

PATTERNS OF TROMBICULID MITE (*HANNEMANIA DUNNI*) PARASITISM AMONG PLETHODONTID SALAMANDERS IN THE WESTERN PIEDMONT OF NORTH CAROLINA

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ABSTRACT: Trombiculid mites are known to parasitize a variety of amphibian species, yet few comparisons of mite parasitism among amphibian species have been made. In this study, we investigated patterns of trombiculid mite parasitism among 3 plethodontid salamanders (*Desmognathus fuscus*, *Eurycea cirrigera*, and *Plethodon cylindraceus*) in the western Piedmont of North Carolina. All 3 salamander species were parasitized by a single species, *Hannemania dunni*. *Desmognathus fuscus* harbored mites more frequently (60.4% of individuals) than *E. cirrigera* (11.1%) or *P. cylindraceus* (14.6%). *Desmognathus fuscus* also had higher parasite loads than *E. cirrigera* or *P. cylindraceus* ($P < 0.001$). Mites on *D. fuscus* were found more frequently on the limbs than other body locations ($P < 0.001$). We found no correlation between salamander size and mite abundance ($P = 0.689$), but salamander collection sites influenced the abundance of mites on *D. fuscus* ($P = 0.002$). We found no effect of season on mite abundance in *D. fuscus* ($P = 0.952$). Salamander habitat preferences and edaphic or climatic differences among study sites may influence patterns of *Hannemania* sp. parasitism of salamanders.

Amphibians serve as hosts to many parasites; a variety of factors, including sex, environment, location, and anthropogenic influences, affect amphibian susceptibility to parasite recruitment (Duellman and Trueb, 1986; Johnson and Chase, 2004; Brown et al., 2006). North American woodland and streamside salamanders (Plethodontidae) are parasitized by trombiculid mites, or chiggers, of the genus *Hannemania* (Anthony et al., 1994; Register, 2001); however, little is known about the patterns of *Hannemania* sp. parasitism among salamander species. Adult *Hannemania* sp. are nonparasitic, but larvae parasitize amphibians (Converse and Green, 2005). Larval trombiculid mites produce saliva that destroys the hosts' epidermal cells; the host amphibian's connective tissue is then deposited as a capsule around the mite, resulting in concentrated redness, inflammation, necrosis, and raised abscesses on the dermis known as trombiculidiasis (Sladky et al., 2000). Depending on the host species, larval mites may reside from 6 mo to more than a year within an individual host (Welbourn and Loomis, 1975).

Hannemania sp. attach to specific locations on salamanders' bodies, particularly the extremities (Anthony et al., 1994; McAllister et al., 1995). By burrowing into the limbs, mites are known to cause deformities and to reduce mobility (Register, 2001). Inhibited mobility can negatively affect foraging ability and defensive behavior (Maksimowich and Mathis, 2000). Furthermore, the nasolabial groove, a structure used for detection of chemical cues for communication in plethodontids, can be infected by *Hannemania* sp., which may result in lowered aggression levels toward conspecifics and reduced success in finding mates (Anthony et al., 1994; Maksimowich and Mathis, 2000).

In this study, we describe *Hannemania* sp. parasitism in salamanders inhabiting the western Piedmont of North Carolina. Specifically, we (1) compare the abundance of *Hannemania* sp. parasites among 3 plethodontid salamander species (*Desmognathus fuscus*, *Eurycea cirrigera*, and *Plethodon cylindraceus*), (2) determine whether specific body locations are preferred for

attachment by *Hannemania* sp., (3) evaluate the relationship between salamander body size and parasite abundance on *D. fuscus*, (4) compare parasite abundance among sample locations, and (5) examine seasonal variation in *Hannemania* sp. abundance on *D. fuscus*.

MATERIALS AND METHODS

Salamanders were collected from southern Iredell, northern Mecklenburg, and western Cabarrus counties, North Carolina (Fig. 1). We sampled *D. fuscus* and *E. cirrigera* at 4 semipermanent, first-order streams that were surrounded by forested (locations d, f, and g), and urbanized (location a) land. We sampled *P. cylindraceus* in six terrestrial habitats composed of mixed hardwood–pine forests (locations a, b, c, d, e, and g). We sampled both terrestrial and stream habitats at locations a, d, and g.

We collected *D. fuscus*, *E. cirrigera*, and *P. cylindraceus* from August to October 2006. We used dipnets to capture semiaquatic salamanders (*D. fuscus* and *E. cirrigera*) in and directly adjacent (2 m) to streams. *Plethodon cylindraceus*, a completely terrestrial species, and some *E. cirrigera* were collected by searching under logs and other cover objects in forested areas. A pair of drift fences (Rice et al., 2001) provided supplemental captures of *E. cirrigera* and *P. cylindraceus* during fall 2006.

We used stereomicroscopy to carefully examine each salamander for the presence of larval *Hannemania* sp. Mites were apparent due to concentrated redness, inflammation, necrosis, and raised abscesses (Sladky et al., 2000). The mites were identified as *H. dunni* (Loomis, 1956) based on larval morphology (W. C. Welbourn, pers. comm.). We recorded the location (head, throat, dorsum, venter, cloacal region, tail, or limbs) and abundance of mites on each salamander examined. To permit easier handling, we anesthetized *D. fuscus* using 1 g of Maximum-Strength Orajel® (Del Pharmaceuticals, Uniondale, New York) per 1 L of dechlorinated water (Cecala et al., 2007). We measured the snout–vent length (SVL) and total length of each salamander to the nearest millimeter, recorded mass to the nearest 0.01 g, and individually marked each salamander using visible implant elastomer (Nauwelaerts et al., 2000; Northwest Marine Technology) to avoid counting individuals on multiple occasions. Because of difficulties in determining the sex of each salamander, the sexes were not recorded. Salamanders were returned to their point of capture within 2 days after capture.

We compared the abundance of larval *H. dunni* between *D. fuscus* and *P. cylindraceus* by calculating mean mite abundance per salamander species, which included individuals with no *H. dunni* infections. We used a General Linear Model (GLM) and a Duncan's multiple comparison test (General Linear Model, SAS, version 9.1; SAS Institute, Cary, North Carolina) to compare the abundance of mites found on body locations of *D. fuscus* and *P. cylindraceus*. To evaluate the relationship between salamander body length and parasite abundance, we used a linear regression with SVL as the independent variable and abundance

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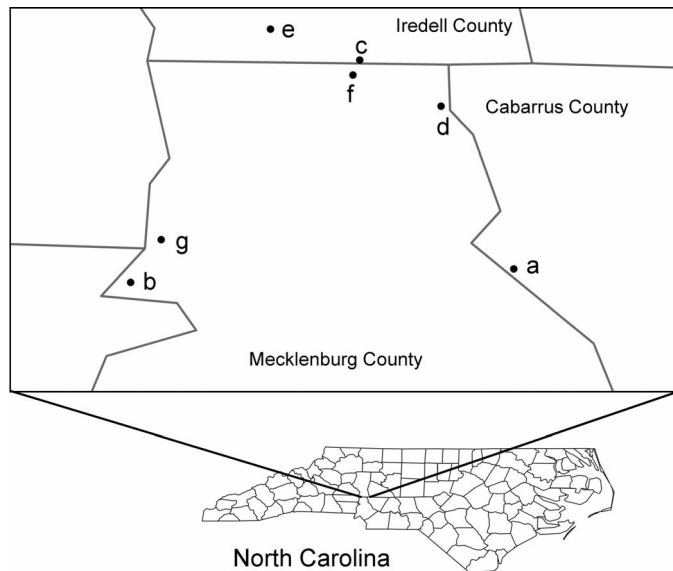


FIGURE 1. Location of salamander collection sites in the western Piedmont of North Carolina. *Desmognathus fuscus* and *Eurycea cirrigera* were collected at 4 semipermanent, first-order streams that were surrounded by forested (locations d, f, and g), and urbanized (location a) land. *Plethodon cylindraceus* were collected at six terrestrial, forested locations (a, b, c, d, e, and g). We sampled both terrestrial and stream habitats at locations a, d, and g.

of mites (i.e., total number on each individual) as the dependent variable. We compared differences in mite abundances among different sample locations by using a GLM and a Duncan's multiple comparison test to compare the number of mites on individual *D. fuscus* and *P. cylindraceus* from 4 and 6 localities, respectively (SAS, version 9.1; SAS Institute). Limited samples of *E. cirrigera* prevented them from being used in any statistical analysis.

We measured seasonal variation of mite abundances in *D. fuscus* inhabiting one of the study sites (location g) by sampling the population monthly from October 2005 through May 2006. During this sampling, only the number of mites (i.e., abundance) for each infected adult salamander was recorded. Data among months were compared using a GLM (SAS, version 9.1; SAS Institute). We evaluated significant results at $\alpha = 0.05$.

RESULTS

Desmognathus fuscus were parasitized by, on average, 3.5 *H. dunni* per individual ($n = 158$ individuals), whereas *P. cylindraceus* ($n = 48$ individuals) and *E. cirrigera* were parasitized by 0.5 and 0.4 *H. dunni* per individual, respectively ($n = 11$ individuals; GLM; $F = 3.04$; $df = 2$; $P < 0.001$; Fig. 2). Most *P. cylindraceus* and *E. cirrigera* lacked parasites (85.4 and 88.9%, respectively), whereas 60.4% of all *D. fuscus* were parasitized. The abundance of mites among salamander body parts also differed among species. *Desmognathus fuscus* harbored the greatest concentration of mites on the limbs and tail, with all other body parts having lower parasite abundance (GLM; $F = 22.7$; $df = 6$; $P < 0.001$; $n = 128$; Fig. 3A). Although mites did not preferentially attach to a particular body location on *P. cylindraceus* (GLM; $F = 1.86$; $df = 6$; $P = 0.088$; $n = 48$; Fig. 3B), the greatest mean concentration of mites was found on the limbs (Fig. 3B). We did not detect *H. dunni* parasitizing salamander nasolabial grooves.

We found no significant relationship between salamander SVL and parasite abundance in *D. fuscus* or *P. cylindraceus*

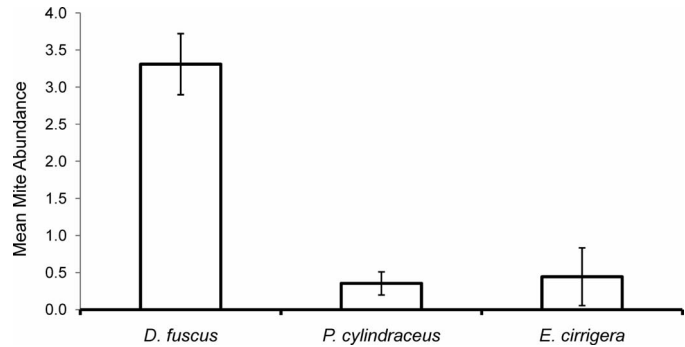


FIGURE 2. Abundance of *Hannemania dunni* on 3 salamander species: *Desmognathus fuscus* ($n = 158$), *Eurycea cirrigera* ($n = 11$), and *Plethodon cylindraceus* ($n = 48$). *Desmognathus fuscus* harbored significantly more mites than either *E. cirrigera* or *P. cylindraceus* (GLM; $F = 3.04$; $df = 2$; $P < 0.001$). Error bars represent ± 1 SE. Samples were collected during fall 2005, spring 2006, and fall 2006.

(linear regression, *D. fuscus*; $R^2 = 0.055$; $P = 0.688$; *P. cylindraceus*; $R^2 = 0.005$; $P = 0.428$). However, we did find significant variation in mite abundance on *D. fuscus* among the sampled localities (GLM; $F = 5.33$; $df = 3$; $P = 0.002$; Fig. 4A). *Desmognathus fuscus* captured at location f had a greater mean abundance per individual than the abundances at other sites (Fig. 4A), but we did not find variation in mite abundance among sample localities for *P. cylindraceus* ($F = 1.89$; $df = 1$; $P = 0.182$; Fig. 4B). The frequency of mite infection of *D.*

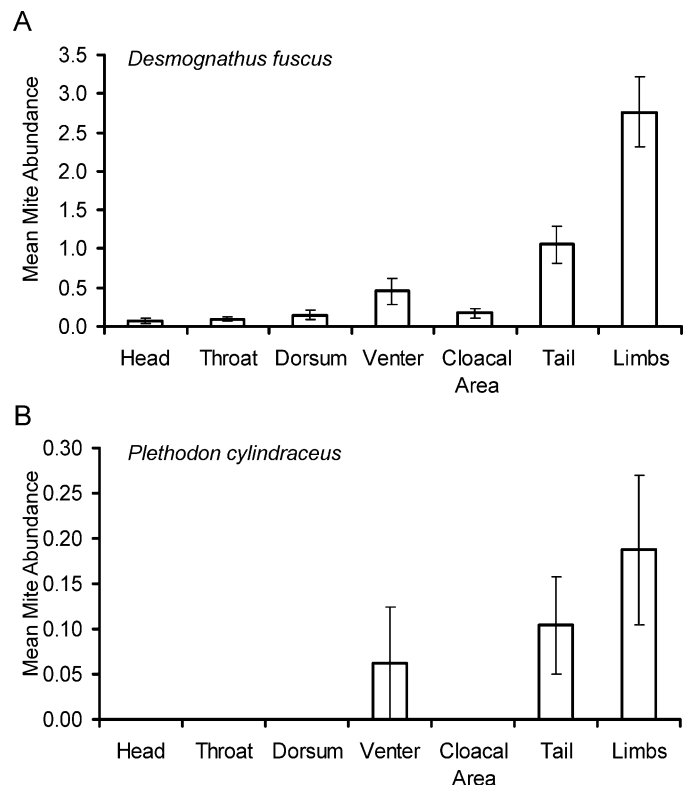


FIGURE 3. Abundance of *Hannemania dunni* on specific body parts of *Desmognathus fuscus* (A; $n = 128$) and *Plethodon cylindraceus* (B; $n = 48$). Although many body parts harbored a number of mites, the limbs and the tail harbored the greatest concentration. Error bars represent ± 1 SE. Samples were only collected in fall 2006.

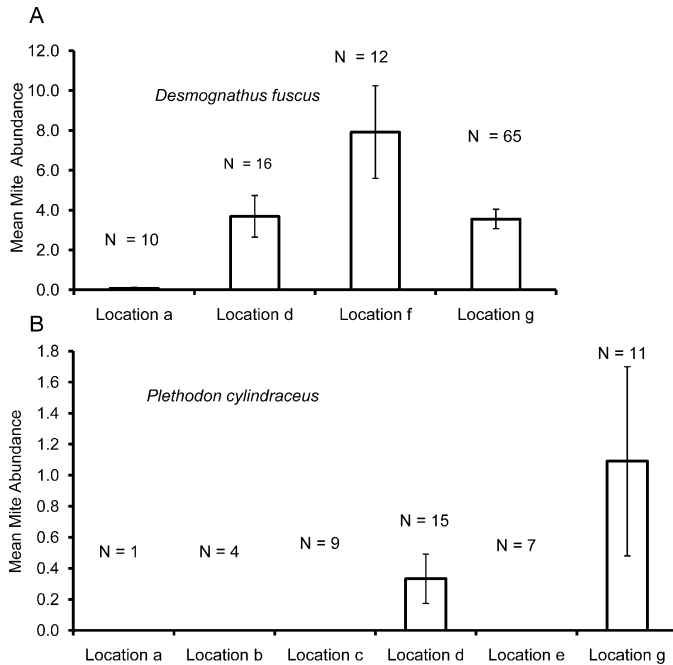


FIGURE 4. Abundance of *Hannemania dunnii* on *Desmognathus fuscus* (A; $n = 103$) and *Plethodon cylindraceus* (B; $n = 47$) at specific sample locations. *Desmognathus fuscus* at location f had a significantly greater density of mites than the other locations. *Plethodon cylindraceus* captured at location g had a greater mean abundance than *P. cylindraceus* at other locations. Error bars represent ± 1 SE.

fuscus did not change from October 2005 through May 2006 (GLM; $F = 0.300$; $df = 7$; $P = 0.952$; Fig. 5).

DISCUSSION

Larval *H. dunnii* seem to be a common ectoparasite on plethodontid salamanders of the western Piedmont of North Carolina, but we found significant variation in *H. dunnii* abundance among the salamander species we studied. Duncan and Highton (1979) also found that fully terrestrial, plethodontid salamanders (i.e., *P. glutinosus*, *P. fourchenis*, and *P. caddoensis*) studied in the Ouachita Mountains of Arkansas and Oklahoma had different prevalences of *H. dunnii* infection; larval mites were rarely found on *P. glutinosus*, yet heavy infestations occurred on other species. In our investigation, *D. fuscus*, the most aquatic of the three species studied, harbored the greatest concentration of mites. These results differ from previous studies describing higher parasitism in more terrestrial amphibian species (Regester, 2001; Converse and Green, 2005).

We found significant variation in mite attachment locations among salamander species. The high abundance of *H. dunnii* on the limbs and tail of *D. fuscus* and *P. cylindraceus* may indicate that mites have a preference for attaching to the extremities, even though there is more surface area on the dorsal and ventral regions of the animal (Anthony et al., 1994; McAllister et al., 1995; Malone and Paredes-Leon, 2005). Green (2001) also noted that embedded mites are most commonly found on the extremities, which may be explained by *Hannemania* sp. selecting their penetration site (Hyland, 1961; Malone and Paredes-Leon, 2005) because the connective tissue of the extremities may allow for a firmer attachment (Malone and Paredes-Leon, 2005).

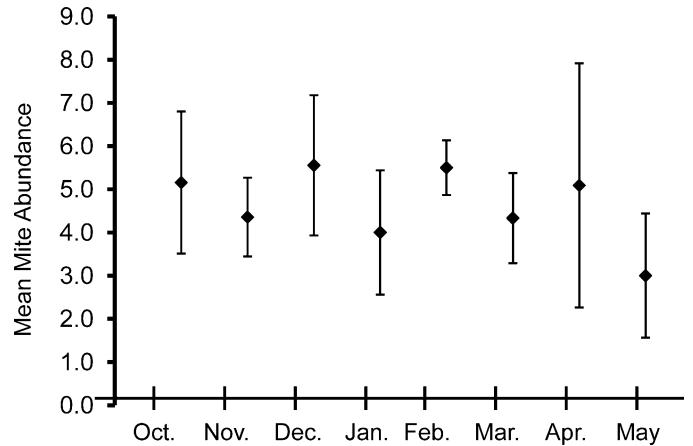


FIGURE 5. Mite abundance for *Desmognathus fuscus* from fall 2005 to spring 2006. Mite abundance did not vary by month ($R^2 = 0.206$; $P = 0.952$). Error bars represent ± 1 SE.

Brown et al. (2006) suggested that limb loss may result from high mite abundance. We did not document a direct link between limb loss and mite attachment in our study, but we witnessed clusters of mites on the extremities that seemed to impair toe and foot motility. Mite attachment to these areas could impair activities such as foraging and courtship (Anthony et al., 1994; Maksimowich and Mathis, 2000; Regester, 2001; Malone and Paredes-Leon, 2005), which could have detrimental impacts on salamander populations.

We also found differences in salamander parasitism among sampling locations. Duncan and Highton (1979) also noted variation in infestation rates by *H. dunnii* among 20 localities in the Ouachita Mountains. Differences in parasitism rates among localities may be the result of different edaphic or climatic microhabitat characteristics, although we noticed no obvious differences among our study sites. Future studies should determine whether microhabitat conditions affect mite distribution or salamander susceptibility to mite infestation.

The present study describes patterns of *H. dunnii* abundance on plethodontid salamanders of the western Piedmont of North Carolina, and provides a greater understanding of the natural history of both larval *H. dunnii* and plethodontid salamanders. We have also detected larval *Hannemania* sp. on anurans, including *Rana sphenoccephala* and *R. palustris*; future studies should investigate patterns of trombiculid mite parasitism on other amphibian species. Furthermore, studies examining the effects of *Hannemania* sp. parasitism on amphibian survivorship or determining climatic and edaphic influences on parasitism rates would be helpful to fully describe the impacts of trombiculid mite parasitism of amphibians.

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