

COMPLEX AND DIVERSE DRIVERS OF PARASITE LOADS IN A COSMOPOLITAN INSECT

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KEY WORDS ABSTRACT

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The goal of parasite epidemiologists is to understand the factors that determine host infection levels. Potential infection determinants exist at many scales, including spatial and temporal environmental variation, among-host differences, and interactions between symbionts infecting the same host. All of these factors can impact levels of parasitism, but frequently only a subset is considered in any host-parasite system. We examined several potential determinants of pinworm infection in wild Australian cockroaches (*Periplaneta australasiae*) from multiple biological scales: (1) habitat; (2) season; (3) cockroach body size, developmental stage, and sex; and (4) interactions between 2 pinworm species (*Leidynema appendiculata* and *Thelastoma* sp.). Over 1 yr, we collected 239 cockroaches from 2 separate rooms in an Illinois greenhouse. We used generalized linear mixed-effects models (GLMMs) to evaluate simultaneously the influence of these factors on pinworm abundance, and nearly all had significant effects. Overall, the abundance of *L. appendiculata* was greater than *Thelastoma* sp., but the relative abundance of the 2 species was reversed in each room (i.e., a taxon × habitat effect). Abundance varied over 4 trapping seasons and increased with cockroach size. Adult cockroaches had more pinworms than nymphs, and there was also a significant taxon × stage effect: adult cockroaches had fewer pinworms than expected for their larger size, and this reduction was greater in *Thelastoma* sp. than in *L. appendiculata*. Cockroach sex had no effect on infection. Although females had more worms than males, this difference could be explained by the larger size of females. Finally, after controlling for all other potential determinants of infection, we found a strong negative association between *Thelastoma* sp. and *L. appendiculata*; cockroaches tended to be infected with either 1 pinworm species or the other. Our work underscores the importance of measuring potential determinants of infection from as many scales as possible. Such approaches are necessary to unravel the complexities of host-parasite interactions.

Factors at many scales can affect parasite transmission, and so determine levels of host infection (reviews in Wilson et al., 2002; Behnke, 2008). Spatially, habitat differences can affect the persistence of free-living stages or the occurrence of alternative hosts. Temporally, seasonal variation in abiotic or biotic factors can alter infection levels. Among-host attributes such as age or sex can affect susceptibility to infection. Finally, within-host interactions can increase or decrease infection levels of interacting parasite species. Given the complexity of host-parasite interactions, most studies focus on one, or a subset, of these potential determinants of infection (e.g., Calegario-Marques and Amato, 2013; Zimmermann et al., 2016). However, to accurately determine which of these factors affect parasitic infection, it is important to examine all of them simultaneously, because their relative importance can vary among parasite taxa (Monello and Gompper, 2009), and variation among the factors may be

correlated (Behnke, 2008; Fenton et al., 2010; Hellard et al., 2015).

Insects, owing to their small size, abundance, and broad geographic distributions, provide excellent models with which to evaluate multiple determinants of parasitic infection at a single time (e.g., Bunker et al., 2013). However, despite their ecological importance, insect parasite communities are studied far less frequently than those of vertebrates. In this paper, we evaluate determinants of infection from multiple scales in a relatively simple host-parasite system: pinworm infections in Australian cockroaches (*Periplaneta australasiae*) from an Illinois greenhouse with 2 distinctive microhabitats.

Cockroaches are an ancient group of omnivorous insects, dating back at least to the early Triassic Period (Bourguinon et al., 2018; Brenner and Kramer, 2018). Juveniles (nymphs) are morphologically similar to adults, and share the same habitat and diet (i.e., they are paurometabolous). There are approximately

4,400 species, but fewer than 20 are considered pests because they inhabit human-made structures (i.e., are peridomestic). Pest cockroaches can serve as intermediate hosts or mechanical vectors for a variety of human infectious agents (Etim et al., 2013; Adenusi et al., 2018; Brenner and Kramer, 2018). *Periplaneta australasiae* is a common greenhouse pest that can cause substantial damage by feeding on plants. It may live up to 2 yr, spending approximately 1 yr passing through 10–12 nymphal instars, and up to 1 yr as an adult (Brenner and Kramer, 2018). The species likely evolved in Africa and was dispersed globally in cargo or slave ships (Rehn, 1945). In temperate regions, it is dependent on the warm environment provided by greenhouses, but in warmer regions, it can live in natural areas.

Cockroaches are hosts to a natural diversity of symbionts (Roth and Willis, 1960; Bracke et al., 1979; Philippe and Deleporte, 1996), including pinworm nematodes in the order Oxyurida (Jex et al., 2007a; Carreno, 2014). Pinworms have served as model systems in studies of community assembly (Schad, 1963), host-parasite coevolution (Brooks and Glen, 1982), and parasite epidemiology (González-Hernández et al., 2014). Oxyurids are distinctive among nematodes because some representatives use vertebrates as definitive hosts (superfamily Oxyuroidea), while others (superfamily Thelastomatoidea) infect invertebrates (Adamson and van Waerebeke, 1992; Carreno, 2014). Members of the largest Thelastomatoid family, the Thelastomatidae, display a wide host range but have been described primarily from terrestrial arthropods, particularly cockroaches (Insecta: Blattodea) and millipedes (Diplopoda: Myriapoda; Jex et al., 2006; Carreno, 2014).

Thelastomatoid nematodes inhabit the lower digestive tract of their invertebrate hosts, where they eat bacteria (Carreno, 2014) or other hindgut contents (Peregrine, 1974). It is presumed that they are generally non-pathogenic to their hosts (Dobrovolny and Ackert, 1934; Zervos, 1988b; but see Müller-Graf et al., 2001). Their life cycle is direct (reviewed in Adamson, 1994): female worms lay eggs that are passed out with cockroach feces, and eggs are accidentally ingested during coprophagy (Dobrovolny and Ackert, 1934; McCallister and Schmidt, 1981).

Ecological aspects of the relationships between pinworms and American cockroaches (*Periplaneta americana*) and German cockroaches (*Blattella germanica*) have been described under laboratory conditions (e.g., Hominick and Davey, 1972a; Adamson and Noble, 1992, 1993; Müller-Graf et al., 2001), but the pinworm fauna of *P. australasiae* has not been fully documented (Carreno, 2017). There are few studies of Thelastomatoid nematodes in naturally occurring host populations (Carreno, 2014). Most field studies are restricted to New Zealand and Australia (Zervos, 1988a, 1988b; Jex et al., 2007a, 2007b), and to our knowledge, only 2 have been performed in North America (Dobrovolny and Ackert, 1934; Carreno, 2017). Here, we examine simultaneously the effects of multiple potential determinants of pinworm infection in wild *P. australasiae* from multiple biological scales: (1) habitat; (2) season; (3) cockroach developmental stage, body size, and sex; and (4) interactions between 2 pinworm species (*Leidynema appendiculata* and *Thelastoma* sp.). We found that nearly all of these factors play a significant role in determining pinworm parasitism in cockroaches, highlighting the importance of evaluating their roles altogether.

MATERIALS AND METHODS

Study site and trapping

Cockroaches were collected from 2 adjacent rooms (“upper” and “lower”) in the Western Illinois University (WIU) Biological Sciences greenhouse (McDonough County, Macomb, Illinois; 40°28′15.77″N; 90°41′13.14″W). These rooms were chosen because they were likely to display microhabitat differences that could affect pinworm transmission. The upper room (~108 m²) is on the ground level, and connected by a 4-m concrete ramp to the lower room (54 m²), which is approximately 2 m below ground level. In the upper room, potted plants are on wooden shelves approximately 60 cm off the floor, and include multiple orchids (Orchidaceae), ferns (Pteridophyta), cycads (Cycadales), bromeliads (Bromeliaceae), and cacti (*Rhipsalis* spp.), as well as other species. Maximum plant height is approximately 1 m above the shelves. Plants in the upper room receive relatively direct sunlight year-round and are watered by spraying approximately weekly. The lower room simulates a tropical forest. Plants are rooted in the soil (floor level) rather than contained in pots. There is also substantial leaf litter and a small stream with a waterfall. There is lower plant diversity in the lower room, which consists mainly of trees that reach the ceiling of the greenhouse. Species include multiple slender lady palms (*Rhapis humilis*), and Burmese fishtail palms (*Caryota mitis*), as well as single individuals of other species. Since there is substantial plant canopy, the interior of the lower room receives less direct sunlight than in the upper room. Plants in the lower room are watered monthly.

Cockroaches were trapped in 2007–2008, during pairs of sessions approximately 1 mo apart in each of the 4 seasons (8 sessions total): spring (March 29–30 and April 26), summer (June 30–July 4 and July 31–August 1), fall (October 5 and November 9–12), and winter (January 3–5 and January 25–29). During this study, we measured 2 environmental variables, temperature and relative humidity, that have been shown to affect the persistence and viability of nematode eggs in general (e.g., Froeschke et al., 2010), and oxyurid eggs in particular (Zervos, 1988a; Jex et al., 2007b). Both variables were recorded hourly in each room with a data logger (Hobo Pro Series RH/Temp, Onset Computer Corporation, Bourne, Massachusetts) during the entirety of individual trapping sessions.

Cockroach traps were made from 473-ml clear plastic water bottles, which were cut in half and had the top inverted into the bottom. Traps were baited with paper towels immersed in sugar solution (122 g in 100 ml), and the inner top portion of the trap was lined with petroleum jelly to prevent cockroaches from escaping. During each trapping session, 10 traps were set in each room, and traps were checked and cockroaches collected every 24 hr. Trapping continued in each session until 40 cockroaches were caught, or 5 days had elapsed, whichever occurred first. Captured cockroaches were killed with ethyl acetate within 24 hr. Morphological and parasitological measurements were taken immediately after death.

Cockroach attributes

We categorized the cockroach developmental stage as nymph or adult by the presence of wings (present in adults only). It was not possible to determine the particular instar to which nymphs belonged; however, cockroach stage does reflect metabolic

differences related to sexual maturity. We also estimated cockroach size by measuring the femur length of the left hind leg. Cockroach femur size increases with nymphal instar (Hominick and Davey, 1972a; Zervos and Webster, 1989; Adamson and Noble, 1992) and, in adults, reflects nutrition acquired as a nymph (Barrett et al., 2009). Sex of adult cockroaches was determined by the presence or absence of stylets on the posterior of the abdomen (Bell et al., 2007). Only males have stylets. It is not possible to sex cockroach nymphs because both sexes are born with stylets, and although females lose their stylets as they age, the timing of this loss has not been determined (Bell et al., 2007).

Pinworm enumeration

Cockroaches were dissected in saline solution, their intestinal tracts were teased apart, and all pinworms were removed. Pinworms were fixed in a solution of hot (100 C) glycerin and ethanol and then mounted on slides for later identification (Pritchard and Kruse, 1982). To measure pinworm population sizes, we counted and identified only adult female pinworms (Hominick and Davey, 1972a; Adamson and Noble, 1992) because: (1) adult females were the most abundant demographic stage in this cockroach population (93% of recovered pinworms were adult females), (2) it is difficult to determine the species of male or juvenile worms in mixed-species infections (Carreno, 2014), and (3) diagnostic features are frequently not visible in small juveniles (Hominick and Davey, 1972a).

Pinworm identification was based on features of the cuticle and upper digestive tract (Adamson and van Waerebeke, 1992; Shah et al., 2011; Carreno, 2017). In the WIU greenhouse, *P. australasiae* was infected by *L. appendiculata* and *Thelastoma* sp. *Leidynema appendiculata* was identified by the presence of a gastric cecum, a blind appendix that extends from the anterior intestine, just posterior to the end bulb of the esophagus. Individuals of *Thelastoma* sp. do not have this structure. Several species of *Thelastoma* infect *P. americana*, but these are difficult to distinguish (Khairul Anuar and Rodzoh, 1978; Adamson and Noble, 1992), so we refer to them here as *Thelastoma* sp., which was distinguished from other thelastomatid pinworms by esophageal structures. *Hammerschmidtella diesingi*, a common inhabitant of *P. americana* (Dobrovolny and Ackert, 1934; Hominick and Davey, 1972a; Adamson and Noble, 1992), was not found in *P. australasiae*. This pinworm is distinguished from *Thelastoma* sp. by its swollen middle esophagus, or “pseudobulb” (Shah et al., 2011). We also did not find *Blattophila perigrinata*, in which the esophagus has a slightly expanded anterior end, and gradually broadens from anterior to posterior (Carreno, 2017).

Statistical analyses

Our definitions of standard measures of parasitism follow Bush et al. (1997). To investigate possible determinants of infection in Australian cockroaches, we focused on pinworm abundance as an overall measure of parasitic infection (e.g., Johansen et al., 2010; Lafferty et al., 2010) because it captures variation in both prevalence and intensity. The abundances of both pinworm species were aggregated, and neither was significantly different from a negative binomial distribution (*L. appendiculata*, variance:mean = 11.09, $k = 0.45$; $X^2 = 26.69$, $df = 17$, $P > 0.05$; *Thelastoma* sp., variance:mean = 6.57, $k = 0.31$; $X^2 = 17.00$, $df =$

15, $P > 0.05$). We analyzed abundance with generalized linear mixed-effects models (GLMMs; Paterson and Lello, 2003; Fenton et al., 2010) implemented via the *lme4* package (Bates et al., 2014) in R (R Development Core Team, 2012). We used Poisson family error terms and a log link function. We also analyzed the pinworm prevalence using GLMMs with binomial family error terms and a logit link function, but this analysis did not reveal any new patterns (data not shown).

In our GLMM analysis, the response variable was the abundance per pinworm species (either *L. appendiculata* or *Thelastoma* sp.); thus there were 2 observations per each individual host. Fixed effects included pinworm taxon (*L. appendiculata* and *Thelastoma* sp.), habitat (upper and lower room), season (spring, summer, fall, and winter), cockroach stage (nymph and adult), and size (femur length). To evaluate the effect of sex on infection, we explored models in which hosts were divided into 3 classes (nymphs, adult males, and adult females), rather than just 2 classes (nymphs and adults). To examine interactions between pinworm species, we included a correlated random effect of parasite taxon over individual cockroaches. The biological interpretation of this random effect structure is that the quality of individual cockroaches as hosts might be different for *L. appendiculata* and *Thelastoma* sp. Statistically, this structure accounted for overdispersion in parasite abundance data (Zwolak et al., 2013; Harrison, 2014) and allowed the measurement of the correlation between the abundance of *L. appendiculata* and *Thelastoma* sp. that is controlled for the variables included as fixed effects. Positive values of the correlation would indicate that abundances of the 2 pinworm species are positively associated within hosts, while negative values would indicate that cockroaches infected with 1 species of pinworm tend to have lower abundance of the other species. The 95% confidence intervals of the correlation coefficient were obtained with parametric bootstrapping (function *sim*, package *arm*).

Because we were interested mainly in species-specific variables influencing parasite loads, our initial model included only 2-way interactions between pinworm taxa and all the remaining fixed effects. Thus, the initial model had the following structure:

$$\text{glmer}\left(\text{abundance} \sim (1 + \text{taxon} | \text{individual}) + \text{taxon} \times \text{habitat} + \text{taxon} \times \text{season} + \text{taxon} \times \text{stage} + \text{taxon} \times \text{size}\right)$$

The final model structure was identified through the elimination of non-significant interaction terms using likelihood ratio tests.

In our results, we report mean abundances and their standard errors based on GLMM estimates. To aid interpretation of these models, we used raw data to calculate prevalences, mean intensities, and their 95% confidence intervals, using Quantitative Parasitology 3.0 (Reiczigel et al., 2013), and these are provided as supplemental tables.

RESULTS

Environmental variation

The upper and lower rooms displayed consistent environmental differences over the duration of this study (Table I). The upper room was significantly warmer across seasons (paired *t*-test on trapping session mean temperatures: $t = 2.60$, $df = 7$, $P = 0.04$), with a mean difference of 0.9 C (Table I). Relative humidity also

Table I. Environmental measurements of the Western Illinois University Biological Sciences greenhouse during 4 seasons. “Upper” and “Lower” refer to 2 rooms in which cockroaches were collected. Mean (and SEM) based on hourly recordings during 8 trapping sessions (2 in each season). U – L = difference between rooms = mean upper room measurement minus mean lower room measurement.

Season	Temperature (C)			Relative humidity (%)		
	Upper	Lower	U – L	Upper	Lower	U – L
Spring	23.4 (0.3)	21.8 (0.1)	1.6	83.3 (0.9)	95.2 (0.7)	-11.9
Summer	25.2 (0.2)	24.0 (0.2)	1.2	80.2 (0.7)	102.7 (0.2)	-22.5
Fall	22.7 (0.2)	22.3 (0.1)	0.4	81.2 (0.9)	87.1 (1.1)	-5.9
Winter	21.0 (0.1)	20.8 (0.1)	0.2	63.9 (0.9)	71.4 (0.9)	-7.5
Mean	23.1 (0.1)	22.2 (0.1)	0.9	77.2 (0.9)	89.1 (0.9)	-11.9

differed significantly ($t = -5.12$, $df = 7$, $P < 0.01$). The upper room was drier than the lower room in every season, with a mean difference of approximately 12% (Table I). In both rooms, there was also significant environmental variation across seasons (1-way ANOVA: Lower room; temperature, $F = 75.6$, $df = 3$, 512 , $P < 0.001$; humidity, $F = 329.62$, $df = 3$, 512 , $P < 0.001$. Upper room; temperature, $F = 86.6$, $df = 3$, 512 , $P < 0.001$; humidity, $F = 103.6$, $df = 3$, 512 , $P < 0.001$). In both rooms, the temperature increased from spring to summer, then decreased in fall and winter (Table I). Relative humidity in the upper room was relatively constant across spring, summer, and fall, but declined in winter, and in the lower room, increased from spring to summer, and then decreased in fall and winter (Table I).

Cockroaches

Over all 4 seasons, we collected and identified pinworms from a total of 239 cockroaches (Table II). Cockroach femur length ranged from 1 to 10 mm, suggesting that we sampled roaches over the entire age range, from early nymphal instars to adults (Adamson and Noble, 1992). Over all seasons, we collected significantly more nymphs than adults (66%:34%; $X^2 = 36.5$, $df = 1$, $P < 0.001$) and more females than males (72%:38%; $X^2 = 23.21$, $df = 1$, $P < 0.001$). Finally, the number of cockroaches collected per trap was higher in the lower room than the upper room ($X^2 = 33.98$, $df = 1$, $P < 0.001$). We collected 156 (65% of all roaches) in the lower room, and 83 roaches (35%) in the upper room.

Infection

The overall prevalence of infection with any pinworm species was 82% (197/239). Of 239 cockroaches, 24% were infected by

Table II. Developmental stage and sex of Australian cockroaches (*Periplaneta australasiae*) collected over 4 seasons in the Western Illinois University Biological Sciences greenhouse.

Season	Demographic category				Total by season
	Nymph	Adult female	Adult male	Total adults	
Spring	22	23	5	28	50
Summer	35	5	1	6	41
Fall	56	11	13	24	80
Winter	50	16	2	18	68
Total	163	55	21	76	239

both species, 37% by *L. appendiculata* alone, and 22% by *Thelastoma* sp. alone.

Overall, there were significant differences in patterns of abundance of the 2 pinworm species (taxon effect in Table III), and several of these were context dependent. The average abundance of *Thelastoma* sp. was 2.01/host (range = 0–29) and of *L. appendiculata* was 2.74/host (range = 0–47). Overall prevalence and intensity measures are provided in Suppl. Table S1. Relative abundance of these pinworm taxa was highly conditional on habitat (taxon × habitat effect in Table III; Fig. 1; Table S2); *Thelastoma* sp. was more abundant than *L. appendiculata* in cockroaches inhabiting the upper room, but the opposite pattern was found in the lower room (Fig. 1), resulting in no significant main effect of habitat. The abundances of both pinworm taxa also differed among seasons (season effect in Table III; Fig. 2; Table S3), with the highest abundance of each species recorded in winter.

The abundance of both pinworm taxa increased with host body size (size effect in Table III; Fig. 3) and was greater in adults than in nymphs (stage effect in Table III; Fig. 1). However, relative to nymphs, cockroach adults actually had fewer pinworms than expected for their larger size (stage effect in Table III), and this pattern was more pronounced in *Thelastoma* sp. than *L. appendiculata* (taxon × stage effect in Table III). As a consequence, relative infection levels in nymphs and adults were more similar for *Thelastoma* sp. than for *L. appendiculata* (Table S4).

Table III. GLMM results: factors influencing the abundance of pinworms (*Leidyneria appendiculata* and *Thelastoma* sp.) infecting Australian cockroaches (*Periplaneta australasiae*). “Taxon” indicates *L. appendiculata* vs. *Thelastoma* sp.; “Habitat” denotes the difference between the upper and the lower rooms of the Western Illinois University Biological Sciences greenhouse; “Season” is the difference between spring, summer, fall, and winter; “Size” is the effect of cockroach body size, based on femur length; “Stage” denotes nymph vs. adult cockroaches.

Variable	X^2	df	P
Taxon	7.80	1	0.005
Habitat	2.05	1	0.152
Season	18.67	3	<0.001
Size	54.92	1	<0.001
Stage	9.12	1	0.003
Taxon × habitat	33.46	1	<0.001
Taxon × stage	3.85	1	0.049

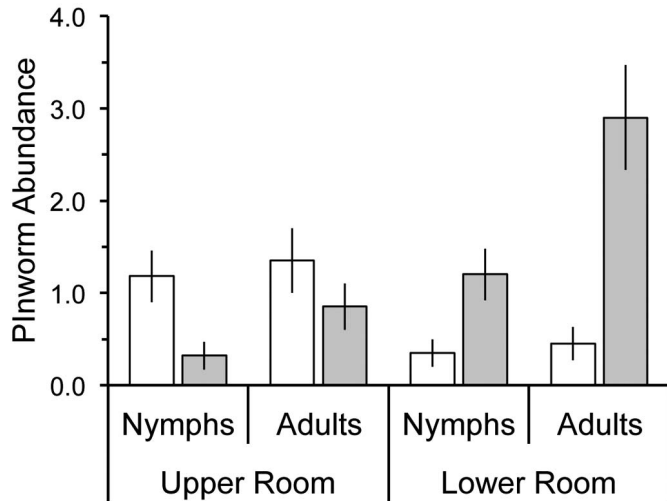


Figure 1. Habitat and developmental stage influence pinworm infection in cockroaches. Abundance of *Thelastoma* sp. (open bars) and *Leidyneina appendiculata* (closed bars) infecting nymph and adult Australian cockroaches (*Periplaneta australasiae*) collected from upper and lower rooms of the Western Illinois University Biological Sciences greenhouse. Bars show median abundance and standard errors. Abundance estimated at mean femur size for each stage (nymph = 5.39 mm, adult = 8.51 mm). Median abundances were estimated using a generalized linear mixed model with a log link function (see “Methods”) and back-transformed to the original scale (the estimate of the mean on the log scale is the median on the original scale).

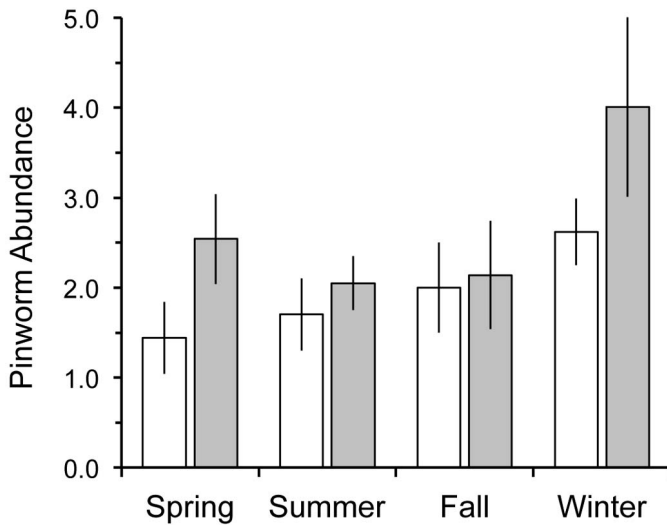


Figure 2. Pinworm abundance varies seasonally in cockroaches. Abundance of *Thelastoma* sp. (open bars) and *Leidyneina appendiculata* (closed bars) infecting Australian cockroaches (*Periplaneta australasiae*) in 4 trapping seasons. Bars show median abundances (estimated as described in Fig. 1 legend) and standard errors. Abundance estimated at grand mean femur size (6.06 mm).

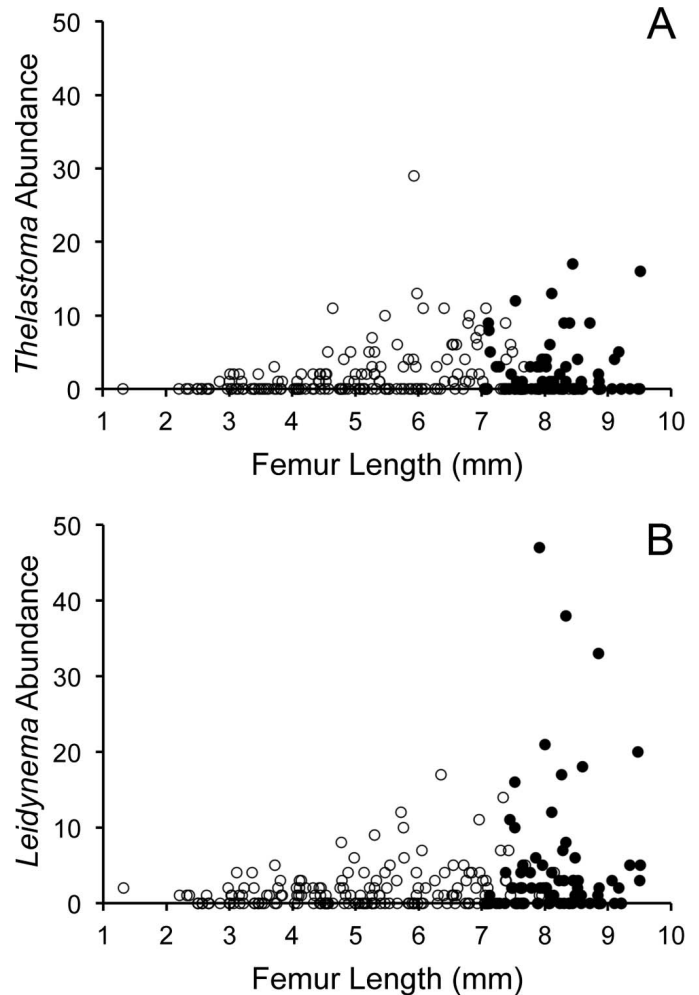


Figure 3. Pinworm infection increases with cockroach size. Abundance of pinworms infecting Australian cockroaches (*Periplaneta australasiae*) as a function of cockroach femur length. (A) *Thelastoma* sp. and (B) *Leidyneina appendiculata*. Open circles, nymphal cockroaches; closed circles, adult cockroaches.

There was no evidence that sex affected pinworm infection: the GLMM with 3 classes of hosts (nymphs, adult females, and adult males) did not perform better than the model with only 2 classes of hosts, nymphs and adults (likelihood ratio test, $\chi^2 = 0.20$, $df = 2$, $P = 0.90$). Finally, after controlling for all of the above effects, there was a strong, negative correlation between the abundance of *L. appendiculata* and the abundance of *Thelastoma* sp. ($R = -0.55$, 95% confidence interval [CI] -0.61 to -0.45). Cockroaches tended to be infected with either 1 pinworm species or the other (Fig. 4).

DISCUSSION

In this study, we provide one of few descriptions of the thelastomatid community infecting *P. australasiae* (Carreno, 2017), which shares pinworm species in common with the congeneric host, *P. americana* (Hominick and Davey, 1972a; Khairul Anuar and Rodzoh, 1978; Adamson and Noble, 1992; Ghosh, 2017). We examined multiple potential determinants of these cockroach-infecting pinworm communities. In addition to factors previously studied in these systems, we examined 2 host

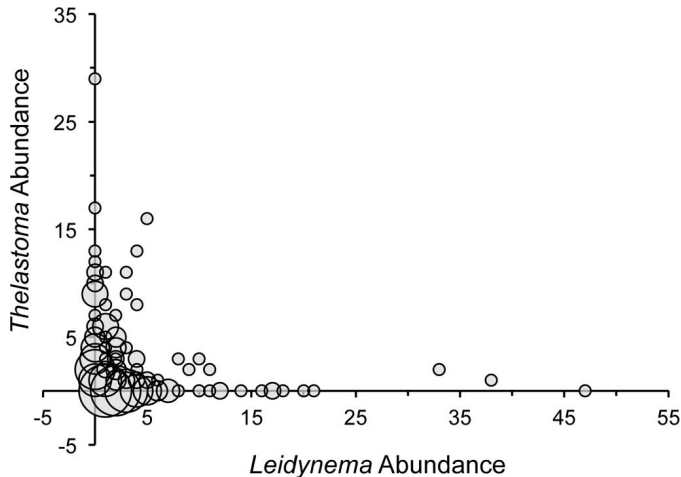


Figure 4. Negative association between 2 cockroach-infesting pinworm species. Relationship between abundances of *Thelastoma* sp. and *Leidyndema appendiculata* infecting Australian cockroaches (*Periplaneta australasiae*). Circle area reflects number of data points for a particular combination of worm counts. (Note: 42 cockroaches infected with neither species [that is, 0, 0] are excluded from graph to improve readability.)

attributes that change with age: size (i.e., femur length) and developmental stage (i.e., sexual maturity). Earlier studies either considered only developmental stage (Khairul Anuar and Rodzoh, 1978; McCallister, 1988), or used size to infer stage (e.g., Zervos 1988a; Adamson and Noble, 1992), so we are the first to disentangle their effects. We also provide the first explicit test of habitat effects on pinworm infections in free-ranging populations of cockroaches. Previous work on the determinants of pinworm communities in cockroaches focused on intraspecific competition (e.g., Hominick and Davey, 1973; Zervos, 1988b; Adamson and Noble, 1993); however, our results indicate that the determinants of pinworm infection in cockroaches are complex, diverse, and represent a variety of scales, including above-host environmental factors (habitat and season), among-host differences (size and stage), and within-host interactions among pinworm species (competition). Furthermore, some of these factors affected both pinworm species in a similar way, but others were taxon-specific.

Habitat effects have not previously been examined in cockroach-pinworm systems. Yet, we found that habitat had significant, taxon-specific effects on pinworm infection levels. This result is not surprising given the general knowledge of host-parasite systems and the fact that habitat differences can influence the probability of host-parasite encounters (Pavlovsky, 1966; Sousa and Grosholz, 1991). Thus, the opposite pattern of abundances of *Thelastoma* sp. and *L. appendiculata* in the 2 investigated habitats could be due to environmental differences between the 2 rooms. For instance, temperature and relative humidity, which differed here, can directly affect the persistence of eggs while outside the host (Zervos, 1988b; Jex et al., 2007b; Froeschke et al., 2010), and optimal climatic conditions can vary among parasite species (e.g., Rossinago and Gruner, 1995). Different habitats may also provide hosts with different diets, which can alter parasite communities (Lange et al., 2014), and have been shown to affect thelastomatid abundance in single-

species (Peregrine, 1974) and mixed-species infections (Hominick and Davey, 1972b).

We also documented significant temporal variation in pinworm abundance, as have others (Zervos, 1988b; Adamson and Noble, 1992). Seasonal changes in parasitism rates are common and can be caused by changes in both abiotic factors and host attributes, such as reproductive status or aggregation patterns (reviewed in Viljoen et al., 2011). However, none of these clearly explain our results. We documented variation in temperature and humidity, but pinworm abundances did not obviously track these changes. Furthermore, cockroaches breed continuously, not seasonally (Brenner and Kramer, 2018), and pinworm abundances did not track cockroach density (as measured by trap success), so these also seem unlikely explanations. More work is needed to determine whether “seasonal” variation in this single-year study is consistent from year to year (e.g., Brunner and Ostfeld, 2008), and thus, whether temporal variation is primarily deterministic or stochastic.

Larger hosts had higher abundances of both pinworm species, a pattern that has been reported for cockroaches and pinworms (McCallister, 1988; Zervos, 1988a, 1988b; Adamson and Noble, 1992, 1993; Morand and Rivault, 1992) and many other host-parasite systems (e.g., Hasu et al., 2007; Lane et al., 2015). This pattern would be produced if larger hosts provide larger habitat or more resources for parasites to develop (Spall and Summerfelt, 1970; Hasu et al., 2007). Alternatively, size may reflect host age and time for exposure (Anderson and Gordon, 1982; Outreman et al., 2007). We were unable to distinguish between size and time effects in this study because we did not know the true age of cockroach hosts (Watkins and Blouin-Demers, 2019).

When taking into account their larger size, adult-stage cockroaches had relatively fewer pinworms than did nymphs, and the magnitude of this effect differed between pinworm species (i.e., the number of *Thelastoma* sp. in adult hosts was relatively lower). In general, cockroach stage has not been found to affect pinworm infection (Zervos, 1988a, 1988b; Adamson and Noble, 1992, 1993; Morand and Rivault, 1992; but see Hominick and Davey, 1972a). As hosts develop, they undergo a variety of changes that can affect parasite infections (Hasu et al., 2007). For example, behavioral changes can alter exposure to parasites (e.g., Schofield and Torr, 2002). However, cockroaches live in gregarious, mixed-family herds in which nymphs and adults forage and rest together in the same microhabitats (Lihoreau et al., 2012). Therefore, they do not exhibit movement or diet differences that are likely to alter their exposure to pinworm infections.

Physiological changes that accompany maturity could also affect parasite loads (e.g., Giefing-Kröll et al., 2015). For example, insect immune responsiveness changes with age (Rheins and Karp, 1985a; Zervos and Webster, 1989; Srygley, 2012), and the development of immunity to pinworms by mammals leads to reduced infections in older hosts (Behnke, 1976; Pritchett and Johnston, 2002). However, the immune response of insects to nematodes is not yet well understood (Castillo et al., 2011), and to our knowledge, there is no evidence that arthropods respond immunologically to gut nematodes (McCallister, 1988; Zervos, 1988a). Other stage-specific differences could also drive differences in pinworm infection, including differences in hormone levels (Rantala et al., 2003; Huang et al., 2013), gene expression patterns (Mitaka et al., 2017), or even microbiome composition

(Näpflin and Schmid-Hempel, 2016; Vicente et al., 2016). Clearly, more work is needed to distinguish these alternatives.

The 1 host attribute that did not affect pinworm abundance was cockroach sex, which is consistent with general patterns among arthropods (Sheridan et al., 2000). Several authors have reported higher pinworm infections in female cockroaches (Hominick and Davey, 1972a; McCallister, 1988; Adamson and Noble, 1992, 1993), but others have not (Dobrovolny and Ackert, 1934; Khairul Anuar and Rodzoh, 1978; Zervos, 1988b; Morand and Rivault, 1992; Ghosh, 2017). Sex differences that can affect parasitism include diet (Hominick and Davey, 1972b), immunological response (Rheins and Karp, 1985b), and hormone levels (Vea et al., 2016). Alternatively, sex differences in infection could be simply due to differences in body size. We found that pinworm infections were higher in females than males (mean abundance [95% CI] for *Thelastoma* sp. in females = 2.3 [1.4–3.6], males = 2.0 [0.9–4.0]; *L. appendiculata* in females = 5.7 [3.6–9.0], males = 2.0 [1.0–3.3]). However, female cockroaches were also significantly larger than males (mean femur size \pm SD for females = 8.36 mm \pm 0.56; males = 7.62 \pm 0.52; $t = 5.25$, $df = 54$, 20 , $P < 0.001$), and the statistical effect of sex disappeared once the differences in body size were taken into account.

There was a strong negative interaction between *Thelastoma* sp. and *L. appendiculata*, a pattern that has also been observed in laboratory colonies of *P. americana* (Adamson and Noble, 1992, 1993). The presence of one species tends to prevent the occurrence of the other (Adamson and Noble, 1993); thus this interaction plays a fundamental role in determining community structure in these cockroaches. Neither species appears innately superior (that is, all cockroaches are not dominated by 1 pinworm species); thus, it appears that this interaction operates via a priority effect (Poulin, 2001), so that whichever species first colonizes a host is somehow able to exclude the other (Adamson and Noble, 1993). It seems likely that these species would compete; they prefer the same habitat (anterior hindgut; Adamson and Noble, 1992) and show no obvious differences in diet (Connor and Adamson, 1998). However, this strong negative interaction contrasts with the large body of data for vertebrates, for which there is no general consensus on the frequency, or importance, of helminth-helminth interactions in wild hosts (Poulin, 2001; Behnke, 2008).

Helminths can negatively affect each other in a variety of ways (Cox, 2001; Lello et al., 2004; Behnke, 2008), either directly, through physical interference or the release of toxic chemicals, or indirectly, through competition for resources, changes in gut physiology, or via the host immune response. In mammalian hosts, shared resource use and immune responses are primary mechanisms determining the outcome of interactions among helminths (Griffiths et al., 2014). Much less is known about how interactions among insect-infecting helminths are mediated.

Zervos (1988a) suggested that direct physical interference among thelastomatids is unlikely because individuals have no obvious weapons, and she never observed agonistic interactions among worms removed from cockroaches. Adamson and Noble (1993) argued that cockroach pinworms do not display resource competition because high pinworm density does not reduce female pinworm fecundity. Given their similar niches (Hominick and Davey, 1972a; Connor and Adamson, 1998), it seems unlikely that either species alters gut physiology in a way that makes the gut uninhabitable for the other species without harming itself. Finally, there have been few studies of immune responses of co-

infected insects (e.g., Honkavaara et al., 2009), and none of these have included gut nematodes, so it seems premature to attribute the negative interaction between *Thelastoma* sp. and *L. appendiculata* to the host's immune response.

The remaining possible mechanism for the priority effect we observed is toxic allelochemicals (Lello et al., 2004; Behnke, 2008) used by 1 species to suppress colonization by the other. Such chemicals have been proposed to explain intraspecific competition in thelastomatid species (Zervos, 1988a). Although no molecules that mediate interactions between animal-infecting nematodes have been identified, their existence has been inferred for plant-infecting nematodes (e.g., McIntyre and Miller, 1976; Umesh et al., 1994).

Parasite epidemiologists strive to understand which factors, from which scales, determine host infection levels. Our most significant result was not that any particular factor mattered, but that almost every one did. The strength of our study was using a GLMM-based approach to simultaneously examine many possible determinants of infection. Doing so allowed us to assess each factor's impact while controlling for the effects of all others so that we were able to avoid spurious correlations with infection (Fenton et al., 2010; Hellard et al., 2015). This approach revealed that although sex had been proposed to be a determinant of infection, its effects might be explained more parsimoniously by body size. In general, our work underscores the importance of measuring potential infection determinants from as many scales as possible (Monello and Gompper, 2009). Such approaches are necessary to unravel the complexities of host-parasite interactions (Paterson and Lello, 2003; Fenton et al., 2010; Hellard et al., 2015), as well as their impacts on the larger communities of which they are a part.

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LITERATURE CITED

- ADAMSON, M. L. 1994. Evolutionary patterns in life histories of Oxyurida. *International Journal of Parasitology* 24: 1167–1177.

- ADAMSON, M. L., AND S. NOBLE. 1992. Structure of the pinworm (Oxyurida: Nematoda) guild in the hindgut of the American cockroach, *Periplaneta americana*. *Parasitology* 104: 497–507.
- ADAMSON, M. L., AND S. NOBLE. 1993. Interspecific and intraspecific competition among pinworms in the hindgut of *Periplaneta americana*. *Journal of Parasitology* 79: 50–56.
- ADAMSON, M. L., AND D. VAN WAEREBEKE. 1992. Revision of the Thelastomatoidea, Oxyurida of invertebrate hosts I. Thelastomatidae. *Systematic Parasitology* 21: 21–63.
- ADENUSI, A. A., M. I. AKINYEMI, AND D. AKINSANYA. 2018. Domiciliary cockroaches as carriers of human intestinal parasites in Lagos metropolis, southwest Nigeria: Implications for public health. *Journal of Arthropod-Borne Diseases* 12: 141–151.
- ANDERSON, R. M., AND D. M. GORDON. 1982. Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology* 85: 373–398.
- BARRETT, E. L. B., J. HUNT, A. J. MOORE, AND P. J. MOORE. 2009. Separate and combined effects of nutrition during juvenile and sexual development on female life-history trajectories: The thrifty phenotype in a cockroach. *Proceedings of the Royal Society B: Biological Sciences* 267: 3257–3264.
- BATES, D., M. MAECHLER, B. BOLKER, AND S. WALKER. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7. Available at: <http://CRAN.R-project.org/package=lme4>. Accessed 28 November 2015.
- BEHNKE, J. M. 1976. *Aspicularis tetraptera* in wild *Mus musculus*. Age resistance and acquired immunity. *Journal of Helminthology* 50: 197–202.
- BEHNKE, J. M. 2008. Structure in parasite component communities in wild rodents: Predictability, stability, associations and interactions or pure randomness? *Parasitology* 135: 751–766.
- BELL, W. J., L. M. ROTH, AND C. A. NALEPA. 2007. *Cockroaches: Ecology, behavior, and natural history*. Johns Hopkins University Press, Baltimore, Maryland, 175 p.
- BOURGUIGNON, T., Q. TANG, S. Y. W. HO, F. JUNA, Z. WANG, D. A. ARAB, S. L. CAMERON, J. WALKER, D. RENTZ, T. A. EVANS, ET AL. 2018. Transoceanic dispersal and plate tectonics shaped global cockroach distributions: Evidence from mitochondrial phylogenomics. *Molecular Biology and Evolution* 35: 970–983.
- BRACKE, J. W., D. L. CRUDEN, AND A. J. MARKOVETZ. 1979. Intestinal microbial flora of the American cockroach, *Periplaneta americana* L. *Applied and Environmental Microbiology* 38: 945–955.
- BRENNER, R. J., AND R. D. KRAMER. 2018. *Cockroaches (Blattaria)*. In *Medical and veterinary entomology*, 3rd ed., G. Mullen and L. Durden (eds.). Academic Press, London, U.K., p. 61–77.
- BROOKS, D. R., AND D. R. GLEN. 1982. Pinworms and primates: A case study in coevolution. *Proceedings of the Helminthological Society of Washington* 49: 76–85.
- BRUNNER, J. L., AND R. S. OSTFELD. 2008. Multiple causes of variable tick burdens on small-mammal hosts. *Ecology* 89: 2259–2272.
- BUNKER, B. E., J. JANOVY JR., E. TRACEY, A. BARNES, A. DUBA, M. SHUMAN, AND J. D. LOGAN. 2013. Macroparasite population dynamics among geographical localities and host life cycle stages: Eugregarines in *Ichnura verticalis*. *Journal of Parasitology* 99: 403–409.
- 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.
- CALEGARO-MARQUES, C., AND S. B. AMATO. 2013. Seasonal influences on parasite community structure of *Turdus rufiventris* (Aves). *Journal of Parasitology* 99: 1–5.
- CARRENO, R. A. 2014. The systematics and evolution of pinworms (Nematoda: Oxyurida: Thelastomatoidea) from invertebrates. *Journal of Parasitology* 100: 553–560.
- CARRENO, R. A. 2017. New species of *Hammerschmidtella* Chitwood, 1932, and *Blattophila* Cobb, 1920, and new geographical records for *Severianoia annamensis* Van Luc & Spiridonov, 1993 (Nematoda: Oxyurida: Thelastomatoidea) from Cockroaches (Insecta: Blattaria) in Ohio and Florida, U.S.A. *Zootaxa* 4226: 429–441.
- CASTILLO, J. C., S. E. REYNOLDS, AND I. ELEFThERIANOS. 2011. Insect immune responses to nematode parasites. *Trends in Parasitology* 27: 537–547.
- CONNOR, S., AND M. ADAMSON. 1998. Niche overlap among three species of pinworm parasitic in the hindgut of the American cockroach, *Periplaneta americana*. *Journal of Parasitology* 84: 245–247.
- COX, F. E. G. 2001. Concomitant infections, parasites and immune responses. *Parasitology* 122(Suppl. S1): S23–S38.
- DOBROVOLNY, C. G., AND J. E. ACKERT. 1934. The life history of *Leidynema appendiculata* (Leidy), a nematode of cockroaches. *Parasitology* 26: 468–480.
- ETIM, S. E., O. E. OKON, P. A. AKPAN, G. I. UKPONG, AND E. E. OKU. 2013. Prevalence of cockroaches (*Periplaneta americana*) in households in Calabar: Public health implications. *Journal of Public Health and Epidemiology* 5: 149–152.
- FENTON, A., M. E. VINEY, AND J. LELLO. 2010. Detecting interspecific macroparasite interactions from ecological data: Patterns and process. *Ecology Letters* 13: 606–615.
- FROESCHKE, G., R. HARF, S. SOMMER, AND S. MATTHEE. 2010. Effects of precipitation on parasite burden along a natural climatic gradient in southern Africa—Implications for possible shifts in infestation patterns due to global changes. *Oikos* 119: 1029–1039.
- GHOSH, J. 2017. A study on the occurrence of pinworms in the hindgut of *Periplaneta americana*. *Journal of Parasitic Diseases* 41: 1154–1157.
- GIEFING-KRÖLL, C., P. BERGER, G. LEPPERDINGER, AND B. GRUBECK-LOEBENSTEIN. 2015. How sex and age affect immune responses, susceptibility to infections, and response to vaccination. *Aging Cell* 14: 309–321.
- GONZÁLEZ-HERNÁNDEZ, M., A. RANGEL-NEGRÍN, V. A. M. SCHOOF, C. A. CHAPMAN, D. CANALES-ESPINOSA, AND P. A. D. DIAS. 2014. Transmission patterns of pinworms in two sympatric congeneric primate species. *International Journal of Primatology* 35: 445–462.
- GRIFFITHS, E. C., A. B. PEDERSEN, A. FENTON, AND O. L. PETCHEY. 2014. Analysis of a summary network of co-infection in humans reveals that parasites interact most via shared resources. *Proceedings of the Royal Society B: Biological Sciences* 281: 20132286. doi:10.1098/rspb.2013.2286.
- HARRISON, X. A. 2014. Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ* 2: e616. doi:10.7717/peerj.616.

- HASU, T., J. C. HOLMES, AND E. T. VALTONEN. 2007. Isopod (*Asellus aquaticus*) size and acanthocephalan (*Acanthocephalus lucii*) infections. *Journal of Parasitology* 93: 450–457.
- HELLARD, E., D. FOUCHET, F. VAVRE, AND D. PONTIER. 2015. Parasite–parasite interactions in the wild: How to detect them? *Trends in Parasitology* 31: 640–652.
- HOMINICK, W. M., AND K. G. DAVEY. 1972a. Influence of host stage and sex upon the size and composition of the population of the thelastomatids parasitic in the hindgut of *Periplaneta americana*. *Canadian Journal of Zoology* 50: 947–954.
- HOMINICK, W. M., AND K. G. DAVEY. 1972b. Reduced nutrition as the factor controlling the population of pinworms following endocrine gland removal in *Periplaneta americana* L. *Canadian Journal of Zoology* 50: 1421–1432.
- HOMINICK, W. M., AND K. G. DAVEY. 1973. Food and the spatial distribution of adult female pinworms parasitic in the hindgut of *Periplaneta americana* L. *International Journal for Parasitology* 3: 759–771.
- HONKAVAARA, J., M. J. RANTALA, AND J. SUHONEN. 2009. Mating status, immune defence, and multi-parasite burden in the damselfly *Coenagrion armatum*. *Entomologia Experimentalis et Applicata* 132: 165–171.
- HUANG, J. H., J. LOZANO, AND X. BELLES. 2013. Broad-complex functions in postembryonic development of the cockroach *Blattella germanica* shed new light on the evolution of insect metamorphosis. *Biochimica et Biophysica Acta* 1830: 2178–2187.
- JEX, A. R., M. A. SCHNEIDER, AND T. H. CRIBB. 2006. The importance of host ecology in thelastomatoid (Nematoda: Oxyurida) host specificity. *Parasitology International* 55: 169–174.
- JEX, A. R., M. A. SCHNEIDER, H. A. ROSE, AND T. H. CRIBB. 2007a. A comprehensive analysis of the biogeography of the thelastomatoid pinworms from Australian burrowing cockroaches (Blaberidae: Geoscaphinae, Panesthiinae): No evidence of coevolution. *Parasitology* 134: 1387–1399.
- JEX, A. R., M. A. SCHNEIDER, H. A. ROSE, AND T. H. CRIBB. 2007b. Local climate aridity influences the distribution of thelastomatoid nematodes of the Australian giant burrowing cockroach. *Parasitology* 134: 1401–1408.
- JOHANSEN, C. E., C. LYDERSEN, P. E. ASPHOLM, AND K. M. KOVACS. 2010. Helminth parasites in ringed seals (*Pusa hispida*) from Svalbard, Norway with special emphasis on nematodes: Variation with age, sex, diet, and location of host. *Journal of Parasitology* 96: 946–953.
- KHAIRUL ANUAR, A., AND S. RODZOH. 1978. The influence of host stage and sex upon the size and composition of the thelastomatids parasitic in the hindgut of *Periplaneta americana* L. and *Neostylopyga rhombifolia* (Stoll). *Medical Journal of Malaysia* 1: 76–85.
- LAFFERTY, K., S. A. HATHAWAY, A. S. WEGMANN, F. S. SHIPLEY, A. R. BACKLIN, J. HELM, AND R. N. FISHER. 2010. Stomach nematodes (*Mastophorus muris*) in rats (*Rattus rattus*) are associated with coconut (*Cocos nucifera*) habitat at Palmyra atoll. *Journal of Parasitology* 96: 16–20.
- LANE, B., T. SPIER, J. WIEDERHOLT, AND S. MEAGHER. 2015. Host specificity of a parasitic fluke: Is *Posthodiplostomum minimum* a centrarchid-infecting generalist or specialist? *Journal of Parasitology* 101: 6–17.
- LANGE, B., M. REUTER, D. EBERT, K. MUYLEAERT AND E. DECAESTECKER. 2014. Diet quality determines interspecific parasite interactions in host populations. *Ecology and Evolution* 4: 3093–3102.
- LELLO, J., B. BOAG, A. FENTON, I. R. STEVENSON, AND P. J. HUDSON. 2004. Competition and mutualism among the gut helminths of a mammalian host. *Nature* 428: 840–844.
- LIHOREAU, M., J. T. COSTA, AND C. RIVAULT. 2012. The social biology of domiciliary cockroaches: Colony structure, kin recognition and collective decisions. *Insectes Sociaux* 59: 445–452.
- MCCALLISTER, G. L. 1988. The effect of *Thelastoma bulhoesi* and *Hammerschmidtella diesingi* (Nematoda: Oxyurata) on host size and physiology in *Periplaneta americana* (Arthropoda: Blattidae). *Proceedings of the Helminthological Society of Washington* 55: 12–14.
- MCCALLISTER, G. L., AND G. D. SCHMIDT. 1981. Diurnal migration of the female of *Thelastoma bulhoesi* (Oxyurata: Thelastomida) in the American cockroach, *Periplaneta americana*. *Proceedings of the Helminthological Society of Washington* 48: 127–129.
- MCINTYRE, J. L., AND P. M. MILLER. 1976. Competitive interaction of *Tylenchorhynchus claytoni* and *Pratylenchus penetrans* in tobacco roots. *Phytopathology* 66: 1427–1430.
- MITAKA, Y., K. KOBAYASHI, AND K. MATSUURA. 2017. Caste-, sex-, and age-dependent expression of immune-related genes in a Japanese subterranean termite, *Reticulitermes speratus*. *PLoS ONE* 12: e0175417. doi:10.1371/journal.pone.0175417.
- MONELLO, R. J., AND M. E. GOMPPER. 2009. Relative importance of demographics, locale, and seasonality underlying louse and flea parasitism of raccoons (*Procyon lotor*). *Journal of Parasitology* 95: 56–62.
- MORAND, S., AND C. RIVAULT. 1992. Infestation dynamics of *Blatticola blattae* Graeffe (Nematoda: Thelastomatidae), a parasite of *Blattella germanica* L. (Dictyoptera: Blattellidae). *International Journal for Parasitology* 22: 983–989.
- MÜLLER-GRAF, C. D. M., E. JOBET, A. CLOAREC, C. RIVAULT, M. VAN BAALEN, AND S. MORAND. 2001. Population dynamics of host-parasite interactions in a cockroach-oxyuroid system. *Oikos* 95: 431–440.
- NÄPFLIN, K., AND P. SCHMID-HEMPEL. 2016. Immune response and gut microbial community structure in bumblebees after microbiota transplants. *Proceedings of the Royal Society B: Biological Sciences* 283: 20160312. doi:10.1098/rspb.2016.0312.
- OUTREMAN, Y., F. CÉZILLY, AND L. BOLLACHE. 2007. Field evidence of host size-dependent parasitism in two manipulative parasites. *Journal of Parasitology* 93: 750–754.
- PATERSON, S., AND J. LELLO. 2003. Mixed models: Getting the best use of parasitological data. *Trends in Parasitology* 8: 370–375.
- PAVLOVSKY, E. N. 1966. Natural nidity of transmissible diseases: In relation to landscape, epidemiology, of zooanthroposes. University of Illinois Press, Champaign, Illinois, 261 p. (Translated from the Russian edition, 1964, by F. K. Pious Jr.; English translation edited by N. D. Levine.)
- PEREGRINE, P. C. 1974. The effects of host diet on *Thelastoma attenuatum* (Nematoda: Thelastomatidae) populations in cockroaches. *Journal of Helminthology* 48: 47–57.

- PHILIPPE, G., AND P. DELEPORTE. 1996. The origin of protistan symbionts in termites and cockroaches: A phylogenetic perspective. *Cladistics* 12: 93–98.
- POULIN, R. 2001. Interactions between species and the structure of helminth communities. *Parasitology* 122: S3–S11.
- PRITCHARD, M. H., AND G. O. W. KRUSE. 1982. The collection and preservation of animal parasites. Technical Bulletin No. 1 of the Harold W. Manter Laboratory. University of Nebraska Press, Lincoln, Nebraska, 141 p.
- PRITCHETT, K. R., AND N. A. JOHNSTON. 2002. A review of treatments for the eradication of pinworm infections from laboratory rodent colonies. *Journal of the American Association for Laboratory Animal Science* 41: 36–46.
- R DEVELOPMENT CORE TEAM. 2012. R: A language and environment for statistical computing. Available at: <http://www.R-project.org/>. Accessed 28 November 2018.
- RANTALA, M. J., A. VAINIKKA, AND R. KORTET. 2003. The role of juvenile hormone in immune function and pheromone production trade-offs: A test of the immunocompetence handicap principle. *Proceedings of the Royal Society B: Biological Sciences* 270: 2257–2261.
- REHN, J. A. G. 1945. Man's uninvited fellow traveller—The cockroach. *Scientific Monthly* 4: 265–276.
- REICZIGEL, J., L. ROZSA, A. REICZIGEL, AND I. FABIAN. 2013. Quantitative Parasitology (QPweb). Available at: <http://www2.univet.hu/qpweb/qp10>. Accessed 20 February 2018.
- RHEINS, L. A., AND R. D. KARP. 1985a. Ontogeny of the invertebrate humoral immune response: Studies on various developmental stages of the American cockroach (*Periplaneta americana*). *Developmental and Comparative Immunology* 9: 395–406.
- RHEINS, L. A., AND R. D. KARP. 1985b. Effect of gender on the inducible humoral immune response to honeybee venom in the American cockroach (*Periplaneta americana*). *Developmental and Comparative Immunology* 9: 41–49.
- ROSSINAGO, C. E., AND L. GRUNER. 1995. Moisture and temperature requirements in faeces for the development of free-living stages of gastrointestinal nematodes of sheep, cattle and deer. *Journal of Helminthology* 69: 357–362.
- ROTH, L. M., AND E. R. WILLIS. 1960. The biotic associations of cockroaches. *Smithsonian Miscellaneous Collections* 141: 1–470.
- SCHAD, G. A. 1963. Niche diversification in a parasitic species flock. *Nature* 198: 404–406.
- SCHOFIELD, S., AND S. J. TORR. 2002. A comparison of the feeding behaviour of tsetse and stable flies. *Medical and Veterinary Entomology* 16: 177–185.
- SHAH, M. M., N. MOHILAL, M. PRAMODINI, L. BINA, AND T. HEMANANDA. 2011. Nematode parasites of invertebrates from Manipur, North East India (Diagnosis, keys and illustration). *Zootaxa* 3107: 1–37.
- SHERIDAN, L. A. D., R. POULIN, D. F. WARD, AND M. ZUK. 2000. Sex differences in parasitic infections among arthropod hosts: Is there a male bias? *Oikos* 88: 327–334.
- SOUSA, W. P., AND E. D. GROSHOLZ. 1991. The influence of habitat structure on the transmission of parasites. In *Habitat Structure. Population and Community Biology Series*, vol. 8, S. S. Bell, E. D. McCoy, and H. R. Mushinsky (eds.). Springer, Dordrecht, the Netherlands, 438 p.
- SPALL, R. D., AND R. C. SUMMERFELT. 1970. Life cycle of the white grub, *Posthodiplostomum minimum* (MacCallum 1921: Trematoda, Diplostomatidae), and observations on host-parasite relationships of the metacercaria in fish. A symposium on diseases of fishes and shellfishes, Special Publication number 5, American Fisheries Society, Washington, D.C., p. 218–230.
- SRYGLEY, R. B. 2012. Ontogenetic changes in immunity and susceptibility to fungal infection in Mormon crickets *Anabrus simplex*. *Journal of Insect Physiology* 58: 342–347.
- UMESH, K. C., H. FERRIS, AND D. E. BAYER. 1994. Competition between the plant-parasitic nematodes *Pratylenchus neglectus* and *Meloidogyne chitwoodi*. *Journal of Nematology* 26: 286–295.
- VEA, I. M., S. TANAKA, T. SHIOTSUKI, A. JOURAKU, T. TANAKA, AND C. MINAKUCHI. 2016. Differential juvenile hormone variations in scale insect extreme sexual dimorphism. *PLoS ONE* 11: e0149459. doi:10.1371/journal.pone.0149459.
- VICENTE, C. S. L., S. OZAWA, AND K. HASEGAWA. 2016. Composition of the cockroach gut microbiome in the presence of parasitic nematodes. *Microbes and Environments* 31: 314–320.
- VILJOEN, H., N. C. BENNETT, E. A. UECKERMAN, AND H. LUTERMANN. 2011. The role of host traits, season and group size on parasite burdens in a cooperative mammal. *PLoS ONE* 6: e27003. doi:10.1371/journal.pone.0027003.
- WATKINS, H. J., AND G. BLOUIN-DEMERS. 2019. Body size, not age, predicts, parasite load in Clark's spiny lizards (*Sceloporus clarkii*). *Canadian Journal of Zoology* 97: 220–224.
- WILSON, K., O. N. BJØRNSTAD, A. P. DOBSON, S. MERLER, G. POGLAYEN, S. E. RANDOLPH, A. F. READ, AND A. SKORPING. 2002. Heterogeneities in macroparasite infections: Patterns and processes. In *The ecology of wildlife diseases*, P. J. Hudson, A. Rizzoli, B. T. Grenfell, H. Heesterbeek, and A. P. Dobson (eds.). Oxford University Press, Oxford, U.K., p. 6–44.
- ZERVOS, S. 1988a. Population regulation in parasitic nematodes (Thelastomatidae) of cockroaches. *New Zealand Journal of Zoology* 15: 333–338.
- ZERVOS, S. 1988b. Population dynamics of a thelastomatid nematode of cockroaches. *Parasitology* 96: 353–368.
- ZERVOS, S., AND J. M. WEBSTER. 1989. Susceptibility of the cockroach *Periplaneta americana* to *Heterorhabditis heliothidis* (Nematoda: Rhabditoidea) in the laboratory. *Canadian Journal of Zoology* 67: 1609–1611.
- ZIMMERMAN, M. R., K. E. LUTH, AND G. W. ESCH. 2016. Microhabitat differences in the benthic substrata affect parasitism in a pulmonate snail host, *Helisoma anceps*. *Journal of Parasitology* 102: 306–311.
- ZWOLAK, R., S. MEAGHER, J. W. VAUGHN, S. DZIEMIAN, AND E. E. CRONE. 2013. Reduced ectoparasite loads of deer mice in burned forest: From fleas to trees? *Ecosphere* 4: 132. doi:10.1890/ES13-00138.1.