliance on field-collected specimens to infer host specificity suggests that experimental infections are needed to better understand atractid life histories. Our goals were to establish the seasonal prevalence and abundance of *C. penneri* from *A. sagrei* on the Florida Southern College campus, examine field host specificity in Polk County, Florida, lizards, and use experimental infections to establish the route(s) of host to host

transmission and physiological and ecological limitations on host specificity.

MATERIALS AND METHODS

Field collections

Anolis sagrei were captured from the Florida Southern College (FSC) campus (28°1′50.7″N, −81°56′51.6″W) from October 2010 to September 2011. Animals were transported to the FSC Biology Department, euthanized, measured for snout-vent (SVL) and total length (TL), and all organs and body cavities were examined for parasites within 48 hr of collection. Most specimens of *C. penneri* were removed and fixed in 70% ethyl alcohol for identification, whereas some specimens were placed in reptile Ringer's solution for up to 24 hr (worms can survive up to 80 hr; G. J. Langford, pers. obs.) before the worms were used in experimental infections (see below). Representative specimens were cleared and temporarily mounted in glycerol for identification (Pritchard and Kruse, 1982). We found 1 species of atractid, *C. penneri*, in our study, which were identified by their lack of a gubernaculum, papillae pattern, and unequal length spicules (see Bursey and Flanagan, 2002). Voucher specimens of juvenile and adult *C. penneri* from *A. sagrei* were deposited in the H. W. Manter Parasitology Collection, University of Nebraska, Lincoln, Nebraska (accession number HWML-67157).

Host-to-host transmission experimental infections in *Anolis sagrei*

Prior to starting experimental infections, lizards were successfully treated for *C. penneri* infection by feeding them 3 or 4 crickets heavily coated with powdered ivermectin; lizards were not treated again but were held for 10 days prior to use in experimental infections. This method resulted in resolving 100% (n = 16) of *C. penneri* infections when tested for efficacy prior to starting experimental infections. Of note, concurrent infections of *Physaloptera squamatae* in the stomach were apparently unaffected by the ivermectin treatment, whereas fenbendazole was ineffective at treating either species of nematode. We chose to treat lizards for infections because acquiring uninfected adults was problematic due to the high prevalence of atractid infection in the wild population of *A. sagrei* (see results); additionally, our efforts to detect infections using fecal examination were ineffective because few, if any, adult or juvenile worms were shed in lizard feces.

To test the potential route(s) of transmission, 40 mature *A. sagrei* that were treated for atractid infections (see below) were divided into 4 equal groups and assigned to time-0 controls; experimental 1: worms pipetted into the host's cloaca; experimental 2: worms pipetted into the host's esophagus; or time-T controls. All time-0 control lizards were necropsied and examined for the presence of *Cyrtosomum* spp. before the start of the experimental infections. Individuals in experimental groups were given ∼20 *C. penneri* obtained from wild *A. sagrei*; after 24 and 72 hr half of the experimental and time-T control groups were dissected, and the number of *C. penneri* was recorded.

Experimental infections that used cloacal pipetting provided a controlled, mechanical substitution for lizard copulation, but mating trials were needed to determine if worms could be transferred from host to host under natural conditions. *Anolis sagrei* were divided into 4 groups and assigned to time-0 control: treated males and females (n = 5); experimental 1: treated female/wild male (n = 10); experimental 2: treated male/wild female (n = 10); and time-T control: treated female/wild female (n = 7). In the experimental and time-T control groups the treated, uninfected lizards were short-term residents (approximately 14 days), and a presumably infected wild lizard (all wild lizards were confirmed to be infected during necropsy after the mating trial) was placed in the residents' cage. Copulation for 4–7 min was witnessed for almost all pairs in the experimental groups, whereas, as predicted, no copulation was seen between females in the time-T controls. Lizard pairs were housed together for 48 hr, after which the experimental and time-T control groups were dissected and the number of *C. penneri* was recorded. All time-0 control lizards were necropsied and examined for the presence of *Cyrtosomum* spp. before the start of the experimental infections.

To confirm mating trials without the use of antihelminthic drugs, worms were fluoresced, and mating trials were repeated with recently captured lizards. Dyes were prepared according to Keeney et al. (2008), who used these dyes to tag trematodes during experimental infections with no apparent harm. Nematodes were fluoresced using a dye concentration

of 50 nM in 10 mL of Ringer's solution in a stender dish (37 × 25mm), to which *C. penneri* and approximately 2 g of fresh lizard feces, i.e., nematode food, were added. After 12 hr of feeding on the lizard feces and dye, the worm's gastrointestinal tract fluoresced brightly, especially the esophagus and esophageal bulb; these worms were used in the mating trial. *Anolis sagrei* were divided into 2 groups: males (n =10) that were infected with 30 worms fluoresced yellow-orange (BODIPY® 558/568 C12) and females (n = 10) that were infected with 30 worms fluoresced green (BODIPY® FL C12). Lizard pairs were housed together for 24 hr for the mating trial, then they were dissected and the number and fluorescent color of *C. penneri* was recorded. Of note, the worm's cuticle never fluoresced, and the dye appeared harmless to the parasites and their host.

To assess the ability of juvenile (<34 mm) *A. sagrei* to serve as host to *C. penneri*, 40 juvenile *A. sagrei* were divided into 4 equal groups and assigned to time-0 controls; experimental 1: worms pipetted into the host's cloaca; experimental 2: pipetted into the host's esophagus; or time-T controls. All time-0 control lizards were necropsied and examined for the presence of *Cyrtosomum* spp. before the start of the experimental infections. Each lizard within an experimental group was given ∼10 *C. penneri* obtained from wild *A. sagrei*, and after 24 and 72 hr half of the experimental and time-T control groups were necropsied, and the number of *C. penneri* were recorded.

To test the potential role of intermediate or transport hosts in *C. penneri* transmission, 50 juvenile worms in fresh lizard feces were placed in a moist, paper towel–lined 9 cm Petri dish with 10 wild land snails (*Polygyra cereolus*) or 10 captive-bred crickets (*Gryllus assimilis*); both were common invertebrates on campus that were seen consuming lizard feces. After 12 hr, snails and crickets were placed individually in clean containers to void their guts for 24 hr, after which each potential host was dissected under a microscope and searched for juvenile *C. penneri*.

Host specificity studies

In addition to *A. sagrei*, 4 species of lizards, common in Polk County, Florida, were chosen for exposure to *C. penneri*, collected from naturally infected *A. sagrei*. *Hemidactylus turcicus* and *A. carolinensis* were also collected from the FSC campus during August–October 2011. *Anolis carolinensis*, *Sceloporus undulatus*, and *Plestiodon inexpectatus* were collected from the Lakeland Highland Scrub Preserve (27°55′58.5″N, −81°55′34.6″W) during August–October 2011 and May 2012. Animals were transported to the FSC Biology Department's Frank Lloyd Wright Greenhouse and maintained on a diet of commercial crickets, *Gryllus* sp. (Ghann's Cricket Farm, Georgia), under natural light and temperature conditions. Each species of lizard was divided into 3 equal groups and assigned to time-0 controls, experimental infections, or time-T controls. All time-0 control lizards were necropsied and examined for the presence of *Cyrtosomum* spp. before the start of the experimental infections. Individual lizards within experimental groups were exposed to ∼20 *C. penneri* via cloacal pipetting, and after 96 hr the experimental and time-T control groups were necropsied, and the number and location of *C. penneri* was recorded. Prior to dissection, any feces produced by lizards in experimental groups were examined for the presence and condition, alive or dead, of nematodes.

Statistical analyses

Ecological measures of nematode infection are reported in accordance with Bush et al. (1997). A *z*-test for equality of proportions was used to compare the prevalence of infection between male and female lizards, and a *t*-test or Kruskal-Wallis non-parametric test was used to measure differences in mean abundance and mean intensity between lizard sexes (Sokal and Rohlf, 1981). Spearman's rank correlation was used to calculate possible relationships between lizard SVL (normalized with the natural log) and mean intensity, and male and female lizards were analyzed separately.

RESULTS

A total of 8,803 *C. penneri* were collected near the junction of the small and large intestines of wild *A. sagrei* (87 males, 42 females) on the FSC campus (Table I). The prevalence of infection ($z = 2.94$, $P < 0.01$), mean abundance ($x^2 = 3.24$, $P = 0.41$), and mean intensity ($x^2 = 2.98$, $P < 0.05$) were significantly

TABLE I. Prevalence (%), mean intensity (MI), and mean abundance (MA) of *Cyrtosomum penneri* recovered from 129 brown anoles, *Anolis sagrei*, collected on the Florida Southern College campus from October 2010 to September 2011.

Life stage	Prevalence %	MI ± 1 SD (range)	MA ± 1 SD
Male	76	126 ± 90 (3–401)	95.2 ± 93.1
Female	48	56.7 ± 46.5 (3–200)	35.3 ± 45.9
Juvenile (<34 mm SVL)	0	—	—
Adult (≥34 mm SVL)	89	100 ± 80.3 (3–401)	88.8 ± 79.1
All hosts	69	100 ± 80.3 (3–401)	68.2 ± 83.2

higher in male than female hosts. A significant positive correlation between host SVL and mean intensity was found for female lizards ($r_s = 0.41$, df = 30, $P < 0.01$), but not for male lizards ($r_s = 0.11$, df = 52, $P = 0.21$). The monthly prevalence of *C. penneri* was maintained at 100% in adult (SVL ≥34 mm) lizards during most of the host's mating season (April–September; Fig. 1), whereas the mean abundance fluctuated throughout the year (Fig. 2). *Cyrtosomum penneri* was not recovered from wild *A. carolinensis*, *H. turcicus*, *P. inexpectatus*, or *S. undulatus* collected on the FSC campus and/or Lakeland Highland Scrub Preserve.

Experimental infections to test host to host transmission of *C. penneri* in adult *A. sagrei* found that all lizards exposed via cloacal pipetting became infected (24 HPE: MI = 16 ± 2.3 [14–20], n = 5; 72 HPE = 19.4 ± 3.1 [18–24], n = 5), whereas lizards exposed via oral pipetting and controls were uninfected (Table II). Similarly, experimental infections of juvenile *A. sagrei* were successful only using cloacal pipetting (P = 7/10; MI = 6.7 ± 1.4 [4–8]). Dead worms were recovered from the gastrointestinal tract of *A. sagrei* following oral pipetting experimental infections of juveniles and adults.

In mating trials, males transferred worms to females during all exposures (MI = 21 ± 9.4 [8–28], n = 10), and females infected males in 8 of 10 exposures (MI = 6.4 ± 6.2 [1–12]); the difference in mean intensity between sexes was significant (t = 5.4, P < 0.001), and controls were uninfected. Trials using fluorescently marked worms confirmed the results of the mating trial; males
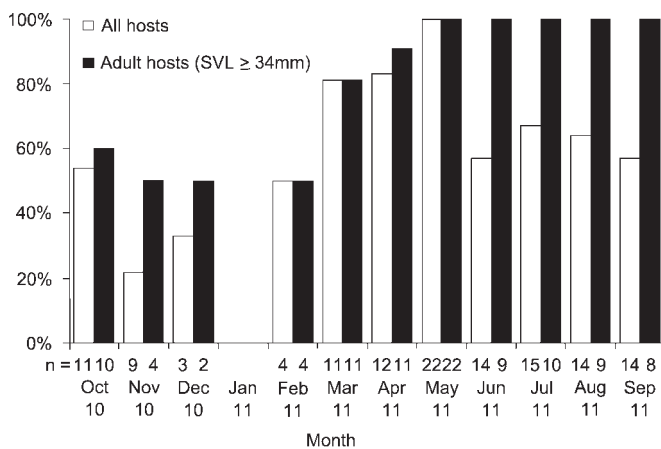
FIGURE 1. Monthly prevalence of *Cyrtosomum penneri* collected from wild *Anolis sagrei* on the Florida Southern College campus in Polk County, Florida. Note that monthly prevalence of *C. penneri* was maintained at 100% in adult (SVL ≥34mm) lizards during most of the host's mating season, April–September.
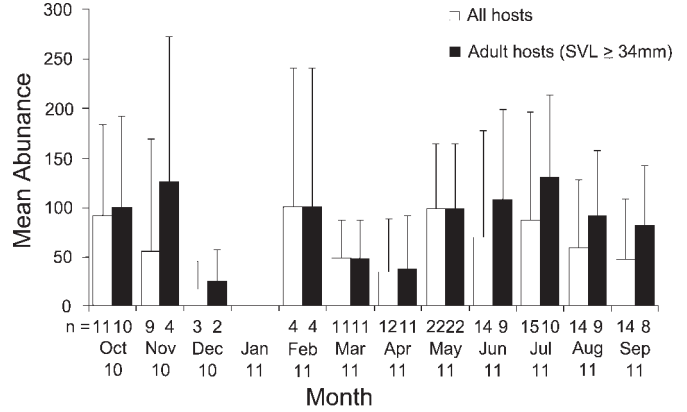
FIGURE 2. Monthly mean abundance + 1 SD of *Cyrtosomum penneri* collected from wild *Anolis sagrei* on the Florida Southern College campus in Polk County, Florida. Note that mean abundance shows no seasonal trend, and that the host population maintains a high number of worms throughout the year.

transferred worms to females during all exposures (MI = 7 ± 2.9 [2–15], n = 10), and females infected males in 7 of 10 exposures (MI = 3.8 ± 2.5 [1–7]); the difference in mean intensity between sexes was significant (t = 4.1, P < 0.01). Of note, *H. tursicus* that were experimentally infected with ~20 nematodes via cloacal pipetting remained infected at the termination of the failed mating trial experiment (P = 7/8; MI = 17.4 ± 6.2 [6–23]).

Experimental infections to test host specificity in *C. penneri* successfully infected *A. sagrei*, *H. turcicus*, and *S. undulatus*. However, *C. penneri* was unable to infect *A. carolinensis* or *P. inexpectatus* (Table II). Recently dead worms were recovered from the gastrointestinal tract of uninfected *A. carolinensis* (2 worms were found in the stomach of 1 host) and *S. undulatus* upon dissection, whereas no worms, dead or alive, were noted in *P. inexpectatus*. Prior to dissection, dead worms were discovered in the feces of all host species and were most abundant in the feces of *A. carolinensis* and *P. inexpectatus*. No time-T or time-0 controls were infected.

Experiments that tested whether land snails and crickets could serve as transport or intermediate hosts found no *C. penneri* infecting or residing on or in these invertebrates.

DISCUSSION

Prior to this study, the route of transmission between lizards was an unsettled issue in atractid biology. Previous researchers

TABLE II. Prevalence, mean intensity, and mean abundance of *Cyrtosomum penneri* in 5 species of laboratory exposed lizards. Nematodes were collected from wild *Anolis sagrei* on the Florida Southern College campus.

Lizard species	Prevalence % (no. infected/ no. exposed)	Mean intensity ± 1 SD (range)	Mean abundance ± 1 SD
Anolis carolinensis	0 (0/9)	—	—
Anolis sagrei	100 (10/10)	18 ± 3.3 (1–18)	18 ± 3.3
Hemidactylus turcicus	78 (7/9)	7.1 ± 5.7 (1–18)	4.9 ± 5.8
Plestiodon inexpectatus	0 (0/9)	—	—
Sceloporus undulatus	38 (3/8)	7.5 ± 3.4 (4–12)	3.7 ± 4.5

have suggested 2 possibilities for transmission of these worms. The first, oral transmission via coprophagy was used to explain the presence of *Atractis sceloperi* in sympatric, yet distantly related species of lizards (Goldberg et al., 1995). The second, venereal transmission via copulation, was first proposed in an atractid nematode that infects tortoises (Petter, 1966); however, Norval et al. (2011) were the first to suggest copulation as a possible mode of transmission in atractids from lizards. Our study is the first to use experimental infections, controlled mating experiments, and data collected from naturally infected hosts to establish venereal transmission between lizards and elucidate the sexual transmission dynamics of an atractid nematode.

In addition to establishing a venereal route of infection, our experimental infection experiments reject coprophagy as a valid mode of transmission for *C. penneri*. Oral infections failed to infect lizards, and we found numerous dead worms in host feces following oral experimental exposure. We propose that adult and juvenile worms are unable to survive passage through the stomach, likely due to the worm's sensitivity to acidic environments; we noted that worms quickly died when placed in reptilian Ringer's solution buffered to pH 4.5 (G. J. Langford, unpub. data). The gastric region of a lizard's stomach is typically below pH 3 (Lehman and Smith, 1988; Ferri et al., 1999), whereas the pH of the worm's typical residence in the intestine is near neutral (Nagy, 1977; Troyer, 1984). In addition, we suggest that fecal transmission is unlikely in *C. penneri* because live worms were rarely found voided in the feces of wild or captive lizards. Our attempts to use crickets and land snails as transport or intermediate hosts failed.

Our assertion that *C. penneri* is transmitted during copulation is supported by the seasonal prevalence data collected on naturally infected *A. sagrei* (Norval et al., 2011; this study). Our study found that prevalence in sexually mature lizards ($\geq$34 mm) was 100% during all months (except April) of the breeding season that occurs from April to September. However, no juvenile lizards (<34 mm) were found infected during the entire study and juvenile lizards that transitioned to adults after the breeding season did not acquire infections until the next breeding season. Thus, we suggest that upon obtaining a SVL of 34 mm, anoles in our population became sexual mature and copulated with older, infected cohorts, which provided their initial infection. Our results support Norval et al.'s (2011) finding that maturing juveniles and seasonal reproduction combine to produce relatively low prevalence in winter and high prevalence in summer. We found no predictable trend for mean abundance, whereas Norval et al. (2011) suggested that seasonal reproduction and maturing juveniles also produced a peak in mean abundance during the summer months. If the June peak in mean abundance found by Norval et al. (2011) is removed, then no trend is present and their data appear similar to our results. We suggest that a seasonal trend in *C. penneri* mean abundance is unlikely because the infrapopulation of this auto-infective atractid should increase, except for nematodes lost during copulation, until the host's death. Given that *A. sagrei* can produce up to 1 egg per week over the reproductive season (Andrews and Rand, 1974), we should expect several, i.e., at least 12, different lizard cohorts to mature, become infected, and play host to an increasing infrapopulation throughout the year, thus creating a non-seasonal mean abundance for a random sample of lizards. Furthermore, we hypothesize that lizards of similar age may host different numbers of worms based on host sex (see below), and both the frequency of mating and number of worms gained and lost during copulation events.

We found a significant relationship between female lizard SVL and an increase in mean abundance, but no relationship in male lizards, whereas Norval et al. (2011) did not find a relationship between mean abundance and host sex. As we discussed above, a consistent increase in worm numbers following initial infection is expected, so a relationship between mean abundance and SVL in adult lizards is not surprising, but why does it not occur in males? And why do males have a significantly higher prevalence, mean intensity, and mean abundance compared to females? From the viewpoint of the worm, differences in host sex hormones could alter its ability to establish in a new host and alter reproductive output, which has been suggested for other species of nematodes that infect lizards (Calisi et al., 2008; Hilsinger et al., 2011) and vertebrates in general (review in Poulin, 1996). Alternatively, or in synergy, some aspect of the host's polygynous mating system may affect the predicted accumulation of this autoinfective atractid in male lizards. In support of this alternative, we found that males transferred more worms than females during copulations in mating trials. Thus, large territorial males, which copulate frequently and presumably transfer large numbers of worms, may reduce *C. penneri* intensity below expectations based on host SVL, whereas infrequent mating may result in high-intensity infections in males. This alternative might explain the variation in worm mean intensity among large males in our population.

Prior to this study, no experimental infections had been conducted on any member of the Atractidae and determinations of host specificity, based on naturally infected lizards, suggest *C. penneri* shows little, if any, host specificity (Gambino and Heyneman, 1960; Goldberg et al. 2003; Bursey et al., 2005; Bursey and Brooks, 2010; Norval et al., 2011). Our results suggest that *C. penneri* displays host specificity among lizards, and specificity does not appear to follow host relationships. For example, the ability of worms collected from *A. sagrei* (Polychrotidae) to infect distantly related hosts, such as *P. inexpectatus* (Scincidae) and *H. turcicus* (Gekkonidae), but not the relatively closely related *A. carolinensis* (Polychrotidae) suggests that these worms are tracking host resources instead of host phylogenies, i.e., they are an example of ecological fitting (see Brooks et al., 2006). In support of ecological fitting, a recent study in Costa Rica found *C. penneri* infecting *Sceloporus variablis* and *Anolis biporcatus*, but not 5 species of sympatric *Anolis* (Bursey and Brooks, 2010). For most lizards it is unclear whether specificity for *C. penneri* is mediated by physiological conditions of the host or limitations on host to host transmission. It should be noted that a lack of clarity in host specificity is not uncommon in helminth-infected herpetofauna (e.g., Bolek and Janovy, 2007; Langford, 2010).

We initially assumed a lack of interspecific mating attempts would be the primary limiting factor for interspecific transmission; however, several genera of North American lizards known to host *C. penneri*, such as *Anolis* (Losos, 2009 and references within), *Crotaphytus* (Montanucci, 1983), and *Sceloporus* (Leache, 2008 and references within) can interbreed within genera in nature. Therefore, opportunities for interspecific transmission within lizard genera are available, whereas transmission between host genera (e.g., *Anolis* and *Sceloporus*) seems less likely and would be difficult to explain. In support of physiological limitations on specificity, we found *A. carolinensis* to be resistant to infection with *C. penneri* during experimental infections and in

nature (Gambino and Heyneman, 1960; Sellers, 1971; this study). This is surprising because *A. carolinensis* and *A. sagrei* copulate in nature (M. Lucas, pers. comm.; G. J. Langford, pers. obs.), and these closely related lizards have similar natural histories, anatomies, and ecologies (Losos, 2009). Both lizards evolved from the anole fauna on Cuba, but *A. carolinensis* likely arrived in Florida during the Pliocene (Glor et al., 2005), whereas *A. sagrei* is a recent invader.

Our finding that *C. penneri* is transmitted by copulation has some interesting implications for the host's reproductive and behavioral biology. *Anolis sagrei* reproduces in a female-defense polygyny, wherein large males (e.g., SVL > 50 mm) establish and maintain territories containing multiple, relatively small females (Schoener and Schoener, 1980). In *Anolis* mating systems, young males are generally thought to have little mating success because they are excluded from females by large territorial males (Losos, 2009). In contrast, our parasitological results suggest that small male lizards are copulating with mature females and becoming infected with a sexually transmitted parasite. Thus, our results provide some support for the female mimicry hypothesis (Orrell and Jenssen, 2003) and/or the "dear enemy" phenomenon (Paterson, 2002) in anoles. This insight into *A. sagrei* reproduction should encourage anologists to reconsider the role of covert and satellite males in anole mating systems where *C. penneri* infects small male lizards. In conclusion, the major contribution of our study is the establishment of copulation as the route of transmission for *C. penneri* between lizards and the discovery of both ecological and physiological host specificity in these worms. This study also provides insight into the host's biology, specifically support for the female mimicry hypothesis in anoles proposed by Orrell and Jenssen (2003). Future comparative studies on *Cyrtosomum* spp. are needed to elucidate the ecological and/or physiological mechanisms of host specificity in different lizard species and genera, which should include helminth surveys of Cuban lizards. It would also be interesting to explore the role of host reproductive strategies on worm transmission and population dynamics. Last, the route(s) of infection for atractids infecting hosts that use external fertilization, e.g., fishes and amphibians, or hosts that lack a cloaca, e.g., mammals, is currently unknown. Thus, it is apparent that more research is needed on the ecology and evolution of atractid nematodes.

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LITERATURE CITED

ANDERSON, R. C. 2000. Nematode parasites of vertebrates: Their development and transmission. CAB International, Wallingford, Oxon, U.K., 650 p.

ANDREWS, R., AND A. S. RAND. 1974. Reproductive effort in anoline lizards. Ecology **55:** 1317–1327.

BAKER, M. R. 1982. Systematic relationships of the Atractidae and Cosmocercidae (Nematoda: Cosmocercoidea): Two new atractids parasitic in amphibians and fish. Canadian Journal of Zoology **60:** 2395–2402.

BOLEK, M. G., AND J. JANOVY, JR. 2007. Evolutionary avenues for, and constraints on, the transmission of frog lung flukes (*Haematoloechus* spp.) in dragonfly second intermediate hosts. Journal of Parasitology **93:** 593–607.

BOWIE, A., AND R. FRANZ. 1974. *Crytosomum mega* sp. n. (Nemotoda: Oxyuroidea) from the lizard *Cyclura carinata.* Journal of Parasitology **60:** 628–631.

BROOKS, D. R., V. LEÓN-RÈGAGNON, D. A. MCCLENNAN, AND D. ZELMER. 2006. Ecological fitting as a determinant of the community structure of platyhelminth parasites of anurans. Ecology **87:** S76–S85.

BURSEY, C. R., AND D. R. BROOKS. 2010. Nematode parasites of 16 lizard species from the Area de Conservación Guanacaste, Costa Rica. Comparative Parasitology **77:** 232–235.

———, AND J. P. FLANAGAN. 2002. *Atractis marquezi* n. sp. (Nematoda: Atractidae) and a revision of *Atractis* Dujardin, 1945, sensu Baker, 1987. Journal of Parasitology **88:** 320–324.

———. S. R. GOLDBERG, AND J. R. PARMELEE. 2005. Gastrointestinal helminths from 13 species of lizards from Reserva Cuzco Amazónico, Peru. Comparative Parasitology **72:** 50–68.

———, S. R. GOLDBERG, S. R. TELFORD, JR., AND L. J. VITT. 2012. Metazoan endoparasites of 13 species of Central American anoles (Sarnia: Polychrotidae: *Anolis*) with a review of the helminth communities of Caribbean, Mexican, North American, and South American anoles. Comparative Parasitology **79:** 75–132.

———, D. REAVILL, AND E. GREINER. 2009. *Pneumoatractis podocnemis* n. gen., n. sp. (Nematoda: Atractidae) from the yellow-spotted Amazon River turtle, *Podocnemis unifilis* (Testudines: Pelomedusidae). Comparative Parasitology **76:** 149–153.

BUSH, A. O., K. D. LAFFERTY, J. M. LOTZ, AND A. W. SHOSTAK. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology **83:** 575–583.

CALISI, R. M., J. H. MALONE, AND D. K. HEWS. 2008. Female secondary coloration in the Mexican boulder spiny lizard is associated with nematode load. Journal of Zoology **276:** 358–367.

CHABAUD, A. G. 1978. CIH keys to the nematode parasites of vertebrates. No. 6. Keys to genera of the superfamilies Cosmocercoidea, Seuratoidea, Heterakoidea and Subuluroidea. Commonwealth Agricultural Bureaux, Farnham Royal, Buckinghamshire, U.K., 71 p.

DA COSTA, S. C. G. 1963. *Rondonia rondoni* Travassos, 1920 (Nematoda, Atractidae). Memorias do Instituto Oswaldo Cruz **61:** 75–88.

FERRI, D., G. E. LIQUORI, AND G. SCILLITANI. 1999. Morphological and histochemical variations of mucous and oxynticopeptic cells in the stomach of the seps, *Chalcides chalcides.* Journal of Anatomy **194:** 71–77.

GAMBINO, J. J., AND D. HEYNEMAN. 1960. Specificity and speciation in the genus *Cyrtosomum* (Nematoda: Atractidae). American Midland Naturalist **63:** 365–382.

GLOR, R. E., J. B. LOSOS, AND A. LARSON. 2005. Out of Cuba: Overwater dispersal and speciation among lizards in the *Anolis carolinensis* subgroup. Molecular Ecology **14:** 2419–2432.

GOLDBERG, S. R., C. R. BURSEY, AND H. CHEAM. 1995. Helminth parasites of three sympatric lizards from Grand Cayman Island, *Anolis conspersus*, *Anolis sagrei* (Polychridae) and *Leiocephalus carinatus* (Tropiduridae). Caribbean Journal of Science **31:** 339–340.

———, ———, AND J. L. CAMARILLO-RANGEL. 2003. Gastrointestinal helminths of seven species of sceloporine lizards from Mexico. Southwestern Naturalist **48:** 208–217.

———, ———, AND R. TAWIL. 1994. Helminth parasites of the bark anole, *Anolis distichus* and the brown anole, *Anolis sagrei* (Polychridae) from Florida and the Bahamas. Caribbean Journal of Science **30:** 275–277.

HILSINGER, K. C., R. A. ANDERSON, AND D. NAYDUCH. 2011. Seasonal dynamics of *Skrjabinoptera phrynosoma* (Nematoda) infection in horned lizards from the Alvord basin: Temporal components of a unique life cycle. Journal of Parasitology **97:** 559–564.

JERKE, H. W. M. 1902. Eine parasitische Anguillula des Pierdes. Archiv für Wissenschaftliche und Praktische Thierheilkunde, Berlin **29:** 113–127.

KEENEY, D. B., C. LAGRUE, K. BRYAN-WALKER, N. KHAN, T. L. F. LEUNG, AND R. POULIN. 2008. The use of fluorescent fatty acid analogs as labels in trematodes experimental infections. Experimental Parasitology **120:** 15–20.

KOLBE, J. J., R. E. GLOR, L. R. SCHETTINO, A. C. LARA, A. LARSON, AND J. B. LOSOS. 2004. Genetic variation increases during biological invasion by a Cuban lizard. Nature **431:** 177–181.

LANGFORD, G. J. 2010. Ecology and relationships of *Rhabdias* spp. (Nematode: Rhabdiasidae) from North American amphibians and reptiles. Ph.D. Dissertation. University of Nebraska, Lincoln, Nebraska, 129 p.

LEACHE, A. D. 2008. Speciation in North American spiny lizards (genus *Sceloporus*): Insights from phylogeny, phylogeography, and hybrid zones. Ph.D. Dissertation. University of California, Berkeley, California, 660 p.

LEHMAN, S., AND A. A. SMITH. 1988. Regional differentiation in the stomach of the green anole. Anatomical Record **220:** 364–368.

LOSOS, J. B. 2009. Lizards in an evolutionary tree: Ecology and adaptive radiation of anoles. University of California Press, Berkeley, California, 528 p.

MONTANUCCI, R. R. 1983. Natural hybridization between two species of collared lizards (Crotaphytus). Copeia **1983:** 1–11.

NAGY, K. A. 1977. Cellulose digestion and nutrient assimilation in *Sauromalus obesus*, a plant-eating lizard. Copeia **1977:** 355–362.

NORVAL, G., C. R. BURSEY, S. R. GOLDBERG, J.-J. MAO, AND K. SLATER. 2011. Origin of the helminth community of an exotic invasive lizard, the brown anole, *Anolis sagrei* (Squamata: Polychrotidae), in southwestern Taiwan. Pacific Science **65:** 383–390.

OLIVER, J. A. 1950. *Anolis sagrei* in Florida. Copeia **1950:** 55–56.

ORRELL, K. S., AND T. A. JENSSEN. 2003. Heterosexual signaling by the lizard *Anolis carolinensis*, with intersexual comparisons across contexts. Behaviour **140:** 603–634.

PATERSON, A. V. 2002. Effects of an individual's removal on space use and behavior in territorial neighborhoods of brown anoles (*Anolis sagrei*). Herpetologica **58:** 382–393.

PETTER, A. J. 1966. Equilibre des espèces dans les populations de nèmatodes parasites du colon des *Tortues terrestres*. Memoires du Museum National d'Histoire Naturelle Série A, Zoologie **39:** 1–252.

PFAFFENBERGER, G. S., T. L. BEST, AND D. DE BRUIN. 1986. Helminths of collared lizards (*Crotaphytus collaris*) from the Pedro Armendariz lava field, New Mexico. Journal of Parasitology **72:** 803–806.

POULIN, R. 1996. Sexual inequalities in helminth infections: A cost of being male? American Naturalist **147:** 287–295.

PRITCHARD, M. H., AND G. O. W. KRUSE. 1982. The collection and preservation of animal parasites. University of Nebraska Press, Lincoln, Nebraska, 141 p.

SCHOENER, T. W., AND A. SCHOENER. 1980. Densities, sex ratios, and population structure in four species of Bahamian *Anolis* lizards. Journal of Animal Ecology **49:** 19–53.

SELLERS, L. G. 1971. Three helminths recovered from *Anolis carolinensis*. Journal of Parasitology **57:** 355.

SOKAL, R. R., AND J. F. ROHLF. 1981. Biometry, 2nd ed. W. H. Freeman and Company, New York, New York, 859 p.

TROYER, K. 1984. Structure and function of the digestive tract of a herbivorous lizard *Iguana iguana*. Physiological Zoology **57:** 1–8.