Journal of the Arkansas Academy of Science

Volume 40 Article 27

1986

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COMPARISON OF THE SYMBIOTIC FAUNA OF THE FAMILY PLETHODONTIDAE IN THE OUACHITA MOUNTAINS OF WESTERN ARKANSAS

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ABSTRACT

During the spring of 1985, 101 salamanders representing six host species (29 *Plethodon ouachitae*, 25 *P. caddoensis*, 6 *P. fourchensis*, 23 *P. serratus*, 13 *Desmognathus brimleyorum*, and 5 *P. glutinosus glutinosus*) were collected from six localities in three counties in Arkansas (Polk, Scott, and Montgomery) and examined for symbionts. With the exception of *Hannemania dunni*, all symbionts recovered from the first five species listed constitute new host records, and the endoparasitic fauna in all species establish new locality records. Examinations revealed one or more species of parasites in 82% of the hosts. Eight species of symbionts (3 nematode, 1 trematode, 1 cestode, 1 protozoan, 1 arthropod, and 1 cystacanth acanthocephalon) were recovered. Conclusions are based on the three host species examined in the largest numbers. *Thelandros magnavulvularis* and *H. dunni* were the most commonly occurring parasites, found in five and four host species respectively. *Cepedietta michiganensis* was restricted to *P. ouachitae* and *Brachycoelium storeriae* to *P. caddoensis*. *Hannemania dunni* was absent in *P. serratus*.

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INTRODUCTION

In general, surveys of the parasitic fauna of Plethodontid salamanders have been isolated studies with small samplings of hosts. The most comprehensive survey was conducted in North Carolina by Rankin (1937). He examined 297 individuals of the genus *Desmognathus* and 32 specimens of the genus *Eurycea*. Rankin's study represents one of the most extensive surveys of the genus *Plethodon;* examinations included 74 *P. cinereus*, 119 *P. glutinosus*, 18 *P. metcalfi*, and 3 *P. yonahlossee*. Working in the Smoky Mountains, Powders (1967) collected 392 specimens of *P. glutinosus* and 988 of *P. jordani*. His examinations concentrated solely on two parasites, *Brachycoelium* and *Cepedietta michiganensis*. All of the diagnosed symbionts we recovered from *P. glutinosus glutinosus* have been reported in that host species by these authors. General surveys and ecological studies of Arkansas salamander parasites are limited. Saltarelli (1977) examined 100 individuals of the genus *Eurycea* in northwestern Arkansas. Rosen and Manis (1976) examined 259 amphibian hosts, but only 14 were plethodontid salamanders.

This study is intended to provide a description and comparison of the parasitic fauna of three species of woodland salamanders (Family Plethodontidae): *Plethodon ouachitae, P. serratus,* and *P. caddoensis*. Two other terrestrial species, *P. glutinosus glutinosus* and *P. fourchensis,* and one aquatic species, *Desmognathus brimleyorum,* were examined in insufficient numbers to generate reliable comparison data. However, the data for the three latter species constitute new documentation and suggest certain relationships between the hosts and their symbionts. Table 1 lists the hosts examined as well as their sex and age distribution.

Specimens were collected at six sites in three counties during April and May, 1985. These collection sites were located on the Rich, Caddo, and Fourche mountain ranges of the western Ouachitas. According to Conant, the ranges of five of the six salamander species examined are restricted to specific regions of the Ouachita Mountains. The range of *P. glutinosus* extends throughout most of the eastern half of the United States. *Plethodon ouachitae, P. caddoensis,* and *P. fourchensis* represent allopatric species, while the three remaining species are sympatric with each other and the allopatric species mentioned above. Specimens of *P. serratus* and *D. brimleyorum* were collected from the same localities as the three allopatric species.

Table 1. Number of hosts examined.

HOST	TOTAL	MALES	FEMALES	JUVENILES
P. ouachitae (P.O.)	29	13	16	0
P. caddoensis (P.C.)	25	8	17	0
P. serratus (P.S.)	23	9	12	2
P. glutinosus glutinosus (P.G.G.)	5	1	4	0
P. fourchensis (P.F.)	6	3	1	2
D. brimleyorum (D.B.)	13	5	6	2
NUMBER OF HOSTS EXAMINED	101	39	56	6

MATERIALS AND METHODS

All specimens were collected under flat rocks and logs except *D. brimleyorum,* which was found in mountain spring run-offs. Specimens were immediately transported to the laboratory in styrofoam chests containing moist leaves and snails. The salamanders were then stored in a refrigerator at 7°C to slow body metabolism and prevent shedding of intestinal parasites. Examinations were completed within 72 hours. Specimens were dispatched in a hot water bath and all internal organs (excluding gonads) were removed through a mid-ventral incision. The intestine was divided linearly and examined in three equal sections. The distribution of chiggers in the integument was mapped on host cards. All examinations were done using a binocular dissecting microscope.

Nematodes were fixed with hot Looss' solution prior to evaporation and temporary mounting in glycerine. Cestodes were straightened by placing them in tap (hypotonic) water in a refrigerator for 24 hours prior to fixing with AFA. They were subsequently stained with Semichon's acetocarmine. Trematodes were flattened under a cover slip and fixed by drawing AFA underneath using porous paper. They were then stained with alum cochineal. The immature acanthocephalon and the protozoa were fixed in AFA, stained with alum cochineal, and mounted whole. Some protozoa found in the gall bladder were fixed *in situ* and later sectioned, mounted, and stained using hematoxylin and eosin. Chiggers were excised and fixed in 70% ethanol and subsequently mounted in CMCP-9AB. With the exception of nematodes and chiggers, all parasites were mounted in Permount. Voucher specimens were prepared for the U.S. National Museum.

RESULTS AND DISCUSSION

One or more of eight species of symbionts were found to infect 83 (82%) of 101 salamanders of the family Plethodontidae. *Plethodon ouachitae* (100%), *D. brimleyorum* (100%), *P. caddoensis* (92%), and *P. fourchensis* (83%) were the most heavily parasitized hosts, while *P. serratus* (48%) and *P. glutinosus glutinosus* (40%) exhibited the lowest prevalence. The most striking difference in intensity was recorded for *H. dunni* in *P. ouachitae* and *P. caddoensis* (Table 2).

The diversity of parasites was greatest in *P. ouachitae* and *D. brimleyorum*, as 6 of the 8 symbiont species were encountered in these hosts. Despite the small sample size, *P. fourchensis* also exhibited high diversity (5 of 8 symbiont species). *Plethodon glutinosus glutinosus, P. serratus,* and *P. caddoensis* harbored 2, 4, and 5 symbiont species respectively (Table 2). Our data suggest that each allopatric salamander species has as many or more symbiont species than the common sympatric species (Table 3).

With one exception, all endoparasites were restricted to either the intestine or the gall bladder. The acanthocephalon was found in the coelom.

Table 2. Prevalance and (mean intensity) of each symbiont.

SPECIES	P.O.	P.C.	P.S.	P.G.G.	P.F.	D.B.
PROTOZOA						
C. michiganensis	48(113)	--------	--------	20(465)	33(286)	--------
TREMATODA						
B. storeriae	--------	20(4.2)	--------	--------	17(7.0)	--------
CESTODA						
Nematotaeniidae	21(1.8)	8(1.5)	26(4.3)	--------	17(8.0)	15(4.0)
NEMATODA						
T. magnavulvularis	14(1.0)	12(1.0)	22(1.0)	--------	33(1.0)	77(4.6)
O. euryceae	38(3.6)	68(3.6)	9(2.0)	--------	--------	15(2.0)
Oxysomatium sp.	3(1.0)	--------	4(1.0)	--------	--------	8(1.0)
Unidentified immature Oxyuroids	14(1.8)	--------	--------	20(4.0)	--------	--------
ACARINA						
H. dunni	100(20)	80(6.2)	--------	--------	17(6.0)	77(21)
ACANTHOCEPHALA	--------	--------	--------	--------	--------	8(1.0)
TOTAL SPECIES	6	5	4	2	5	6

TREMATODA

Brachycoelium storeriae Harwood, 1932

The only trematode species recovered in this study was *Brachycoelium storeriae,* which was collected from the anterior third of the small intestine of *P. caddoensis* and *P. fourchensis.* It infected 5 (20%) of 25 *P. caddoensis* and 1 (17%) of 6 *P. fourchensis* examined. The average number of flukes per infected host was 4.2 and 7.0 respectively; the range varied from 1 to 15 flukes per host.

There has been much controversy surrounding the assignment of species in the genus *Brachycoelium.* Rankin (1938) reduced all North American species of this genus to the single species *B. salamandrae.* In contrast, Parker (1941) and Cheng (1958) recognized 7 and 10 distinct species of *Brachycoelium* respectively. More recently, Cheng and Chase (1961) and Couch (1966) proposed that 13 species represent the genus. Despite slight differences in size, we have concluded that all our specimens most closely resemble *B. storeriae* as described by Harwood (1932).

NEMATODA

Thelandros magnavulvularis (Rankin, 1937) Schad, 1960

This nematode was recovered from all host species, excluding *P. glutinosus glutinosus,* and was restricted to the posterior third of the intestine. *Desmognathus brimleyorum* was most heavily parasitized, with a prevalance of 77%; the four other infected species exhibited markedly lower prevalences and intensities of infection (Table 2). Although this nematode ranged from 1 to 19 parasites per infected host, only 4 (17%) of 24 infected hosts had more than two specimens. Only two male specimens of *T. magnavulvularis* were recovered, both from *D. brimleyorum.* This is similar to the experience of other investigators. However, most females were observed shedding fertile eggs during recovery, which may indicate that males are short-lived.

Table 3. Comparison of the prevalence of symbionts of the three allopatric species with that of their common sympatric species.

SPECIES	Plethodon serratus		
	Rich Mtn.(12)*	Caddo Mtns.(7)	Fourche Mtns.(4)
CESTODA			
Nematotaeniidae	25	--	75
NEMATODA			
T. magnavulvularis	33	29	--
O. euryceae	17	--	--
Oxysomatium sp.	--	14	--

SPECIES	P. ouachitae(29)	P. caddoensis(25)	P. fourchensis(6)
PROTOZOA			
C. michiganensis	48	--	33
TREMATODA			
B. storeriae	--	16	17
CESTODA			
Nematotaeniidae	21	8	17
NEMATODA			
T. magnavulvularis	14	12	33
O. euryceae	38	68	--
Oxysomatium sp.	3	--	--
ACARINA			
H. dunni	100	80	17

* NUMBER OF HOST EXAMINED

Ozwaldocruzia euryceae Reiber, Byrd, and Parker, 1940

This trichostrongyloid nematode is found in the anterior third of the intestine. It has been reported in only three species of the genus *Eurycea* (Saltarelli, 1977); therefore, new host records are established for *P. ouachitae, P. caddoensis, P. serratus,* and *D. brimleyorum.* It was most prevalent in *P. caddoensis,* infecting 17 (68%) of 25 hosts. Infections varied from 1 to 12 specimens per host with an average of 3.6.

Oxysomatium sp.

A total of three specimens (1 male and 2 females) was recovered, two from *P. ouachitae* and *D. brimleyorum* at Rich Mountain, and one from *P. serratus* at Caddo Mountain. Fischthal (1955) reported *O. americana* in *F. fuscus,* and Landewe (1963) found *Oxysomatium sp.* in *P. cinereus.* The species designation of our specimens is uncertain at this point. Unpublished work (Johnson) has shown *Ambystoma maculatum* in this region to be more commonly infected with *Oxysomatium.*

CESTODA: Nematotaeniidae

Representatives of the family Nematotaeniidae were recovered from the anterior two-thirds of the intestine of all host species excluding *P. glutinosus. Plethodon serratus* exhibted the highest prevalence of infection (26%), with an average of 4.3 specimens per host. The other host species infected by nematotaeniids exhibited prevalences ranging from 8-21% and intensities ranging from 1.5 to 8. The average number of tapeworms per infected host was 4.4.

Immature forms of Nematotaeniidae were collected from all host species except *P. glutinosus glutinosus* and *D. brimleyorum.* This was the only cestode form represented in *P. caddoensis,* although the infections exhibited low prevalence (8%) and low intensity (1.5). *Plethodon ouachitae, P. serratus,* and *P. fourchensis* were the host species in which both mature and immature nematotaeniids were found.

Cylindrotaenia americana is the most frequently reported nematotaeniid in *Desmognathus* and *Plethodon.* This cestode has been

recovered by Dunbar and Moore (1979) from *D. monticola, D. ochrophaeus, P. glutinosus, P. richmondi,* and *P. cinereus.* Rankin (1937) reported a proteocephalid cestode, *Crepidobothrium cryptobranchi,* from these two genera.

Our specimens have almost all of the morphological characteristics of the family Nematotaeniidae, including the four unarmed suckers, cylindrical body, and distinct posterior segmentation. However, the four recorded genera representing this family also exhibit two or more parauterine organs. Our specimens quite vividly show a progression of eggs from a degenerating uterus into a single parauterine organ in each succeeding posterior segment. Because these specimens do not fit into other families characterized by a single parauterine organ, we have tentatively placed them in the family Nematotaeniidae pending further identification.

PROTOZOA

Cepedietta michiganensis Woodhead, 1928

Cepedietta (=Haptophrya) michiganensis, a large ciliate (1.1 - 1.6 mm), was recovered from the gall bladder and intestine of *P. ouachitae.* In this host, intestinal infections never occurred in the absence of parasites in the gall bladder. However, one individual from *P. glutinosus glutinosus* and *P. fourchensis* exhibited extremely large infections (>450) of the intestine but not the gall bladder. The ciliates from the two different organs could not be distinguished morphologically. In gall bladder sections, the protozoans appeared to be randomly distributed and were not attached to the epithelium. Although these organisms could be found throughout the intestine, they were concentrated in the anterior third.

Cepedietta michiganensis undergoes asexual reproduction by binary fission. Powders (1967) reported chains of up to seven individuals. Woodhead (1928) and Puytorac (1963) state that chains of six are the maximum and occur rarely. We observed a maximum of only two individuals in tandem.

ACARINA

Hannemania dunni Sambon, 1928

This ectoparasite was first reported in *P. ouachitae* by Dunn and Heinze (1933). Pope and Pope (1951) confirmed this observation and reported a prevalance of 83% and an average of 10-15 per infected host. The latter authors also note the absence of this parasite from *P. glutinosus glutinosus* taken from the same locations on Rich Mountain. Large infestations of *H. dunni* were recorded for *P. ouachitae and P. fourchensis* by Duncan and Highton (1979). They also reported variable infestations in *P. caddoensis* and very rare occurrences on the sympatric *P. serratus.* Our observations as to host distribution conform well to those recorded by the above authors.

Hannemania dunni was recovered from all species except *P. serratus* and *P. glutinosus glutinosus.* Although only 17% of *P. fourchensis* were infected, chiggers were recovered from at least 77% of the hosts of the other infected species. The most prevalent infection occurred in *P. ouachitae,* in which 100% of the hosts were infected with an average of 20 chiggers.

The average number and distribution of *H. dunni* appeared to be species dependent. In all infected species, this parasite was usually dorsal in location. In *P. ouachitae,* an average of 20 chiggers was distributed primarily on the appendages and on the mid-body region to a lesser extent (Fig. 1). *Desmognathus brimleyorum* also displayed a high intensity of infection of *H. dunni.* An average of 21 chiggers was distributed almost entirely on the feet of this host. In a few individuals, severe infestation of the appendages resulted in tissue destruction and loss of toes. This host also seemed to have more chiggers located ventrally in comparison with other host species (Fig. 2). In *P. caddoensis,* an average of 6 chiggers was randomly distributed over the entire body, but absent in the feet (Fig. 3).

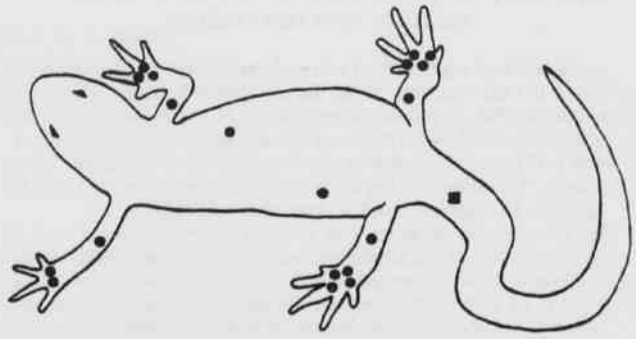

Figure 1. Average number and distribution of *Hannemania dunni* in *Plethodon ouachitae.*

Legend:
• represents one chigger on dorsal surface
■ represents one chigger on ventral surface

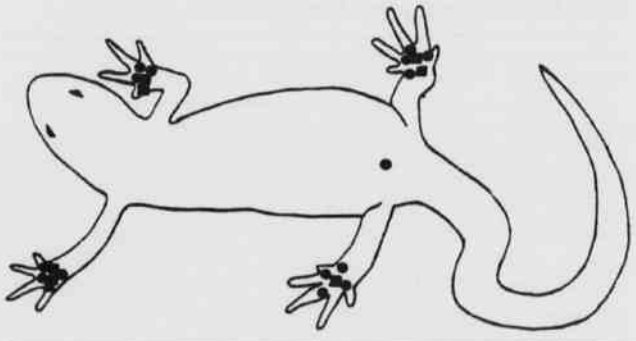

Figure 2. Average number and distribution of *Hannemania dunni* in *Desmognathus brimleyorum.*

Legend:
• represents one chigger on dorsal surface
■ represents one chigger on ventral surface.

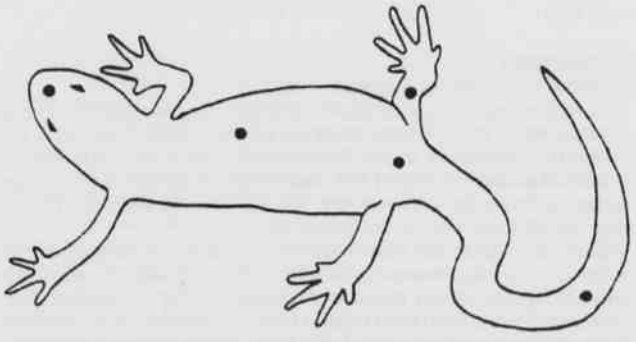

Figure 3. Average number and distribution of *Hannemania dunni* in *Plethodon caddoensis.*

Legend:
• represents one chigger on dorsal surface

CONCLUSIONS

No information concerning the endosymbionts of five of the six host species we examined has been published previously. Thus, our study provides new documentation of eight species of symbionts in six species of plethodontid salamanders from the Ouachita Mountains of western Arkansas. Furthermore, all endosymbiont recoveries represent new locality and host records.

With the exception of *T. magnavulvularis,* which was found in the posterior third of the intestine, and the acanthocephalon in the coelom, all endoparasites were collected from the anterior two-thirds of the intestine. Data regarding the single ectoparasite species recovered con-

firm the work done by others and provide further quantification of the distribution of the chiggers on the infected hosts. Two sympatric species lacked these symbionts.

The three allopatric species exhibited both higher prevalences and intensities of infection in comparison with their common sympatric species, *P. serratus*. These data indicate that, evolutionarily, the allopatric species probably did not acquire their symbionts from this sympatric host.

Comparison of the three host species examined in the largest numbers, two allopatric and one sympatric, shows exclusive infections of certain symbionts. *Brachycoelium storeriae* and *C. michiganensis* were restricted to *P. caddoensis* and *P. ouachitae* respectively, and *H. dunni* was limited to the two allopatric species. The common symbionts of these three major hosts were the nematodes *T. magnavulvularis* and *O. euryceae* and the nematotaeniid cestode.

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