Parasites of *Desmognathus brimleyorum* (Caudata: Plethodontidae) from the Ouachita Mountains of Arkansas and Oklahoma

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ABSTRACT: Forty-one juvenile and adult Ouachita dusky salamanders, Desmognathus brimleyorum, were collected from Arkansas and Oklahoma and examined for parasites. Thirty-two (78%) were infected with 1 or more parasites, including 25 (61%) with Chloromyxum salamandrae, 1 (2%) with Brachycoelium salamandrae, 2 (5%) with Cylindrotaenia americana, 8 (20%) with Mesocestoides sp. tetrathyridia, 9 (22%) with Batracholandros magnavulvaris, 3 (7%) with Desmognathinema nantahalaensis, 4 (10%) with Hedruris pendula, 6 (15%) with Omeia papillocauda, 1 (2%) with unidentified Ascaridoidea larvae, 1 (2%) with an acanthocephalan cystacanth, and 28 (68%) with larval Hannemania sp. In addition, 10 (24%) salamanders harbored an intraerythrocytic inclusion, thought to represent a rickettsia or virus of undetermined taxonomic status. Several new host and distributional records are documented for parasites of D. brimleyorum, including the first report of Mesocestoides sp. in a caudate amphibian worldwide.

KEY WORDS: Cylindrotaenia americana, Mesocestoides sp., Desmognathus brimleyorum, Hannemania sp., Chloromyxum salamandrae, Batracholandros magnavulvaris, Omeia papillocauda, Brachycoelium salamandrae, acanthocephalan cystacanth, Desmognathinema nantahalaensis, Hedruris pendula.

The Ouachita dusky salamander, *Desmognathus brimleyorum* Stejneger, 1894, is a large, robust amphibian that is restricted in range to the Ouachita uplift of central Arkansas and southeastern Oklahoma (Conant and Collins, 1991). This semi-aquatic salamander is found in seepages around rocky and gravelly streams where it hides under rubble and leaf litter. To our knowledge, there is only 1 previous published report on helminths of *D. brimleyorum* (Winter et al., 1986). We report new host and distributional records on several parasites from *D. brimleyorum* from Arkansas and Oklahoma, including the first record of *Mesocestoides* sp. from a caudate amphibian.

Materials and Methods

During March and May 1994, 41 (29 male, 12 female) juvenile and adult ($\bar{x} \pm SE$ snout-vent length [SVL] = 58.0 \pm 2.8, range 19–93 mm) *D. brimleyorum* were collected by handraking streamlets in Polk County, Arkansas (N = 37), and LeFlore County, Oklahoma (N = 4), and examined for parasites. Of the 41 *D.*

brimleyorum, 22 were considered juveniles with SVL's of ≤62 mm (Trauth et al., 1990). Salamanders were placed in bags containing stream water and transported on ice to the laboratory within 48 hr. Specimens were sacrificed by prolonged immersion in a dilute chlorotone solution. Methods for salamander necropsy and preparation and staining of blood smears, helminths, myxozoans, and coccidial isolation follow McAllister and Upton (1987) and Upton et al. (1995). Mites were gently teased from capsules, fixed in 70% ethanol, dehydrated, heated to 60°C for 5-10 min in lactophenol, and mounted in Hoyer's medium. Voucher specimens of hosts are deposited in the Arkansas State University Museum of Zoology (ASUMZ 19494-19520, 19522-19524, 19769-19780). Specimens of parasites are deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705, as follows: Chloromyxum salamandrae (USNM 83947), intraerythrocytic inclusion (USNM 83944), Brachycoelium salamandrae (83945), Cylindrotaenia americana (USNM 84048), Mesocestoides sp. tetrathyridia (USNM 83943), Batracholandros magnavulvaris (83948), Desmognathinema nantahalaensis (USNM 83950, 84047), Hedruris pendula (USNM 83951, 84046), Omeia papillocauda (USNM 83949), Ascaridoidea larvae (USNM 83952), acanthocephalan cystacanth (USNM 83946), and Hannemania sp. (USNM 83953).

Table 1. Parasites of Desmognathus brimleyorum from Arkansas and Oklahoma.

Parasite	Prevalence*	Mean intensity ± 1 SE (range)	Locality
Intraerythrocytic inclusion‡§	10/41 (24)	72	1, 2
Protozoa			
Chloromyxum salamandrae§	25/41 (61)	=	1, 2
Trematoda Brachycoelium salamandrae§	1/41 (2)	$2.0 \pm - (-)$	1
Cestoidea			
Cylindrotaenia americana	2/41 (5)	$1.0 \pm 1.0 (1)$	1
Mesocestoides sp.§	8/41 (20)	Car - Agriconines	1
Nematoda			
Ascaridoidea (larvae)§	1/41 (2)	$1.0 \pm - (-)$	1
Batracholandros magnavulvaris	12/41 (27)	$2.6 \pm 0.7 (1-8)$	1
Desmognathinema nantahalaensis§	3/41 (7)	$3.0 \pm 2.0 (1-7)$	1
Hedruris pendula§	4/41 (10)	$6.3 \pm 2.3 (2-12)$	1
Omeia papillocauda\	6/41 (15)	$2.6 \pm 0.7 (1-6)$	1
Acanthocephala			
Unidentified cystacanth	1/41 (2)	$1.0 \pm - (-)$	1
Acari			
Hannemania sp.	28/41 (68)	_	1, 2

^{*} Number infected/number examined (percent).

Results and Discussion

Thirty-two of 41 (78%) *D. brimleyorum* were infected with 1 or more parasites (Table 1). No coccidia were found in the feces or intestinal contents of salamanders. All helminth parasites were found exclusively in *D. brimleyorum* from the Rich Mountain (Arkansas) site, whereas the modest sample from Kiamichi Mountain (Oklahoma) harbored only the intraerythrocytic inclusion, *C. salamandrae*, and *Hannemania* sp. (Table 1).

Intraerythrocytic inclusions of an unknown classification (Fig. 1) were found in nearly one-fourth of all salamanders examined (10 adults [4 male, 6 female]; 75.3 ± 2.5 , 67-92 mm). No infections were observed in juvenile salamanders or any salamanders collected in May. These organisms resembled a frog intraerythrocytic virus or rickettsia reported from *Rana catesbeiana*, *R. clamitans*, and *R. septentrionalis* in Canada (Barta and Desser, 1984; Barta et al., 1989; Gruia-Gray and Desser, 1992). McAllister et al. (1993) reported similar intraerythrocytic inclusions

thought to be Aegyptianella (syn. Cytamoeba) bactifera (Labbé, 1894) Barta, Boulard, and Desser, 1989, in Plethodon albagula from Arkansas. In addition, Rankin (1937a) reported A. bactifera in Desmognathus fuscus fuscus, D. ochrophaeus, D. imitator, D. monticola, and D. quadramaculatus from North Carolina. Ultrastructural examination will be necessary to determine the identity of this enigmatic organism in D. brimleyorum.

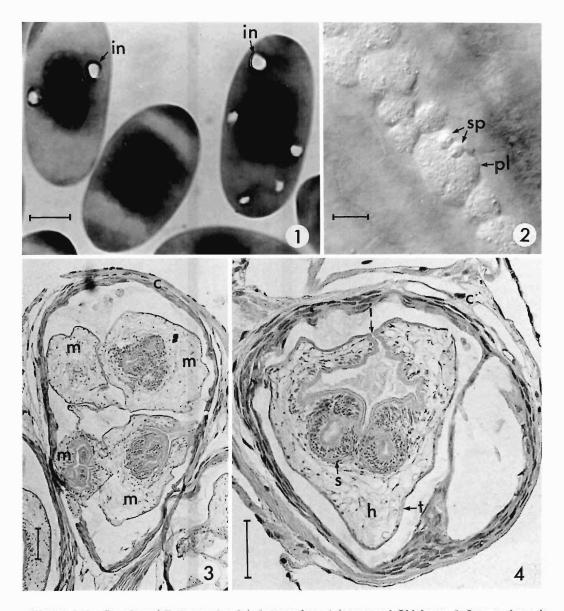
Adhering to the gall bladder epithelium in 14 (64%) of the juvenile (10 male, 4 female; 52.1 ± 1.7 , 40–62 mm) and 11 (58%) of the adult (7 male, 4 female; 72.9 ± 2.7 , 65–83 mm) *D. brimleyorum* were myxozoan plasmodia of *Chloromyxum salamandrae* Upton, McAllister, and Trauth, 1995 (Fig. 2). Two distinct types of plasmodia were observed in *D. brimleyorum*, including a dendritic sheet-like form of *C. salamandrae* in 13 (52%), a compact form in 7 (28%), and a mixture of both forms in 5 (20%) salamanders. The Ouachita dusky salamander is a new host of *C. salamandrae*. Other hosts of this

[†] Localities: 1 = 1.0 km S of Rich Mountain, off State Hwy 272, Polk County, Arkansas; 2 = N face of Kiamichi Mountain, off State Hwy 259, LeFlore County, Oklahoma.

[‡] An intracellular parasite of unknown classification thought to be rickettsial in nature and may represent Aegyptianella bactifera (Labbé, 1894) Barta, Boulard, and Desser, 1989.

[§] New host record.

^{||} New distributional record.



Figures 1-4. Parasites of Desmognathus brimleyorum from Arkansas and Oklahoma. 1. Intraerythrocytic inclusion (in); scale bar = $50~\mu m$. 2. Chloromyxum salamandrae plasmodia (pl) and spores (sp) adhering to gall bladder epithelium; scale bar = $20~\mu m$. 3. Mesocestoides sp. tetrathyridia encapsulated in mesenteries showing 4 metacestodes (m) in single host-derived fibrous capsule (c); scale bar = $200~\mu m$. 4. Single tetrathyridium of Mesocestoides sp. showing structure of parasite within capsule (c). Note the solid cellular hindbody (h), deep invagination canal (i), well-developed tetracetabulate scolex (s), distinct tegument (t), and absence of an apical organ; scale bar = $200~\mu m$.

myxozoan include Eurycea multiplicata griseo-gaster and E. multiplicata multiplicata from Arkansas and E. neotenes from Texas (Upton et al., 1995).

Two specimens of the plagiorchid trematode, Brachycoelium salamandrae (Frölich, 1789) Du-

jardin, 1845, were found in the small intestine of a single adult male *D. brimleyorum* (92 mm SVL). *Brachycoelium elongatum* Cheng, 1958, was reported previously in *Desmognathus fuscus conanti* from Arkansas by Rosen and Manis (1976). Therefore, *D. brimleyorum* represents a

new host record for *B. salamandrae*. Other dusky salamanders have been reported to harbor *Brachycoelium* spp., including *D. ochrophaeus*, *D. monticola*, and *D. quadramaculatus* from North Carolina (Rankin, 1937a; Goater et al., 1987) and Tennessee (Dunbar and Moore, 1979) and *D. fuscus* from Georgia (Byrd, 1937; Parker, 1941), Illinois (Dyer et al., 1980), New York (Fischthal, 1955a), North Carolina (Rankin, 1937a), Pennsylvania (Fischthal, 1955b), and Tennessee (Dunbar and Moore, 1979). McAllister et al. (1995a, b, c) previously reported *B. salamandrae* from Arkansas in *E. multiplicata griseogaster*, *Rana palustris*, and *Rana sylvatica* (respectively).

Rankin's (1938) review of the genus *Brachy-coelium* reduced all known species to synonymy with *B. salamandrae*, a view not universally accepted (see Dyer and Brandon, 1973). McAllister et al. (1995a) suggested adopting a conservative approach until an exhaustive revision has been completed of this morphologically variable genus.

Two immature cyclophyllidean cestodes, most closely matching the description of Cylindrotaenia americana (Jewell, 1916) were found in the small intestine of 2 juvenile D. brimleyorum (male and female, 45 and 40 mm SVL) collected in Polk County. In an unpublished thesis, Bouchard (1953) reported 1/11 (9%) D. brimleyorum from Oklahoma to harbor C. americana. Other species of Desmognathus have been reported previously as hosts of C. americana, including D. fuscus fuscus from New York and North Carolina, D. ochrophaeus and D. monticola from North Carolina and Tennessee, and D. quadramaculatus from North Carolina (see McAllister, 1991). Cylindrotaenia americana has been reported previously from Arkansas in P. albagula (McAllister et al., 1993). However, Jones (1987) considers C. americana to be an anuran parasite, whereas Cylindrotaenia idahoensis (Waitz and Mehra, 1961) Jones, 1987, has been reported only in plethodontid salamanders. Jones (1987) further suggests a reexamination of material from salamander hosts to determine whether or not the material is indeed C. americana.

Winter et al. (1986) reported immature nematotaeniid cestodes in *D. brimleyorum* from Arkansas. However, because these cestodes were considered to contain a single parauterine organ, the authors tentatively placed them in the family Nematotaeniidae, without generic designation. Currently, no known genera within the Nematotaeniidae have fewer than 2 uterine capsules

per segment (Jones, 1987); therefore, Winter et al. (1986) may have observed a single parauterine complex per segment, as by definition in species of *Cylindrotaenia*, consists of 2 parauterine organs joined basally and sharing a common uterine mass (Jones, 1987).

Numerous tetrathyridia of Mesocestoides sp. were found in 5 (23%) juvenile (4 male, 1 female; 58.2 ± 2.0 , 53-62 mm) and 3 (16%) adult (3 male; 68.7 ± 0.9 , 67-70 mm) salamanders. These parasites were encapsulated in the mesenteries of their hosts, either in groups (Fig. 3) or as solitary worms (Fig. 4). All the tetrathyridia appeared healthy, as did the surrounding tissue outside the host-derived capsule. Each tetrathyridium possessed a single tetracetabulate scolex. which lacked hooks, and a rostellum, or apical organ, which was invaginated into a solid hindbody (i.e., lacking a primary lacuna). The tetrathyridia showed no evidence of asexual proliferation, thus conforming to the usual pattern for the genus (Conn, 1990). Furthermore, the presence of groups of tetrathyridia occurring in single host capsules (Fig. 3), but lacking morphological evidence of proliferation, supports the interpretation of Conn (1990), McAllister and Conn (1990), and McAllister et al. (1992) that such groups result from multiple encapsulation rather than asexual activity within a capsule.

This is the first definitive report of Mesocestoides from any salamander species; however, proteocephalan metacestodes have been reported from salamanders, including several species of Desmognathus. Rankin (1937a) reported "proteocephalid cysts" from D. fuscus in North Carolina; Dunbar and Moore (1979) reported "plerocercoids . . . probably of the order Proteocephalidea" from D. monticola in Tennessee; Goater et al. (1987) reported "proteocephalan plerocercoids" from D. quadramaculatus, D. monticola, and D. ochrophaeus in North Carolina. It is possible that some or all of these were actually Mesocestoides; because proteocephalideans have a tetracetabulate acetabulum and solid hindbody, some potential for misdiagnosis exists. The distinguishing characteristic is the presence of an apical organ only in proteocephalideans. However, Rankin (1937a) and Dunbar and Moore (1979) identified their specimens only on the basis of tetracetabulate scoleces. Tetrathyridia have been reported from 10 anuran species in North America, including 4 bufonids, 5 ranids, and 1 hylid (McAllister and Conn, 1990; McAllister et al., 1995b, c). Tetrathyridia have also been reported from lizards in Arkansas (Mc-Allister et al., 1991, 1992).

Two larval and 7 adult (3 male, 4 female) seuratoid nematodes, $Desmognathinema\ nantahalaensis\ Baker$, Goater, and Esch, 1987, were found in the small intestine of 3 juvenile male $D.\ brimleyorum\ (56.0\pm2.6,51-60\ mm)$. This nematode was originally described from desmognathine salamanders in North Carolina (Baker et al., 1987) and reported recently from $E.\ multiplicata\ griseogaster$ and $Eurycea\ lucifuga$ in Arkansas (McAllister et al., 1995a). $Desmognathus\ brimleyorum$ is a new host and fifth species of plethodontid salamander known to harbor this worm. The Polk County site is approximately 160 km SE of the nearest previously recorded locale for $D.\ nantahalaensis$ in Arkansas.

An unknown species of Ascaridoidea larvae was found encapsulated in the dorsal body wall musculature of a juvenile male (56 mm SVL) D. brimleyorum. Goater et al. (1987) reported similar larvae in D. monticola, D. ochrophaeus, and D. quadramaculatus from North Carolina. The present finding represents a new host record for D. brimleyorum.

Twenty-five specimens (10 male, 15 female) of the habronematoid nematode, Hedruris pendula (Leidy, 1851) Chandler, 1919, were found in the stomach of 1 juvenile male (62 mm) and 3 adult male (69.7 \pm 0.3, 69–70 mm) salamanders. Specimens of H. pendula from D. brimleyorum were only about one-half the size reported for the species (Baker, 1986); however, they matched the description in every other detail, including possessing mature ova without lateral projections and the appropriate ratio for measurements of the distance from end of body to anus. Desmognathus brimleyorum is a new host and Arkansas a new locality for H. pendula. The species has been reported previously in other North American vertebrates (see Baker, 1987). A similar species, H. siredonis Baird, 1858, has been reported from various salamanders, including D. fuscus from Georgia (Baker, 1986, 1987).

A total of 16 (7 male, 9 female) seuratoid nematodes, *Omeia papillocauda* Rankin, 1937, were found in the stomach of 2 juvenile (male and female, 57 mm SVL) and 4 adult (2 male, 2 female, 76.8 \pm 5.5, 67–92 mm) *D. brimleyorum*. A new host and locality record is documented for *O. papillocauda*. This nematode was described from *Desmognathus* spp. and *Gyrinophilus porphyriticus danielsi* in North Carolina

(Rankin, 1937b). It exhibits little host specificity and is a common parasite of numerous plethodontid salamanders from North America (see Baker, 1987). In addition, survey data indicate that prevalence of infection with *O. papillocauda* can vary widely depending on the host species and geographic locality and has been reported to range from 4 to 8% in *D. ochrophaeus*, 13 to 30% in *D. quadramaculatus*, and 20 to 40% in *D. monticola* (Dunbar and Moore, 1979; Baker et al., 1987; Goater et al., 1987; Joy et al., 1993).

A single male and 22 female oxyurid nematodes, Batracholandros magnavulvaris (Rankin, 1937) Petter and Quentin, 1976, were found in the rectum of 11 male and 1 female (64.5 \pm 4.0, 51–92 mm) D. brimleyorum. Of the infected salamanders, 6 (50%) were juveniles (54.8 \pm 1.1, 51-58 mm) and 6 (42%) were adults (75.2 \pm 4.2, 65-92 mm). In addition, the smallest infected salamander (51 mm SVL) had 4 worms, whereas the largest (92 mm SVL) had a single B. magnavulvaris. Mean intensity of B. magnavulvaris was slightly higher in smaller salamanders, as juveniles had 2.5 ± 0.7 (range 1-4) worms per host whereas adults had 1.9 ± 0.8 (1–6) worms per host. Also, there was a 2-fold difference in prevalence of infection depending on the month of collection, as 34% of D. brimleyorum collected in mid-March (N = 29 examined, 57.2 \pm 3.6 mm) versus only 17% collected in late May (N = 12 examined, 59.8 ± 4.0 mm) harbored B. magnavulvaris. This is probably the result of salamanders congregating for courtship and breeding in March-April. Prevalence data can vary greatly, as Winter et al. (1986) reported 77% of the D. brimleyorum they examined had B. magnavulvaris with a mean intensity of 4.6 (range 1-19). Similarly, prevalence in other desmognathine hosts and locales can be variable and has been reported to range from 6 to 27% in D. fuscus, 38 to 50% in D. monticola, 14 to 25% in D. ochrophaeus, and 7 to 85% in D. quadramaculatus (Fischthal, 1955a; Dunbar and Moore, 1979; Dyer et al., 1980; Goater et al., 1987; Joy et al., 1993). This nematode exhibits little host specificity and infects other plethodontids (Muzzall, 1990) as well as salamandrids (Rankin, 1937b; Baker, 1987).

A single acanthocephalan cystacanth was recovered from the body musculature of a juvenile (53 mm SVL) male *D. brimleyorum*. Winter et al. (1986) and McAllister et al. (1993) previously have reported cystacanths in *D. brimleyorum* and *P. albagula* from Arkansas, respectively. Cysts

of Acanthocephalus acutulus Van Cleave, 1931, have been reported from various salamanders, including D. fuscus and D. quadramaculatus from North Carolina (Rankin, 1937a). In addition, larval Centrorynchus conspectus Van Cleave and Pratt, 1940, has been reported from the colon of D. quadramaculatus and D. monticola in North Carolina (Goater et al., 1987).

The most common parasite of D. brimleyorum were larval intradermal mites, Hannemania sp. Twenty-eight infected salamanders measured 62.3 ± 2.4 (range 40–92 mm SVL), whereas the 13 uninfected salamanders were 48.7 \pm 6.6 (19– 93 mm). Unengorged and partially engorged larvae were encapsulated primarily on the appendages and digits by host dermal connective tissue causing nodular projections of the digital skin. Specific identity of Hannemania sp. was not possible because only larvae were found. However, H. dunni Sambon, 1928, was reported previously by Winter et al. (1986) on D. brimleyorum from Polk County, Arkansas. Prevalence was reported to be 77% and intensity averaged 21 chiggers/ host (Winter et al., 1986). Hannemania dunni was described from D. fuscus fuscus from an unnamed locality in the southeastern United States (Sambon, 1928) and has been reported on Desmognathus auriculatus from Cass, Lee, and McLennon counties, Texas, and D. brimleyorum from Montgomery and Polk counties, Arkansas, and LeFlore and Woods counties, Oklahoma (Loomis, 1956). Hannemania sp. has also been reported in Arkansas on E. multiplicata griseogaster (McAllister et al., 1995a) and R. palustris (McAllister et al., 1995b).

In addition to the parasites of *D. brimleyorum* already mentioned, Winter et al. (1986) reported *Oswaldocruzia pipiens* (syn. *O. euryceae* Reiber, Byrd, and Parker, 1940) and an *Oxysomatium* sp. Railliet and Henry, 1916, from this host species. However, the latter genus is known only from Old World amphibians and reptiles (Baker, 1987), and other reports from North America (Walton, 1927; Fischthal, 1955a; Landewe, 1963) are doubtful. Most likely the parasite is *Cosmocercoides* sp.

In summary, 8 new host and 2 new distributional records are reported for parasites of *D. brimleyorum* from Arkansas. The parasite community of our sample of *D. brimleyorum* is variable yet somewhat similar when compared to other desmognathine salamanders. Goater et al. (1987) surveyed 4 species of desmognathine salamanders and reported isolationist parasite in-

fracommunities that they correlated with host diet, size, and habitat preferences. Host range, diet, and parasite life cycles are important in determining what species are present and how intense the infection may be in a given host. Our survey tends to support Aho's (1990) contention of a depauperate noninteractive community structure observed in helminth communities of most amphibians and reptiles.

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