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Surveys of Basking Turtles in the Rivers of Northeastern Oklahoma, with Emphasis on *Graptemys geographica* (Common Map Turtle)

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Abstract: I used binoculars and a spotting scope with a built-in digital camera to survey basking turtles at 29 sites on tributary drainages of the Arkansas River in four counties of northeastern Oklahoma and one county of southeastern Kansas. The predominant species recorded were *Graptemys ouachitensis* (Ouachita map turtle; 57% of all turtles) and *Trachemys scripta* (slider turtle; 25%), typical of results for rivers with similar assemblages of turtle species in the central United States. There were two notable results of the surveys. First, I photographed a male *Graptemys geographica* (common map turtle) twice at a site on the Spring River in Ottawa County; the record is only the third locality reported for the species in Oklahoma and is the first vouchered locality since the initial report of the species in Oklahoma in 1927. Prospects for finding additional localities for *G. geographica* in eastern Oklahoma are discussed, based on records in adjacent Kansas, Arkansas, and Missouri. Second, I observed *Apalone spinifera* (spiny softshell) at a higher frequency compared to basking turtle surveys that have been conducted elsewhere within its range.

Introduction

Management of biodiversity requires that data on occurrence, relative abundance, and absolute abundance be collected over time, to allow assessment of trends. This requirement represents a daunting task given the diversity of species and myriad habitats that exist. Because resources to support data collection are limited, efficiency is paramount. The need for rapid and efficient assessment is especially imperative when anthropogenic threats endanger biodiversity.

Many species of aquatic turtles are habitual baskers that sun themselves on emergent in-stream deadwood and along shorelines. In North America, the behavior is especially well-developed among the six genera of deirochelyine emydids and the trionychid genus *Apalone* (Lindeman 1998, 1999; Selman and Qualls 2009). Trapping aquatic turtle species is labor- and equipment-intensive, particularly in rivers. As a low-cost, high-yield alternative, surveying basking turtles with high-power binoculars or a spotting scope allows collection of large amounts of data from multiple sites relatively quickly (Vogt 2012). Basking surveys are particularly effective when information is sought on the distribution and relative abundance of rare species (Lindeman 1997, 1998, 1999; Selman and Qualls 2009).

In recent years, concerns over the exploitation of turtles for international trade in meat and live pets have placed a premium on information concerning the general distribution and abundance of turtle species, whether rare or common. In May 2008, the Oklahoma Department of Wildlife Conservation (ODWC) announced a three-year moratorium on commercial harvest of turtle species from public waters in order to study issues related to the international trade in turtles (ODWC 2014). The announcement cited a commercial harvest by licensed trappers of nearly 64,000 turtles in Oklahoma during 2007 and a lack of data by which to assess the population impacts of the harvest.

Table 1. Results of August 2009 turtle surveys in northeastern Oklahoma. Percent frequency of occurrence is percent of sites at which occurrence was detected over 1-3 surveys. Photographic vouchers (catalog numbers in the University of Florida Museum of Natural History) refer to species at a site marked with asterisks.

			Total	Total turtles observed in combined surveys						Photographic
Drainage	Site	County, state	surveys	*G. geographica*	*G. ouachitensis*	*G. pseudogeographica*	*P. concinna*	*T. scripta*	*A. spinifera*	vouchers
Spring River	Old Hwy. 96, E of Crestline	Cherokee, KS	1		4		4	2	1	
	Empire Lake spillway, SE 70th St., Lowell	Cherokee, KS	1					4	1	
	Hwy. 400, N of Baxter Springs	Cherokee, KS	2		10		3	2	2	161176
	Hwy. 166, Baxter Springs	Cherokee, KS	3		11*		4	14	8	161177
	Bicentennial State Park, E 040 Rd.	Ottava, OK	3	3*	2		2	8		
	Josephine Smith State Park, E 057 Rd.	Ottava, OK	3		25	1			1	
	Mocassin Bend, Hwy. 10 E of Miami	Ottava, OK	2		4			2		
Neosho River	E 060 Rd., W of Commerce	Ottava, OK	1		6*		2	5	1	161178
	Riverview Park, Hwy. 125, Miami	Ottava, OK	3		52*		1	8		161179
	Twin Bridges State Park, Hwy. 137	Ottava, OK	1		1			2		
	Hwy. 10 overlook, SW of Kellyville[a]	Ottava, OK	1		1			1	3	
	Hwy. 59, NW of Grove[a]	Delaware, OK	1		3		2	5	1	
Lost Creek	S645 Rd., Wyandotte	Ottava, OK	1				1	8		
Elk River	Hwy. 10, SE of Turkey Ford[a]	Delaware, OK	1		1			2		
Honey Creek	Honey Creek State Park, Park Rd. off Hwy. 59[a]	Delaware, OK	1					1		
Verdigris River	E 0030 Rd. SE of South Coffeyville	Nowata, OK	2		9	1			3	
	Hwy. 10, E of Lenapah	Nowata, OK	1			1	1	5		
	Hwy. 28, SE of Delaware	Nowata, OK	2		28*	3*		5*		161180-82
Big Creek	Hwy. 28, SE of Childers	Nowata, OK	2		11	4	1	2		
	N4190 Rd., S of Childers	Nowata, OK	1			2				
Caney River	W3500 Rd., SE of Ramona	Washington, OK	1		4					
	W3200 Rd., NE of Ramona	Washington, OK	2		5*			1		161183
	W3000 Rd., S of Oglesby	Washington, OK	1		5			1		
	W2650 Rd., W of Oglesby	Washington, OK	2		17		5	9	3	
	W2400 Rd., S of Bartlesville	Washington, OK	1		5	1		5		
	Hwy. 123, Bartlesville	Washington, OK	1		2			1		
	W1300 Rd., NW of Dewey	Washington, OK	1		6	1		4		
	W1100 Rd., SW of Copan	Washington, OK	2		4	1		1	3*	161184
Little Caney River	W1100 Rd., SW of Copan	Washington, OK	2		1	1*		13	1	161185
Total (N = 377)				3	216	16	23	95	24	
Percent frequency of occurrence				3%	79%	34%	31%	72%	34%	
Relative abundance				1%	57%	4%	6%	25%	6%	

[a]Impounded habitats of the Grand Lake O' the Cherokees reservoir

The moratorium was extended by two years in April 2011 (ODWC 2014).

The Oklahoma Natural Heritage Inventory (2014) lists *Graptemys geographica* (common map turtle) as a Category II Species of Special Concern. Category II species are those "identified by technical experts as possibly threatened with extirpation, but for which additional information is needed." There are only two previous records of *G. geographica* in Oklahoma. Ortenburger (1929) captured five specimens (OMNH 7272−7274 and 7276;

FMNH 13162) on the Elk River in Delaware County in 1927, six miles northwest of the town of Grove. That sampling site is now part of the Grand Lake O' the Cherokees Reservoir, constructed by damming the Neosho River in 1940. Riedle et al. (2009) captured a specimen of *G. geographica* (unvouchered) on Spring Creek near its confluence with the Neosho River in Mayes County in 1998. No other information exists on the species' occurrence in Oklahoma.

Both Oklahoma localities for *G. geographica* are tributary streams that drain southward into the mainstem Arkansas River. An extensive specimen database for the genus *Graptemys* (Lindeman 2013) suggests that there has been little collecting activity for riverine turtles in the northeastern corner of Oklahoma. Farther upstream in northern tributaries of the Arkansas drainage in Kansas, records for *G. geographica* exist for the Verdigris, Caney, and Spring drainages (KU 3267 and 3285, mapped by Collins 1993, plus recent sight records of Taggart et al. 2014). Hence the low number of localities for *G. geographica* in Oklahoma may be in part an artifact of low sampling effort in the state's northeastern streams.

I surveyed basking turtles in rivers in four counties of northeastern Oklahoma and one county in southeastern Kansas, with an emphasis on evaluating the status of *G. geographica* in Oklahoma. I report data on relative abundance, with emphasis on a new record for *G. geographica* in Oklahoma and prospects for finding additional localities for the species in the state.

Methods

I observed turtles 31 July−3 August 2009 from bridges and roadside pull-offs on the Spring, Neosho, Verdigris, and Caney rivers and their tributaries in Ottawa, Nowata, Washington, and Delaware counties, Oklahoma, and Cherokee County, Kansas. Surveys were conducted during warm, sunny conditions between 0900 and 1800 h. I used 18× Canon image-stabilizer binoculars and a Barska DSS60 spotting scope with 15−45× zoom magnification and built-in digital camera to identify turtles to species. Most turtles were basking when observed, but I also recorded several that were active at the water's surface. When I was able to get a good image, I took voucher photographs that have been deposited in the Florida Museum of Natural History Herpetology Department photographic archive.

Results

I identified 377 turtles in 46 counts made at 29 sites (Table 1). The two predominant species, *Graptemys ouachitensis* (Ouachita map turtle; N = 216, 57% relative abundance) and *Trachemys scripta* (slider turtle; N = 95, 25%), each occurred at more than 70% of sites. Four other species (*Apalone spinifera*, spiny softshell; *Pseudemys concinna*, river cooter; *Graptemys pseudogeographica*, false map turtle; and *G. geographica*) each occurred at between 1 and 10 sites and each comprised no more than 6% of the total sample. Turtles not identified (because they were too far away, or because my view of identifying characteristics was obstructed, or because they jumped into the water before I could identify them) were a small proportion of all turtles seen (<10%) and are not included among totals.

All three observations of *G. geographica* were of a male basking on the same fallen tree on the Spring River in Bicentennial State Park. I photographed the turtle on the first and third occasions (Fig. 1). Examination of head markings in several photographs taken each day suggest it was the same animal. The second observation was of a male that emerged briefly on the same branch where the male was photographed on the third observation, hence it is possible that all three observations were of the same animal.

On the upper Grand Lake O' the Cherokees Reservoir, turtle numbers were low overall, except in two small coves adjacent to the bridge on Hwy. 59. Predominant species were *T. scripta* and *G. ouachitensis*, the same species that predominated on the streams farther north.

Figure 1. A male *Graptemys geographica* basking in the Spring River at Bicentennial State Park, Ottawa County, Oklahoma. The upper picture was taken on 1 August 2009 and the lower picture on 3 August 2009.

Discussion

The record of *G. geographica* for Bicentennial State Park is only the third locality record in Oklahoma and the first to be vouchered since the species was first recorded in the state 82 years earlier by Ortenburger (1929; Fig. 2). The total historical record for the species in Oklahoma thus consists of Ortenburger's five specimens from a portion of the Elk River in Delaware County that is now submerged by impoundment, a specimen captured in Spring Creek near its confluence with the Neosho River in Mayes County (Riedle 2001, Riedle et al. 2009), and my photographed specimen from the Spring River in Ottawa County. All the records are from tributaries of the Arkansas drainage that flow southward toward the mainstem river. Additional records are from further upstream in the Verdigris, Caney, and Spring drainages in southeastern Kansas and Jasper County in southwestern Missouri (Fig. 2; Collins 1993; Daniel and Edmond 2013; Taggart et al. 2014).

Clearly *G. geographica* is an exceptionally rare species in Oklahoma. All species of *Graptemys* are habitutal baskers, and while their absolute abundance in basking surveys may vary seasonally, dramatic changes in relative abundance of species in turtle assemblages have not been described (Lindeman 2013), hence it is unlikely that the low relative abundance of the species was a result of the short time-frame of the present study. The total number of recorded specimens of three species of *Graptemys* from the Arkansas River and its northern tributaries in northeastern Oklahoma, compiled from a combination of museum collections, the trapping study of Riedle et al. (2009), and the present basking surveys, is 454 (Table 2), with *G. ouachitensis* being strongly predominant (89% of all records).

In other parts of its shared range with *G. ouachitensis* and *G. pseudogeographica*, the preferred habitat of *G. geographica* has been described as being rocky streams (DonnerWright et al. 1999, Fuselier and Edds 1994). Further searches for *G. geographica* in northeastern Oklahoma should concentrate on more extensive searches on the Spring River, the Elk River above the Grand Lake O' the Cherokees Reservoir, and the mainstem Arkansas River.

The Spring River was a target of special interest in the present study because of a record of the species several river kilometers further upstream in Jasper County, Missouri (Fig. 2; USNM 55698, collected in 1906; Daniel and Edmond 2013). The Spring River's rocky stream bottom would seem to offer a more suitable habitat than the muddier Neosho and Verdigris rivers to the west, based on habitat studies of *G. geographica* in Kansas, Minnesota, and Wisconsin (DonnerWright et al. 1999, Fuselier and Edds 1994). Subsequent to the surveys reported herein, in 2013 a *G. geographica* was photographed near the confluence of the Spring River and Shoal Creek in Cherokee County, Kansas, ca. 16 river km upstream of the Bicentennial State Park site where I recorded the species (Taggart et al. 2014). In my 15 visits to 7 sites

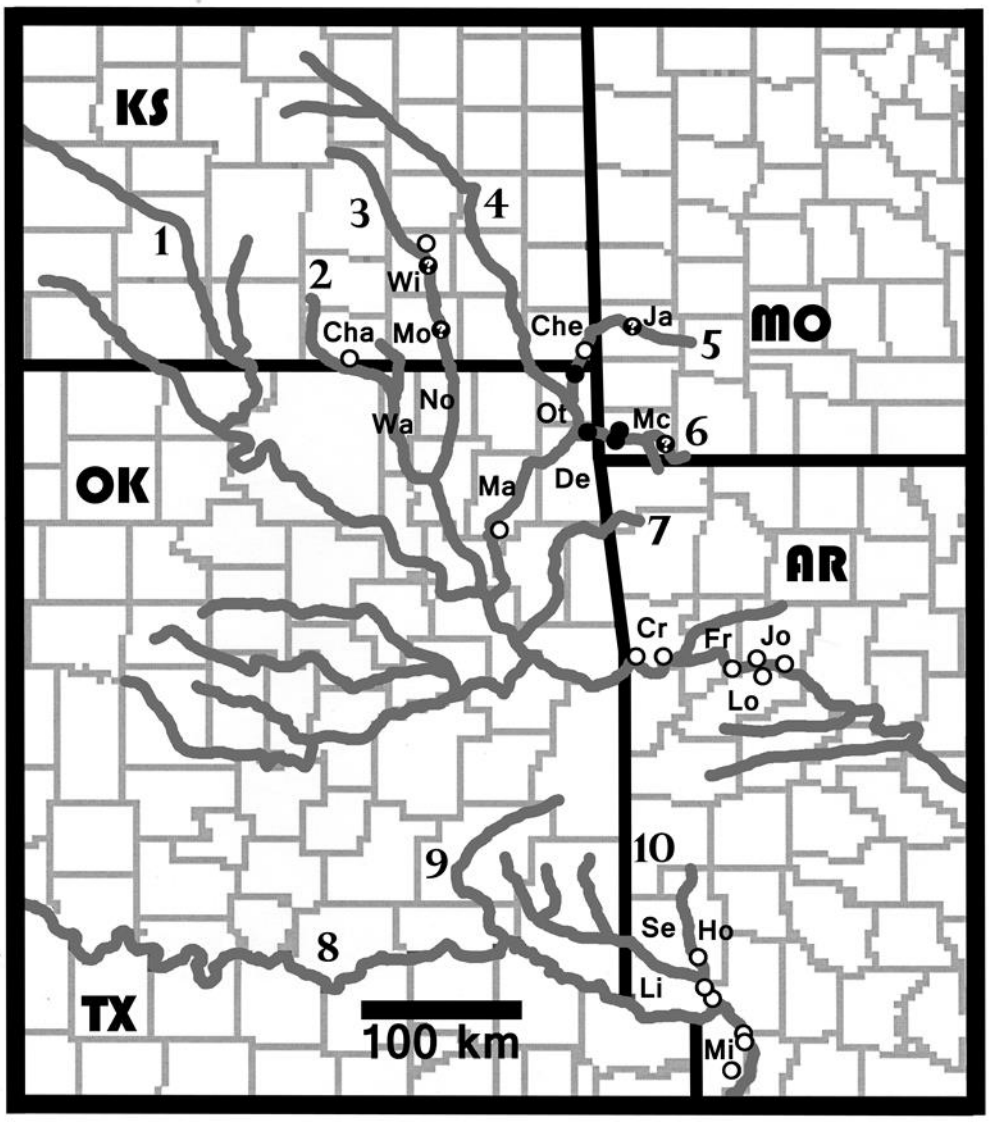

Figure 2. Detail of the Arkansas River drainage and the Red River drainage in eastern Oklahoma and adjacent states, showing the three Oklahoma localities for *Graptemys geographica* and localities for the species in adjacent portions of Kansas, Missouri, and Arkansas. Solid symbols represent vouchered specimens with precise localities, symbols with question marks are vouchered specimens with imprecise localities, and open symbols are unvouchered records from trapping or visual surveys. Labelled counties are as follows: Oklahoma, De = Delaware, Ma = Mayes, No = Nowata, Ot = Ottawa, and Wa = Washington; Kansas, Cha = Chatauqua, Che = Cherokee, Mo = Montgomery, and Wi = Wilson; Missouri, Ja = Jasper and Mc = McDonald; and Arkansas, Cr = Crawford, Fr = Franklin, Ho = Howard, Jo = Johnson, Li = Little River, Lo = Logan, Mi = Miller, and Se = Sevier. Rivers discussed in the text are numbered as follows: 1 = mainstem Arkansas, 2 = Caney (with Big Caney and Little Caney tributaries), 3 = Verdigris, 4 = Neosho, 5 = Spring, 6 = Elk, 7 = Illinois, 8 = mainstem Red, 9 = Kiamichi, 10 = Little (with Glover and Mountain Fork tributaries in Oklahoma and Saline tributary in Arkansas).

Table 2. Records of *Graptemys* from the Arkansas River and its major northern tributaries in northeastern Oklahoma.

	G. geographica				*G. ouachitensis*				*G. pseudogeographica*			
Drainage	Museum specimens	Riedle et al. (2009) trapping	Present basking surveys	Percent of all records	Museum specimens	Riedle et al. (2009) trapping	Present basking surveys	Percent of all records	Museum specimens	Riedle et al. (2009) trapping	Present basking surveys	Percent of all records
Arkansas River[a]	—	—	—	—	9	70	—	95	—	4	—	5
Illinois River	—	—	—	—	2	8	—	67	5	—	—	33
Spring River	—	—	3[b]	7	—	7	31	88	—	1	1	5
Elk River	5	—	—	83	—	—	1	17	—	—	—	—
Neosho River	—	1	—	1	2	33	62	95	—	4	—	4
Verdigris River	—	—	—	—	2	15	48	83	2	—	11	17
Caney River	—	—	—	—	—	62	49	87	—	12	4	13
Total	5	1	3	2	15	195	191	89	7	21	16	10

[a]Includes sites on smaller tributaries near their confluences with the Arkansas River.

[b]All three observations are believed to be of a single male (see text).

on the Spring River, including sites I visited in southeastern Kansas, I observed *G. geographica* at only one site (three times, but possibly all the same animal), hence even in the Spring River the species does not appear to be abundant.

There are also several specimen records for *G. geographica* further upstream in the Elk River and its tributaries in McDonald County, Missouri, that date from 1936−1977 (Fig. 2; KU 91327; AUM 12410−12418; CM 61498−61499, 87510−87518, and 87525). A short segment of the Elk River in Oklahoma (ca. 3 km), between Ortenburger's (1929) historical locality and the localities for the Missouri specimens, is not impacted by impoundment and may still harbor a small population of *G. geographica* within Oklahoma. Impoundments do not appear to be good habitat for the species (e.g., Lindeman 1998, 1999).

The Arkansas River mainstem has not been extensively surveyed for turtles in Oklahoma, except in the vicinity of the Robert S. Kerr Reservoir near the Arkansas border (Riedle et al. 2008). Across the border downstream in the state of Arkansas, there are numerous records of *G. geographica* in small tributaries near their confluences with the Arkansas River mainstem (Fig. 2; 34 specimens captured from six creeks in Crawford, Franklin, Johnson, and Logan counties, Trauth et al. 2004). The records are based on turtle surveys conducted by the Arkansas Game and Fish Commission in 1993 and were not vouchered with specimens or photographs (S. Trauth pers. comm. 2009). Survey efforts along the mainstem Arkansas and lower reaches of its tributary creeks in Oklahoma may prove similarly fruitful for finding additional localities for *G. geographica* in the state.

It is possible that *G. geographica* may also be found in the southeastern corner of Oklahoma. Trauth et al. (2004) mapped localities for *G. geographica* in the Red and Little river drainages of Arkansas that extend to within about 30 km of the Oklahoma state line (Fig. 2; also unvouchered specimens from the 1993 surveys, S. Trauth pers. comm. 2009). Records of *Graptemys* in the Red and Little drainages in southeastern Oklahoma to date (N = 62) include no *G. geographica*, however (Table 3), thus the species is probably at best a rare component of the turtle assemblage in southeastern Oklahoma streams.

The predominance of *G. ouachitensis* and *T. scripta* in northeastern Oklahoma is similar to what I reported for the Tennessee River and its major impoundment, Kentucky Lake, in western Kentucky, where virtually the same turtle fauna occurs (Lindeman 1998, 1999). In replicated basking surveys, 49% of turtles identified to species in Kentucky were *T. scripta* and 29% were *G. ouachitensis*. Also similar to the present study, in Kentucky *G. pseudogeographica* was considerably less abundant (17%) than *G. ouachitensis* and *G. geographica* was much rarer yet, being sighted only once. In trapping studies, *G. ouachitensis* likewise outnumbers *G.*

Table 3. Records of *Graptemys* from the Red River and its major northern tributaries in southeastern Oklahoma[a].

	G. geographica			*G. ouachitensis*			*G. pseudogeographica*		
Drainage	Museum specimens	Riedle et al. (2009) trapping	Percent of all records	Museum specimens	Riedle et al. (2009) trapping	Percent of all records	Museum specimens	Riedle et al. (2009) trapping	Percent of all records
Red River	—	—	0	18	5	100	—	—	0
Little River	—	—	0	1	1	67	1	—	33
Mountain Fork River	—	—	0	10	—	77	2	1	23
Glover River	—	—	0	11	2	81	3	—	19
Kiamichi River	—	—	0	1	1	100	—	—	0
Uncertain[b]	—	—	0	3	—	60	2	—	40
Total	—	—	0	44	9	85	8	1	15

[a]Choctaw, McCurtain, and Pushmataha counties

[b]Specimens from McCurtain County with no further locality data

pseudogeographica in most reported cases (reviewed in Lindeman 2013). In northern Louisiana, however, *G. ouachitensis* outnumbered *G. pseudogeographica* in basking counts on only one of the three drainages where they were observed to co-occur and the latter was seen at more of the lotic survey sites (74% vs. 23%; Carr 2001). *Graptemys pseudogeographica* is more mollusk-dependent in its diet while *G. ouachitensis* is a narrow-headed species that feeds on softer-bodied invertebrates and algae (Lindeman 2000a, b, 2013). It is typical that species of narrow-headed *Graptemys* are substantially more abundant than their sympatric broader-headed congeners (Coleman and Gutberlet 2008, Godwin 2003, Ilgen et al. 2014, Lindeman 1999, Selman and Qualls 2009, Shively 1999, Shively and Jackson 1985).

The high incidence of *A. spinifera* (6% of turtles identified, seen at 34% of sites) is unprecedented in basking surveys within its range. The species was less than 1% of turtles seen in western Kentucky and two rivers in southern Mississippi (Lindeman 1998). It was recorded at 20% of sites on the Pearl drainage but only 5% of sites on the Pascagoula and Tennessee drainages (P.V. Lindeman unpubl. data), despite greater replication of survey effort (i.e., most sites were visited eight times) than in the present study. In northern Louisiana, <1% of turtles seen at river and bayou sites were *A. spinifera* and it was seen at 5% of sites (Carr 2001). In the Mobile Bay drainages of Alabama, <1% of turtles seen were *A. spinifera* and it was seen in 11% of surveyed river reaches (Godwin 2003). In basking surveys on the Bogue Chitto River in southeastern Louisiana, *A. spinifera* and *Apalone mutica* LeSueur (smooth softshell) were not differentiated, but together the two species constituted <3% of turtles seen (Shively 1999). Similarly, the two species constituted <5% of turtles seen and were observed at 21% of survey sites on the Pascagoula drainage in southeastern Mississippi (Selman and Qualls 2009, W. Selman pers. comm. 2010). Further studies would be necessary to determine whether the late-season, warm-weather timing of my Oklahoma surveys contributed to the high incidence of *A. spinifera* or whether the species is simply more abundant in upper Arkansas drainage tributaries than elsewhere.

Visual surveys provide rapid, wide-ranging assessments of the status of turtle species that commonly engage in basking, such as *Graptemys* spp. (Vogt 2012). To generate sample sizes of captured turtles comparable to those of the present four-day study, several weeks of trapping would have been necessary,

given the time-intensive nature of putting in a boat to set and check traps at a site. Naturally, trapping studies are essential to estimates of abundance and collection of data on various biological parameters of a species, but relying solely on trapping, given limited time and resources available, may limit the number of sites sampled, thereby possibly causing locality records for rarer species to be missed. Future studies employing visual surveys should investigate species-specific detection probabilities and their environmental correlates as well as inherent detection biases among species and their impact on relative abundance data.

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Late Pleistocene Remains of an American Black Bear (*Ursus americanus*) and Two Small Vertebrates from an Oklahoma Ozark Cave

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Abstract: The serendipitous discovery of fossil bear bones in a cave in northeastern Oklahoma prompted us to excavate and describe the fossils and their geologic, chronologic, and biogeographic context. We recovered the partial skeleton of a subadult (about 1-2 year-old) male *Ursus americanus* (American black bear) in CZ-9 Cave, Cherokee County, Oklahoma, in late Pleistocene fill sediments within the cave. The locality is at the western edge of the Ozark Highlands. A sample of enamel from a tooth of the bear yielded an AMS radiocarbon age of 10,958 ± 35 years before present. This is the first directly-dated occurrence of a black bear in the late Pleistocene of the western Ozark Highland and in Oklahoma. Two vertebrae of an unidentified snake were found in the same layer as the bear's remains and may be approximately contemporaneous. Fragmentary jaws and teeth of a large species of *Blarina* (short-tailed shrew) occurred in the same sedimentary unit as those containing the bear and also in another unit above them. This large shrew differs from the related smaller species *Blarina hylophaga* (Elliot's Short-tailed Shrew) that currently occupies the same region of eastern Oklahoma, but the fossils cannot be identified to species. The shrew fossils, too, are probably of late Pleistocene age; they may pertain to an ancestral population of larger body size than the extant local species and are about the size of extant *Blarina brevicauda* (Northern Short-tailed Shrew). They indicate a biogeographic or evolutionary change since the late Pleistocene in the short-tailed shrew inhabiting the Oklahoma portion of the Ozark Highland.

Introduction

The American black bear, *Ursus americanus* Pallas, is known in the historic and recent fauna of Oklahoma, including the western part of the Ozark Highland. However, these bears are poorly known as confirmed Pleistocene fossils in this area although they are common as Holocene fossils in other parts of the Ozarks (Hawksley 1986, Graham and Lundelius 2010). We report herein a late Pleistocene occurrence of a black bear and two associated microvertebrates from a cave in the western Ozark Highland in Oklahoma.

Ursine bears (family Ursidae, subfamily Ursinae) are known as fossils or subfossils from several localities in Oklahoma. Previous Quaternary fossil records include three that were originally reported as brown bears or grizzly bears (*Ursus arctos* Linnaeus or *U. horribilis* Ord). Smith and Cifelli (2000) reviewed the Oklahoma records. Stovall and Johnston (1935) reported two bear skulls, one of which originated near Lawton, Comanche County (Oklahoma Museum of Natural History [OMNH] 9569). The other was found

near Cheyenne, Roger Mills County in western Oklahoma. The second specimen was recently in the Black Kettle Museum, Cheyenne, Oklahoma (J. P. Thurmond, in litt.) and was cataloged as Museum of the University of Oklahoma [MUO] 546. Unfortunately, the Black Kettle Museum no longer exists and the disposition of this loaned specimen is unknown; the skull was not returned to the OMNH and its present whereabouts are unknown. Both skulls were found in Quaternary alluvium. As was common practice at the time, Stovall and Johnston (1935) named a new subspecies "tentatively identified as *U[rsus] horribilis oklahomensis*" based on these specimens. However, Graham (1991) showed that these specimens actually represented black bears, *U. americanus*, not brown bears, *Ursus arctos.* Czaplewski et al. (1994) further noted that *Ursus horribilis oklahomensis* was a *nomen nudum*; no type specimen was designated and no diagnosis was given, and few measurements were provided. Thus, the name *Ursus horribilis oklahomensis* has no validity. Stovall (1936) reported a skull and associated right and left femora (OMNH 10735) as *U. horribilis nelsoni* from Quaternary lacustrine deposits near Lenora, Dewey County (OMNH locality V689) in west-central Oklahoma; the specimen actually represents *U. americanus* (pers. observ.). Stangl and Dalquest (1986) and Stangl et al. (2014) reported a subfossil (undated but probably Holocene in age) lower jaw of *U. americanus* from the Red River, Tillman County. Martin and Meehan (2003) listed a metatarsal III from the Burnham site (late Pleistocene) in Woods County as *Ursus* cf. *U. americanus*. Several unpublished black bear specimens from Oklahoma are housed in the OMNH VP collection, including a cranium from near Buffalo, Harper County, a crushed cranium from Rosedale, McClain County, and pieces from sand and gravel bars along the Arkansas and Canadian Rivers. In addition, William Caire (pers. comm.) indicated that the University of Central Oklahoma has a specimen from the Selman Cave System, Woodward County, and there is a partial skeleton in the museum at Northwest Oklahoma State University collected from a sand dune deposit of probable Holocene age near Mooreland, Woodward County. None of these specimens has been radiometrically dated, so all can be considered Holocene or possibly late Pleistocene in age. In the late Holocene at least two Oklahoma archaeological localities are reported to contain Black Bear remains, the Pohly site (34My54), Mayes County, and Bryson-Paddock (34Ka5), Kay County. The Pohly site is dated by relative dating only, with cultural materials indicating an age range for the site of 2,950 to 55 years before present (ybp; Ray 1965). The Bryson-Paddock site has several radiometric dates ranging from 290 ± 70 radiocarbon ybp (rybp) to modern (Bell 1984). To these Quaternary records we add another fossil black bear, represented by a partial skeleton and the first occurrence to be dated directly to the late Pleistocene.

In the late Pleistocene during the full-glacial (approximately 18,000 rybp), the southwestern portion of the Ozark Highland including what is now in eastern Oklahoma was covered with boreal forest based on pollen cores analyzed by Delcourt and Delcourt (1987, 1991); this forest was dominated by spruce and jack pine and included few deciduous trees. During the late glacial about 12,000 rybp, prairie was established in the eastern Great Plains including eastern Oklahoma, and oak-hickory forest with deciduous broadleaf trees (oak, ash, elm, hickory, hornbeam) moved into the southeastern Ozark Highland as conifers declined there by about 14,000 to 10,000 rybp (Albert and Wyckoff 1981, Bryant and Holloway 1985, Delcourt and Delcourt 1991). Whether black bears remained continuously in the Ozark Highland during the Pleistocene glaciations is not yet addressed by genomic studies of these mammals. There are late Pleistocene fossil records of black bears in the Ozarks, but these are spotty and not well dated radiometrically (Graham and Lundelius 2010). Blaine Schubert (pers. commun.) feels there are likely more Pleistocene occurrences in the Ozarks than published records would suggest, but these are as yet unpublished and undated. There are no previous records of black bears from the Ozarks in Oklahoma, but there are two in Arkansas (Hurricane River Cave and

Peccary Cave) and several in Missouri. Changes in the climate and burning of the habitat after human arrival probably resulted in a mix of open prairies and woodlands in the Holocene that could have affected the distribution of black bears. Many plants of the Pleistocene boreal forest retreated northward at the same time as indigenous peoples and lightning-caused fires kept parts of the habitat open (Buckner 1989, Foti and Glenn 1991, Masters et al. 1995).

In the 1500s when Hernando de Soto entered the Ozark region, he recorded a land dominated by prairies with trees restricted to the drainages (Beilmann and Brenner 1951). After the invasion of the region by Europeans and the decline of the native peoples, suppression of fires resulted in the present-day closed-canopy oak-hickory forest in the Oklahoma Ozarks (Tyrl et al. 2002). The resulting near-disappearance of herbivorous prey animals such as elk, bison, and pronghorns from Oklahoma, as well as subsequent fragmentation and conversion of the forest habitat for agricultural, grazing, logging, and urban development, contributed to the disappearance of predators such as bears and wolves, which were also removed by humans because they were considered a threat. Black bears were occasional in Oklahoma in the early 1800s, including among other instances a sighting along the Grand River (Neosho River) in 1823 (Tyler and Anderson 1990) near CZ-9 Cave. Black bears were extirpated from Oklahoma by 1915 and nearly extirpated from adjacent Arkansas by the 1940s (Clark and Smith 1994). However, they reentered eastern Oklahoma forests during the mid-1980s, and genetic studies show they came from remnant Arkansas or Missouri black bear populations and individuals that were reintroduced in the Arkansas Ozark and Ouachita mountains in the 1950s-1960s from populations in Manitoba and Minnesota (Csiki et al. 2003, Faries et al. 2013, Smith and Clark 1994, Van Den Bussche et al. 2009). In Oklahoma, black bear distribution and abundance have increased over the last two decades in the Ozarks and Ouachita Mountains (Jackson et al. 2014).

Methods

In the process of conducting biodiversity surveys in Ozark caves in northeastern Oklahoma during July 2005, WLP noticed bear bones in CZ-9 Cave. The bones appeared in a small collapse of sand and silt from a bank of stratified sediments in a small, low passage. The cave is located along a small tributary of the Neosho River near its confluence with the Verdigris and Arkansas rivers in Cherokee County, Oklahoma. In this part of Oklahoma, the Neosho River and Arkansas River form the western boundary of the Ozark Highland. Thus CZ-9 Cave is at the very western boundary of the Ozark Highland physiographic region (Fig. 1). In historic times, vegetation of the region near the cave was predominantly oak-hickory forest on the uplands, with some post oak-blackjack oak (cross timbers) forest, and bottomland forest along the river floodplains. The authors visited the cave in February and July 2006 to excavate and collect the bones.

CZ-9 Cave (Fig. 1) formed within the Pitkin Formation, which is of Mississippian age. The Pitkin Formation is composed primarily of limestone with thin black shale interbeds. The formation is about 12 m thick in an area 3 km northwest of CZ-9 cave, based on a measured section in Huffman (1958). The cave has a mapped length of 600+ m and shows two levels of horizontal development. Small vertical shafts connect the horizontal passages to one another.

The cave entrance opens at about 180 m elevation. The bear bones were recovered in the lower level horizontal passage 88 m from the entrance. The fossil site within the cave is recorded as Oklahoma Museum of Natural History (OMNH) locality V1510. The bones were in a passage with a low ceiling varying from about 0.5 to 1 m high and 1 to 10 m wide (Figs. 1, 2). This passage drains northward toward the entrance and was barely damp to dusty when we visited it during the very dry winter and summer of 2006. Large deposits of damp, weakly consolidated or unconsolidated sediments formed banks on either side of the stream crawlway. Given the radiometric date on the bear, these sediments probably represent late Pleistocene aggradation within the cave. The sediment banks reached a thickness ranging from halfway to the ceiling

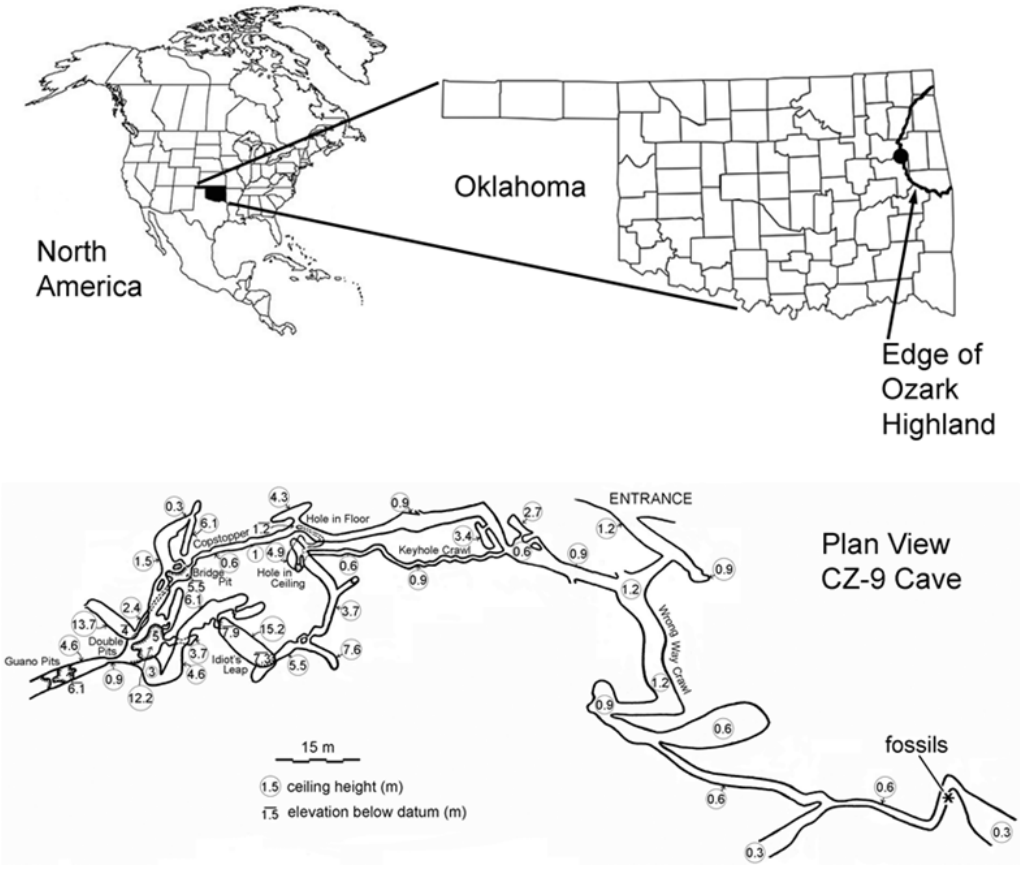

Figure 1. Locator maps (top) and plan view (bottom) of CZ-9 Cave, Cherokee County, Oklahoma, with location of Black Bear and other fossils indicated. North is at top. Black dot indicates location of CZ-9 Cave at western edge of Ozark Highland. Ceiling heights and elevations are in meters (m). Map produced by, and courtesy of, the Tulsa Regional Oklahoma Grotto (TROG).

to within a few cm of the ceiling in some places, such that this portion of the cave was nearly filled with Pleistocene deposits except where the stream channel cut through to form the crawlway. Sediments are currently being removed from the lower level of the cave by episodic underground drainage; in part, this erosion contributed to exposing the cross-sectional profile of sediments and the fossils described in this report.

Along this part of the cave passage most of the sediments that formed the walls of the crawlway showed a weathered surface that was blackish and pitted on its top surface near the ceiling, and was encrusted with whitish and pale yellow carbonate and probably other minerals on its vertical walls facing the crawlway. A small portion of the west bank of the sedimentary fill had collapsed along the stream-channel crawlway, exposing well-stratified sandy to clayey beds in a clean profile. Several black bear bones occurred in the collapsed sediment including a femur shaft lacking proximal and distal epiphyses, a carbonate-encrusted partial hand or foot with one ungual phalanx, and two other fragments of long bones. Cross-sections of other bones were visible in the layered sediment in the wall of the passage (Fig. 3).

We used small folding shovels and trowels to clean the stratigraphic profile and render the strata more visible, as well as to remove the

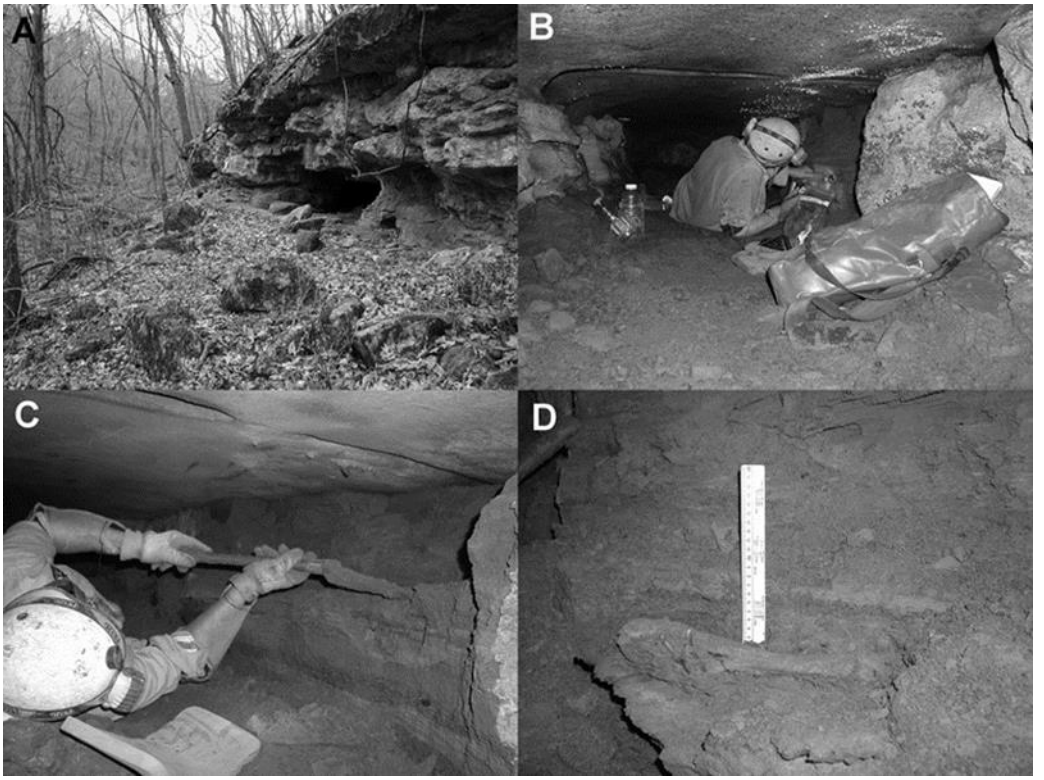

Figure 2. Photographs of CZ-9 Cave, Cherokee County, Oklahoma. A, cave entrance in Pitkin Limestone, winter aspect (February 2006); view is approximately west. B, view into the cave of the passage at the bear bone-containing deposit. C, removing overburden in the deposit; note the faint channel cut-and-fill directly below the shovel head. D, detail at the left (south) side of the deposit showing partly exposed (mud-covered) long bone and two adjacent canines in nearly upright positions; ruler is 200 mm long.

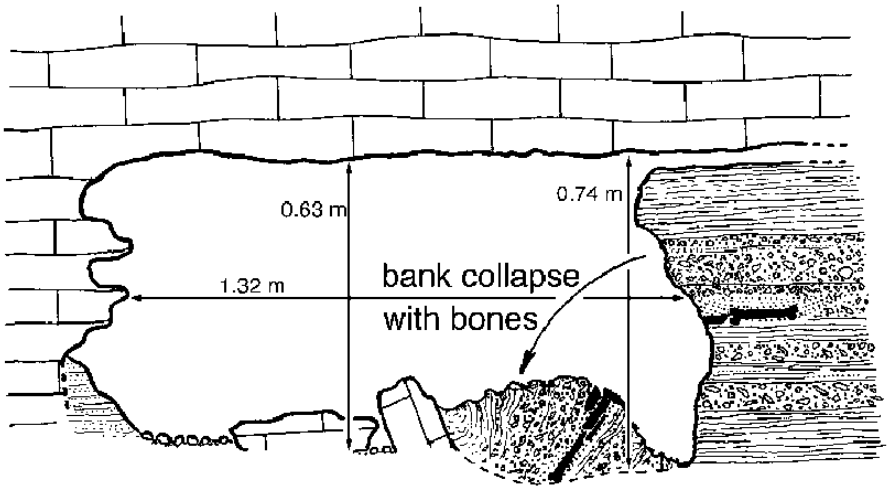

Figure 3. Cross-section of passage at the site of the bear bones as originally discovered. Curved arrow indicates fall of deposits; solid black indicates bear bones exposed by the collapse. Passage heights and width are indicated. View is into the cave.

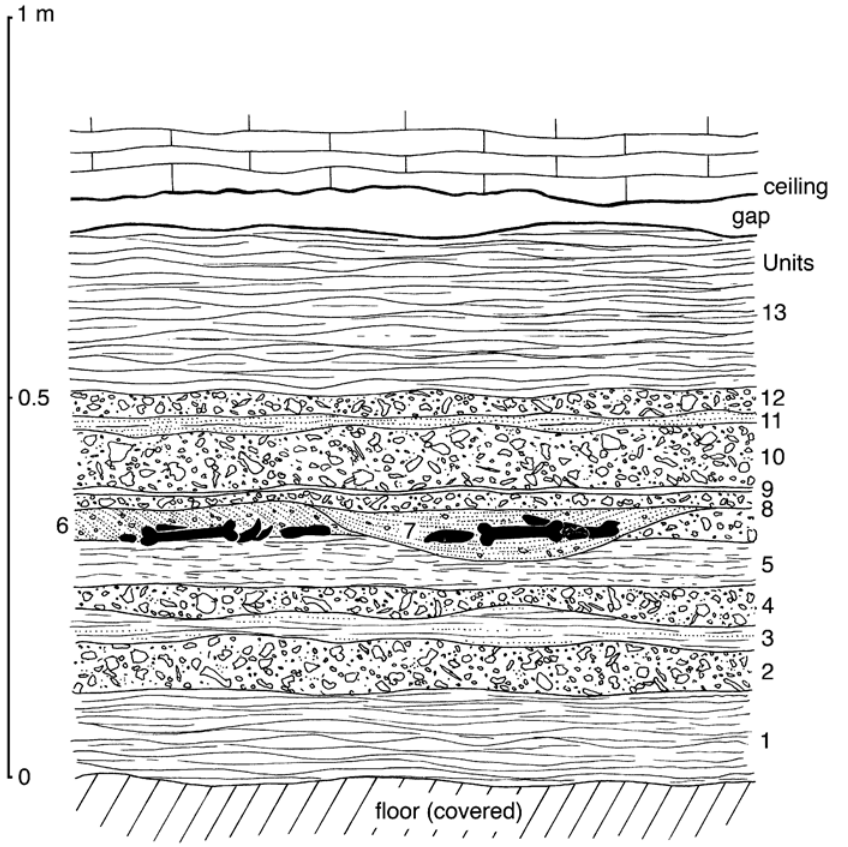

Figure 4. Profile of deposit containing black bear bones in CZ-9 cave. Entrance of cave is toward the right and approximately north. View is to the west. Stratigraphic units are indicated by numbers corresponding to those in the measured profile in text. Solid black indicates bear fossils.

fossils from the intact sediments in the wall of the passage and in the bone-containing sediment that had collapsed from the wall onto the floor of the cave passage. We photographed, sketched, and measured the cleaned profile, in which the bones occurred about halfway up the deposit in a small channel cut-and-fill and adjacent sandy foreset beds. Above and below the sandy beds containing bear bones were deposits of gravels, sands, silts, and clays indicating periodic flooding events. These are documented in the description (below) and sketch of the profile (Figs. 4, 5). Several long bones and a few loose teeth from the bear were found in the channel fill (Unit 7) and foresets (Unit 6) including two incisors, an m3, three of the canines, an articulated manus and partial pes, a femur, and fragments (Fig. 6). Sediment samples dug from the deposit adjacent to the bear bones were bagged and later screenwashed using nested wooden boxes with metal screen bottoms consisting of a coarse mesh of window screen (ca. 1.2 mm openings) on the inner box and a fine mesh (0.6 mm) on the outer box. Fine sediments of clay, silt, and fine sand were washed away in water tanks. The resulting concentrate of coarse sand, pebbles, and fossils in the bottom of the screens was dried and picked under a dissecting microscope to sort out and save the small fossils contained within it. The fossils were cleaned, cataloged, and are curated in the Vertebrate Paleontology collection of the

OMNH. Fossils were identified by comparison with recent specimens preserved in the collections of the OMNH and by consulting relevant literature sources. The taxonomic identifications are provided in the Systematic Paleontology section of Results.

In order to estimate the geological age of the cave deposit, we submitted enamel and dentine fragments of the broken left upper canine from the black bear specimen. The sample was sent to Rafter Radiocarbon Laboratory, New Zealand, for accelerator mass spectrometry (AMS) radiocarbon dating. The tooth was photographed before and after sampling for dating. Processing of the tooth enamel and dentine by Rafter included washing with sodium hypochlorite and acetic acid, etching in HCl, grinding with pestle and mortar, and finally treating through a phosphoric acid sequence. Because of the condition of the dentin, only the enamel fraction of the sample was ground and dated; the carbon/nitrogen ratio was not determined.

Results

Geologic age of the bear and cave deposit

The radiocarbon age yielded by AMS dating of the bear tooth is 10,958 ± 35 rybp (sample result archived as NZA 37942). This places the black bear fossils in the latest part of the Pleistocene epoch, specifically during the time of the deglaciation following the last glacial maximum, which is sometimes called the Wisconsinan glacial. This radiometric date gives an approximate age to the stratigraphic unit (Unit 6) in which the bear skeleton was found. Stratigraphic units lower in the profile are assumed to be relatively older and units above that containing the bear are likely somewhat younger. No datable materials were recovered in the other stratigraphic units. In the absence of datable materials from the other units, we assume the adjacent units are also of latest Pleistocene age.

Description of stratigraphic profile

Units were numbered from bottom to top (Fig. 4). Colors were recorded when the matrix was still damp. Colors given Munsell Soil Color codes were recorded under sunlight; those without Munsell codes were recorded under caving lights in the cave.

Unit 1. Silt; medium brown; thickness 130 mm.

Unit 2. Gravel consisting of clay-pebble and other gravel-sized clasts in a silty and sandy matrix; dark brown and gray; thickness 55-65 mm. Many of the clasts are very dark gray (charcoal gray, clearly derived from the Pitkin Formation black shales), and freshly broken ones at the face of the profile or crushed with the fingers appear black but smear grayish.

Unit 3. Clays, silts and sands; mostly pale buff but mixed with some grayish, yellowish, and tan, with rust-colored (limonite?) streaks; thickness 20-45 mm.

Unit 4. Gravel, dark gray to grayish brown; similar in composition and color to Unit 2; thickness 15-45 mm.

Unit 5. Silt; medium brown, grayish, and rust-tan; thickness 60 mm.

Unit 6. Sand with some gravel; mixed red, black, brown, buff, and gray. Foreset-like cross-bedding advancing to the right (toward the cave entrance); thickness 40 mm. Contained two snake vertebrae and several of the bones of the black bear (see Fig. 6). Bones were lightly to moderately encrusted with rusty dark brown carbonate and sand, but the teeth were not so encrusted. Two canine teeth were sitting tips-upward against the end of a long bone.

Unit 7. Sand with some silt and a little gravel, filling a small channel cut into Units 5 and 6; pale buff (Munsell color: 5YR 4/2 to 7.5 YR 4/2 or 5/2) with limonite streaks and stains; thickness at deepest point 75 mm. Bear fossils occurred within the channel fill at a level above the middle of its deepest point and at the same level as the bones in Unit 6. The fossils in this unit included a canine tooth, an unworn m3, and a large unidentified fragment. Bones were heavily encrusted with carbonate; teeth were not encrusted.

Unit 8. Gravel with some sand; similar to Units 2 and 4; thickness 5-25 mm.

Unit 9. Clay; pale buff and gray brown; thickness 5 mm.

Unit 10. Gravel; similar to Units 2, 4, and 8; Munsell color: 5YR 4/4 to 10 YR ¾; thickness

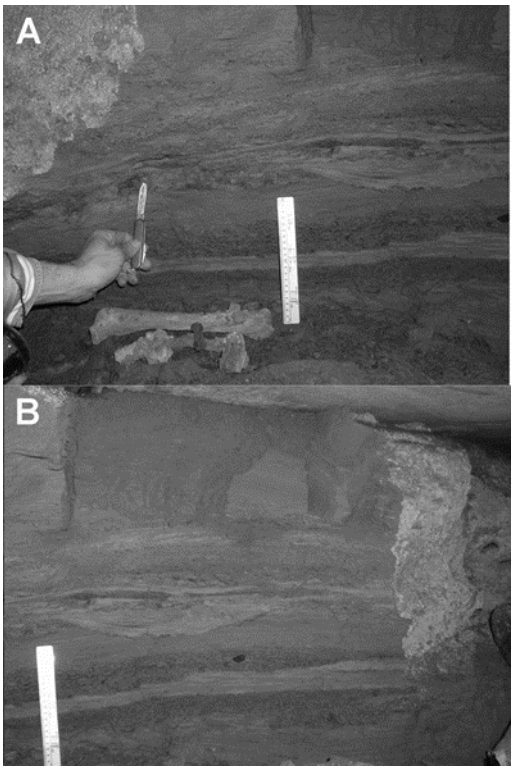

Figure 5. Photographs of same profile as diagrammed in Fig. 4, after collapse and before beginning of excavation. A, left (south) end of deposit before excavation. Pocketknife tip points to the exposed cross-section of a long bone broken in the collapse and buried in the foreset beds of Unit 6. Note also the sandy channel cut-and-fill (Unit 7, at right center) that contained some of the bear bones, and loose bones on floor (at lower left) that were pulled from the collapsed material. Cave entrance is toward the right (and north); Ruler at bottom center is 200 mm long. B, right (north) end of deposit before excavation. The sandy channel cut-and-fill shows at left center. Note carbonate-encrusted pre-collapse face of the deposit at right, and limestone ceiling of passage across the top of the photo. About 195 mm of the ruler is showing.

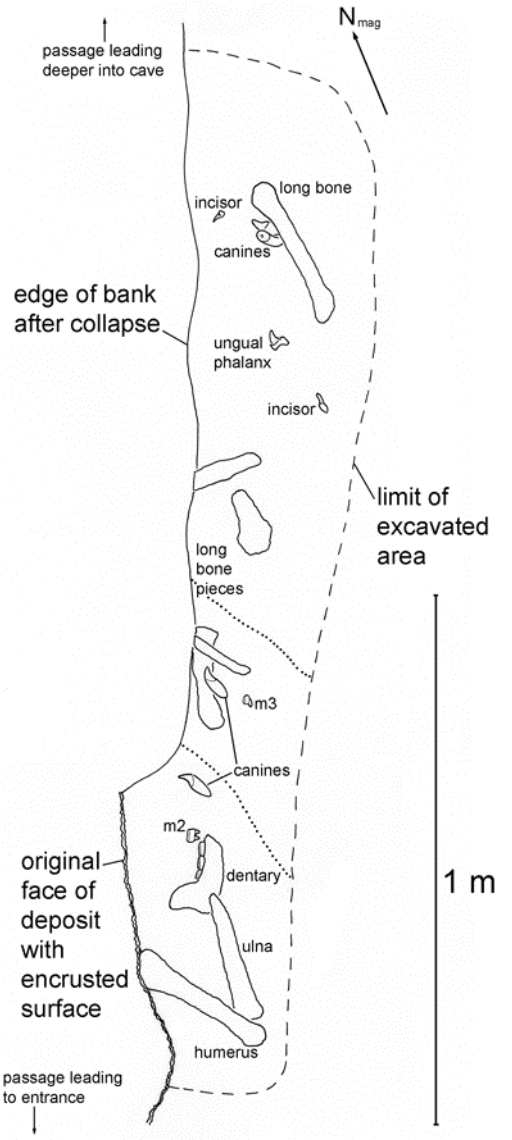

Figure 6. Plan view of excavated portion (dashed line) of the late Pleistocene sedimentary deposit showing relative positions of black bear bones in place. Dotted lines indicate approximate boundaries of sandy channel fill. Most bones were heavily encrusted with gravel and not identifiable in situ, but enamel tooth crowns were not so encrusted.

80 mm. Screenwashing of a sample of this unit yielded a shrew jaw fragment.

Unit 11. Clay and sand; pale buff; thickness 5 mm.

Unit 12. Gravel; dark brown and gray (Munsell color: 5YR 4/4 to 10 YR ¾); similar to Units 2, 4, 8, and 10; thickness 30 mm.

Unit 13. Silt; medium brown at top and bottom, orange or rust-colored zone above the middle (Munsell color: about 5YR 4/3);

thickness 210 mm. Top of deposit with blackish crust on top of the pitted surface.

Total thickness about 705 mm.

There is a gap of 3-5 cm between the top of the deposit and the limestone ceiling at this and most of the deposits along this passage of the cave. Some of the weathered cross-sectional faces of the Quaternary fill banks have thin white and yellow carbonate deposits forming a surface rind on them. Other areas have thick carbonate crusts, especially over the clay layers.

In the process of excavating the bear bones, we removed overburden from the fill bank in levels corresponding to the units in our measured profile, saving samples to be screenwashed. We sampled matrix for screening from Units 13, 10, 9, 8, 7, and 6. A few more teeth and small bones of the bear were recovered by screenwashing from the same sandy layers (Units 6 and 7) in which the larger bear bones occurred, as well as two types of small vertebrates. Specimens are preserved in the Vertebrate Paleontology collection of the OMNH.

Systematic paleontology

In addition to the vertebrates described below, a single plant fossil, one-half of a carbonate-containing endocarp of *Celtis* sp. (hackberry) was recovered from a screenwashed sample of Units 6 and 7. The age of the specimen is unknown.

Class Reptilia

Order Squamata (Lizards and Snakes)

Family undetermined

Material. Two partial trunk vertebrae (OMNH 73939) from a snake or possibly two different snakes were found by screenwashing the sediments from Unit 6. The specimens are incomplete, waterworn, and preserve insufficient morphological characters for generic identification (Fig. 7).

Discussion. Descriptive terminology for snake vertebrae follows LaDuke (1991) and Holman (2000); classification follows Collins (2006) and the Center for North American Herpetology (2012). In the somewhat more complete, smaller of the two vertebrae (Fig. 7A-D) the neural arch is depressed, the neural spine is thin where preserved at its base, the zygosphene appears to have a concave anterior dorsal profile, the prezygapophyseal and postzygapophyseal articular facets are oval to ovoid, the hemal keel is gladiate to spatulate in ventral view, and the subcentral grooves are shallow and weak. In the much less complete, larger vertebra (Fig. 7E-H) the neural arch is moderately vaulted; the hemal keel is robust and spatulate in ventral view. Subcentral grooves are absent. The phylogenetic appropriateness of these morphological features has not been assessed across the various taxa of snakes. Other morphological characters in both vertebrae are broken or obscured.

The CZ-9 Cave snake vertebrae clearly do not pertain to the families Boidae (Boas), Crotalidae (pitvipers), or Leptotyphlopidae (slender blind snakes). They also lack the hypapophysis of Natricidae (water snakes) and Elapidae (coral snakes) vertebrae. The bones probably represent a member of either the Colubridae (harmless egg-laying snakes) or Dipsadidae (rear-fanged snakes); both families encompass a large diversity of genera and species. The fossils contain no reliable criteria on which to base a familial or generic identification.

Class Mammalia

Order Soricomorpha

Family Soricidae

Blarina Gray (short-tailed shrews)

Blarina species indeterminate

Material. OMNH 73938, right i1 and associated m1-m2 in a small fragment of dentary (Fig. 8A-D); and 73937, partial left dentary with m3 (Fig. 8E-H).

Discussion. The only mammal other than the black bear recovered by screenwashing the stratigraphic units at CZ-9 was a short-tailed shrew, *Blarina* sp. The right lower incisor and the right m1-m2 came from screenwashed concentrate of Units 5 and 6, below and in the

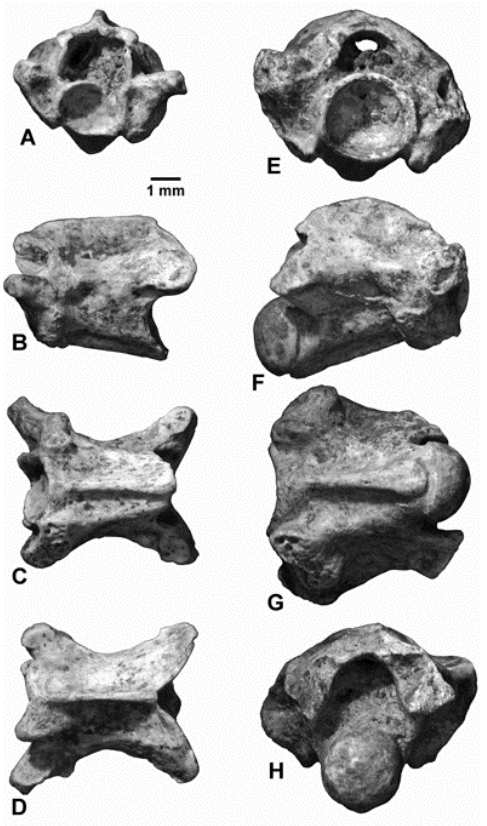

Figure 7. Snake vertebrae (OMNH 73939) from CZ-9 Cave, Cherokee County, Oklahoma. A-D, smaller partial mid-trunk vertebra in (A) anterior, (B) left lateral, (C) ventral, and (D) dorsal views. E-H, larger partial mid-trunk vertebra in (E) anterior, (F) right lateral, (G) ventral, and (H) posterior views.

same unit as some of the bear bones. The dentary was found in screenwashed concentrate from Unit 10, a thick gravel unit above the bear bones. The separate instances and preservation indicate that the material represents at least two individual shrews.

Measurements (in mm) of the shrew specimens as defined by Carraway (1995) are: height of coronoid process, 6.2; length of coronoid-condyloid processes, 5.7; length of i1, 6.2; length of m1, 2.27; width of m1, 1.62; height of pigmented portion of m1, 1.10; height of unpigmented portion of m1, 0.85; length of m2, 1.90; width of m2, 1.35; length of m3, 1.60; width of m3, 0.87. Quantitatively, the CZ-9 shrew specimens are larger than *Blarina carolinensis* (southern short-tailed shrew) and similar in size to *Blarina brevicauda* (northern short-tailed Shrew). However, *Blarina hylophaga* (Elliott's short-tailed shrew) and *B. brevicauda* overlap in size and thus the two cannot be differentiated on this basis.

The falciform lower incisor has two denticulations (terminology of Carraway, 1995). The partial dentary bone includes the ascending ramus and the horizontal ramus forward to the level of the posterior alveolus of m1 (with empty alveoli for m1 and m2) and laterally the posteriormost edge of the i1 alveolus; the angular process is broken off (Fig. 8E, H). The mental foramen is situated below the posterior alveolus of m1. In those features that are preserved in the available specimens, by comparison with the extinct early Pleistocene (Irvingtonian land mammal age) species *Blarina ozarkensis* (as diagnosed by Graham and Semken, 1976; sometimes considered a subspecies of *B. brevicauda*), the CZ-9 Cave shrew has less inflated cingula on the lower molars and a less reduced talonid on m3.

George (2012) critically re-examined morphological characters used by previous authors to distinguish late Pleistocene and recent shrews and searched for apomorphic morphological characters that could be used to differentiate complete or fragmentary fossils of North American shrews, including three of the four *Blarina* extant species recognized on the basis of molecular genetics (the fourth being *Blarina shermani* [Sherman's short-tailed shrew] recently described and based on a geographically restricted population in Florida [Benedict et al. 2006]). George (2012) found several synapomorphies supporting the genus *Blarina* as distinct from other shrew genera and showing that the genus was monophyletic. However, he had difficulty using these characters to distinguish among the three recent species of *Blarina* that he studied. Because of polymorphisms within the genus, late Quaternary fossils of *Blarina* are virtually impossible to assign to a species (George 2012).

We scored the qualitative morphological character states: (1) that are available or unobscured by concreted matrix in the CZ-9

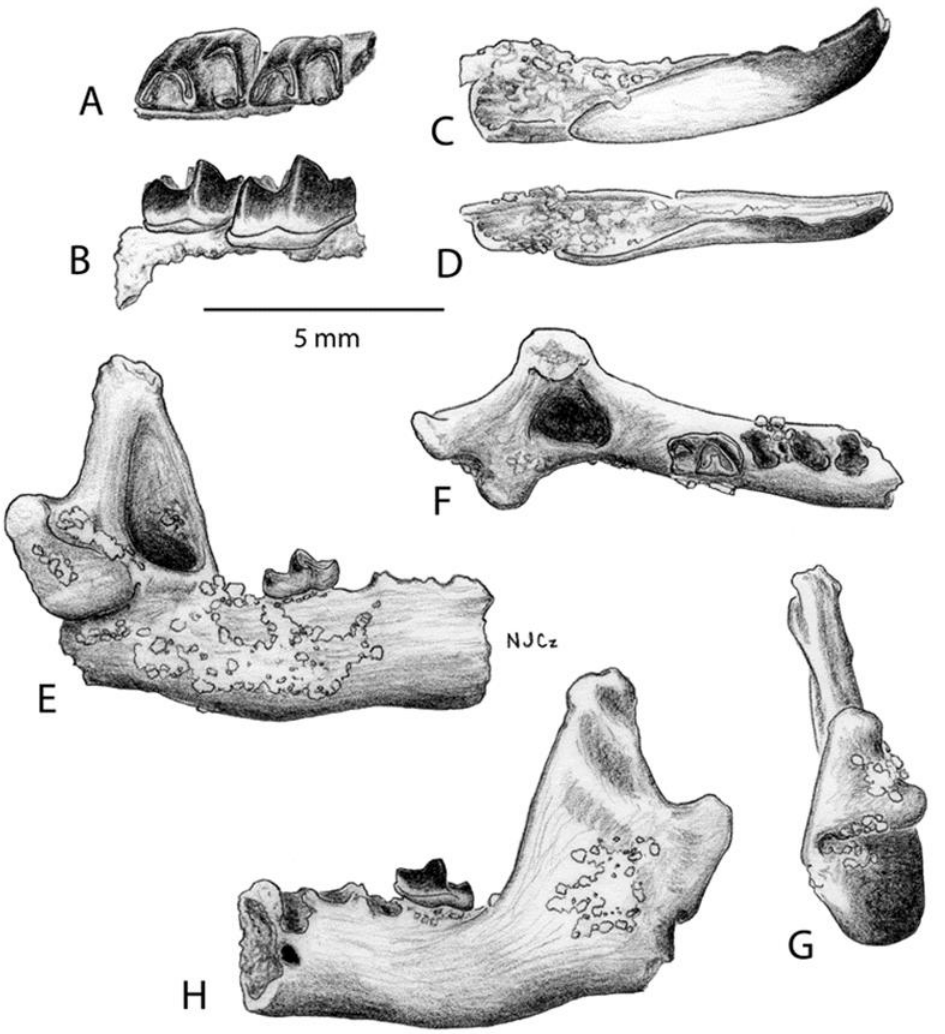

Figure 8. Short-tailed shrew (*Blarina* sp.) fossils recovered from CZ-9 Cave, Cherokee County, Oklahoma. A-B, OMNH 73938, right m1-m2 in small fragment of dentary in A, occlusal view, and B, labial view. C-D, OMNH 73938, right i1 in C, labial view, and D, occlusal view. E-H, OMNH 73937, left dentary with m3 in E, lingual view, F, occlusal view, G, posterior view showing mandibular condyles, and H, labial view.

Cave specimens, and (2) which do not exhibit identical states for all three species of *Blarina* as determined by George (2012). Character states for the CZ-9 specimens (listed here following George's [2012] character and state numbers in parentheses) are: (1:1) teeth pigmentation: heavy; (3:1) interdenticular spaces on i1: shallow; (5:3) posterior extent of i1 in labial view: past metaconid of m1 (as judged on the posterior alveolus rim and m1 alveoli preserved in the dentary OMNH 73937); (7:0) upturning of distal tip of i1: strong; (9:1) talonids of m1 and m2: equivalent in size to trigonids; (12:1) cusps on talonid of m3: two, both hypoconid and entoconid; (13:1) coronoid spicule: strong; (14:1) tip of coronoid process relative to base: wide; and (22:1) size of internal temporal fossa of dentary: medium. These character states occur in all three species *B. brevicauda*, *B. hylophaga*, and *B. carolinensis*, precluding species assignment of the CZ-9 Cave fossils.

Figure 9. Black bear teeth and jaw bones recovered from Cz-9 Cave in labial view. Top row, from left to right: right P3, right C1, right I3, left I3, left C1 (lingual side of this tooth was sampled for AMS radiocarbon date, NZA 37942). Middle row: coronoid process of right dentary, right m3, right m2, right m1, right p4, right c1, right i3, right i2, left i1, left i2, left i3, left c1. Bottom row: horizontal ramus of right dentary (in two pieces), left dentary (in two pieces) with m1-m3.

Order Carnivora

Family Ursidae

Ursus americanus (American black bear)

Material. OMNH 73400 (Figs. 9, 10, 11), partial skeleton including the following elements: five of the upper teeth (left and right I3s, left and right C1s, and right P3), partial right dentary (in 3 pieces, poorly preserved), left i1, left and right i2s, left and right i3s, left and right c1s, right p4, right m1, right m2, right m3, and partial left dentary (in two pieces) with m1-m3, left humerus (missing proximal epiphysis), left ulna (missing distal end), right radius (missing proximal end), partial left radius (3 pieces of the shaft), nearly complete and articulated right manus, left ilium fragment, distal portion of baculum, left femur diaphysis and separate distal epiphysis, patella, left calcaneum, right astragalus and navicular (articulated), left and right cuboids, right ectocuneiform, left metatarsals III, IV, and V, several phalanges, and other fragments.

Discussion. All of the bones belong to one black bear skeleton and were recovered from Units 6 and 7. Based on the presence of a partial baculum, the individual is a male. The skeleton was partly disarticulated. Major limb elements and smaller teeth and bones were scattered, but the bones of one hand were mostly articulated, and several of the bones of one foot were partially articulated. Water transport of the bones before burial and exposure to flowing water during burial appears to have been minimal judging from the condition of the bones, which show little or no wear caused by water-transported sediment. However, the bone is poorly preserved and many elements are broken or soft and friable. The teeth are somewhat better preserved than the bone. Externally the tooth enamel is free of clinging matrix, while internally the dentin is chalky and friable. In places where small amounts of rock matrix had adhered to the enamel, portions of teeth were broken during preparation. The partial left dentary bone contains the m1-m3 but is broken anteriorly ahead of the m1 and posteriorly at the condyloid and angular processes; the dentary depth (in mm) on the labial side of the bone is 35.8 below the m1, 37.0 below the m2, and 39.9 below the m3. Measurements of the individual teeth are provided in Table 1. Widths of m1, m2, m3, and length of m3 of the CZ-9 Cave black bear are larger or near the large end of the observed range for modern black bears from Newfoundland, Alaska, New York, and California (Miller et al. 2009). However, compared to another study (Wolverton and Lyman 1998), length and width of the m3

Table 1. Measurements (in mm) of the teeth of a Pleistocene American black bear, OMNH 73400, from CZ-9 Cave, Cherokee County, Oklahoma.

Tooth	Anteroposterior diameter			er
	Left	Right	Left	Right
I3	12.0	11.8	8.7	7.9
C1	18.7	19.3	13.6	13.5
P3	---	5.0	---	4.0
i1	5.3	---	3.3	---
i2	7.0	7.3	4.7	5.1
i3	6.8	6.9	7.2	7.4
c1	19.1	18.8	12.8	12.8
p4	---	9.3	---	5.9
m1	21.0	20.8	9.6	9.7
m2	21.6	22.1	13.7	13.6
m3	15.4	15.9	12.6	12.5
m1-m3	57.1			

from CZ-9 Cave are somewhat above the averages but well within the observed ranges of these measurements for the same tooth in recent black bears from across the United States. In bears, tooth size is not strongly correlated with body size. The size of the CZ-9 black bear is not unusual.

Ontogenetic age of the bear. The skeleton represents a young adult based on the condition of the teeth and bones. All of the permanent teeth were in place, with no evidence of deciduous teeth, which black bears usually lose before reaching 1 year of age. The amount of wear on the canines and incisors indicates an ontogenetic age of at least 1-2 years (Heffelfinger 1997). The I3s are slightly worn at their apices; all lower incisors are worn on their apices. For all four canines, the permanent teeth are fully erupted and the pulp cavities of the canine roots are completely closed. The tips of the main cusps are unworn, but there are small contact wear facets where the upper and lower canines pass by one another (on the posterior surfaces of the lowers and on the anterior surfaces of the uppers). This interdental contact with the upper canine wore through the enamel to expose a small area of dentin on the posterior surface of the right lower canine, but not on the left. The P3 and p4 are not worn; the lower molars show small areas of exposed dentin especially on the more prominent cusps. In the available molars, there is sufficient wear on the enamel of the higher ridges and cusps on the m1s and m2s to expose very small areas of dentin, but the m3s show no exposed dentin. Among the postcranial bones, several long bones have partly fused or unfused epiphyses. These sutures were differentially weathered during burial, possibly enhancing their apparentness. In the humerus, the proximal epiphysis was unfused and is missing; the distal epiphysis is incompletely fused to the diaphysis. The right radius distal epiphysis is incompletely fused. All epiphyses of the left femur were completely unfused; the proximal ones (head and greater trochanter) are missing but the separate distal epiphysis was recovered. In the left calcaneum, the epiphysis on the tuber calcanei is incompletely fused. All of the epiphyses of the metapodials and phalanges of the manus and recovered bones of the pes are partly fused. In the right manus the distal epiphyseal line is indistinct on metacarpals I, II, and III; it is somewhat more

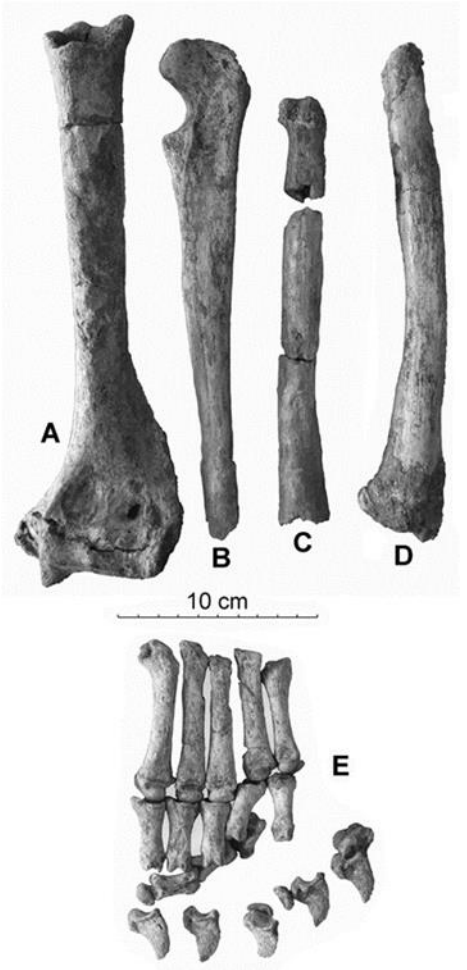

Figure 10. Black bear forelimb elements recovered from Cz-9 Cave. A, left humerus (anterior view); B, left ulna (medial view); C, left radius (anterior view); D, right radius (anterior view); and E, articulated manus, with bones shown in their relative positions as found (metacarpals and first phalanges in anterior view, ungual phalanges in lateral view, second phalanges and sesamoids somewhat scattered).

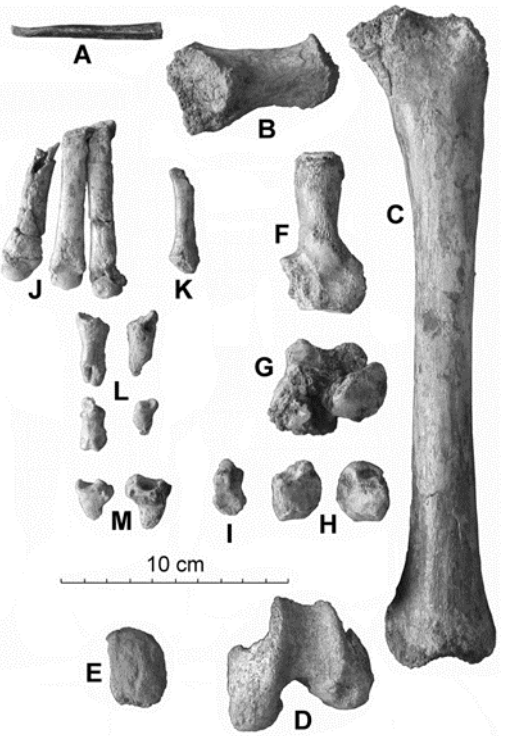

Figure 11. Black bear pelvis, baculum, and hind limb bones from Cz-9 Cave. A, distal portion of baculum (lateral view); B, left ilium (lateral view); C, left femur diaphysis (anterior view); D, left femur distal epiphysis (distal view); E, patella (anterior view); F, left calcaneum (proximal view); G, semi-articulated and partly concreted right astragalus and navicular (proximal view); H, left and right cuboids; I, right ectocuneiform; J, left metatarsals III, IV, and V (dorsal view); K, first(?) metatarsal or metacarpal (dorsal view); L, first and second phalanges (dorsal view); M, ungual phalanges (lateral views).

obvious on metacarpal IV and is quite distinct on metacarpal V. According to Marks and Erickson (1966), metacarpal fusion is complete in male black bears by age 1-2 years, but fusion of the radial epiphyses is not complete until 6-8 years. Marks and Erickson (1966) also note that the length of the baculum in male bears is correlated with age. However, the baculum from the CZ-9 Cave black bear is broken proximally and only 67 mm of the distal portion of the bone is preserved. Given this evidence, the CZ-9 Cave bear was probably between 1 and 2 years old when it died.

Discussion

Because bears often den in protected places such as caves, and because caves sometimes provide an environment favorable for burial and fossilization, the finding of a young black bear in CZ-9 Cave is not unexpected. Nevertheless, such records are not common in Oklahoma and the CZ-9 specimen serves as voucher for the occurrence of the species in

Oklahoma prior to their recent historical extermination and re-entry. Lee and Vaughan (2003) noted that in Virginia, 1- and 2-year-old male black bears were the sex and age-class likely to disperse; subadult females did not disperse in their study. Suitable dens are often reused by black bears (Alt 1984).

Black bears' genetics based on recent populations suggest a historical pattern in North America showing a long regional isolation that separated western from eastern forms beginning in the early Pleistocene, followed by hybridization upon re-contact after the last deglaciation (Wooding and Ward 1997). The isolation in part was probably related to the glacial separation of western from eastern forests that would have been occupied by the bears. The occurrence of a black bear in CZ-9 Cave at 10,958 ± 35 rybp (= 12,959 to 12,654 ybp calibrated date with 95% confidence interval), during the last deglaciation, suggests the species' continued presence in a late Pleistocene forest refugium in the Ozark Highland during the last glacial period, or else indicates a rapid recolonization of the Ozarks from forests farther east or north.

Numerous records of short-tailed shrews exist from the late Pleistocene (late Wisconsinan glacial period) through the Holocene of North America (Graham and Lundelius 2010) as well as earlier fossil species in the Pliocene and early Pleistocene (Hibbard 1957, Repenning 1967). Although historically most Quaternary fossils of short-tailed shrews have been identified in the literature as *Blarina brevicauda*, George (2012) showed that phylogenetically important characters do not support these species identifications. Because of the difficulty of identifying scrappy remains of recently-differentiated and closely-related species of shrews and other small mammals, most of these Pleistocene remains are likely identifiable to genus *Blarina* only.

In recent historic times, the species now known as Elliot's short-tailed shrew, *B. hylophaga*, is the species of *Blarina* occupying northeastern Oklahoma (Brant and Orti 2002) as well as the eastern half of Oklahoma (Caire et al. 1989) and parts of adjacent states. The small-bodied species *B. carolinensis*, the southern short-tailed shrew, today occurs in the extreme southeastern corner of Oklahoma, as well as adjoining parts of Texas, Arkansas, and eastward. Northern short-tailed shrews, *B. brevicauda*, occur today from south-central Canada to the mid-latitudes of the eastern United States (Wilson and Ruff 1999). This large-bodied species today is found north of the Platte River, Nebraska, and in eastern Missouri (Brant and Orti 2002, Reid 2006). As noted above, Sherman's short-tailed shrew, *B. shermani*, occurs only in a small area of Florida.

Like the CZ-9 shrews, some Pleistocene fossil shrews were larger than modern ones of the same geographic areas (e.g., Schubert 2003). This phenomenon caused Hibbard (1943) initially to nominate a species *Blarina fossilis* but later to relegate this extinct taxon to a subspecies *B. brevicauda fossilis* (Hibbard 1957). Rapid changes in climate, like that of the last deglaciation between about 12,000 and 10,000 rybp, can cause rapid shifts in the geographic ranges and contribute to the speciation and extinction of species (e.g., Blois and Hadly 2009, Chen et al. 2011, Schloss et al. 2012, Semken et al. 2010). Short-tailed shrew body size probably changed dynamically across eastern North America as climate, environmental conditions, and geographic ranges of shrew populations changed during the Pliocene, Pleistocene and Holocene, ultimately resulting in the differentiation of the modern named forms. Because the fossils from CZ-9 Cave are of large body size and are probably near the same geologic age as the black bear fossils (late Pleistocene), they provide evidence of this change in the species and body size of *Blarina* inhabiting the Oklahoma Ozarks since the time of deposition of the CZ-9 Cave sediments and fossils, even if they cannot be assigned to a species.

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Macroinvertebrate Community Structure and Physicochemical Conditions of Three Southeastern Oklahoma Springs

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Abstract: Pontotoc Ridge Nature Preserve (PRNP) is located in southeastern Pontotoc County, Oklahoma. This area consists of 2,900 acres of assorted vegetation with several springs, all of which emerge from the Arbuckle-Simpson Aquifer. Three springs, two located within the Nature Preserve and one on adjacent property, were surveyed during this study. Aquatic macroinvertebrates and physicochemical data were collected on a seasonal basis, beginning January 2011 and ending January 2012. With the exception of 16 dissolved oxygen readings and six orthophosphate readings, the physicochemical data meet standards that support and allow for aquatic life. A total of 127,048 individuals, representing 114 taxa, were collected throughout the course of this study. Smith Spring was the most diverse with an average of 44.8 taxa, followed by Canyon Spring with an average of 32.4 taxa, and Cave Spring with an average of only 21 taxa. Canyon Spring was the most populated (84,339 individuals), followed by Smith Spring (38,837 individuals), and Cave Spring (3,873 individuals). The April 2011 collection contained both the largest number of individuals, 34,368, as well as the highest number of taxa, 74, found. Sorenson's similarity indices for combined collections between springs were similar, with the average indices above 0.425. Similarity indices for comparisons between upper and lower collection sites were lower, with average indices no greater than 0.349. Shannon's species diversity values were generally under 2.0, with a few exceptions in Cave Spring and Smith Spring, having averages no greater than 1.785. The results of this investigation indicate these springs are in nearly pristine condition and they play an important role in the Pontotoc Ridge ecosystem.

Introduction

Springs are described as naturally occurring sources of emerging groundwater that have unique properties unto themselves, such as discrete habitats with relatively constant conditions (van der Kamp 1995). Although they are limited in terms of their dimensions and do not have homogeneous environments (Cantonati et al. 2006), they have been described as having mosaic structures that have the potential to support numerous microhabitats (Springer and Stevens 2008). Due to this and the relative consistency of groundwater conditions, springs often support a very dense and diverse fauna (Lock and Williams 1981).

Although springs display unique and interesting ecosystems, studies of macroinvertebrate communities in these environments have been scarcely conducted in the United States and even fewer have taken place in Oklahoma. An investigation by Matthews et al. (1983) examined whether macroinvertebrate compositions could be useful indicators of groundwater quality, but this proved unsuccessful, as similarity values between the springs were very low. Varza and Covich (1995) concluded macroinvertebrate abundance varied in Buckhorn Spring, most likely due to limited food availability and predation by crayfish. Bass (2000) reported 39 taxa of macroinvertebrates from a one-time sampling effort in two springs

of the Pontotoc Ridge Nature Preserve (PRNP) during 1995. Gaskin and Bass (2000) sampled seven springs across the state to establish any potentially occurring patterns and make inter-spring faunal comparisons. Their data suggested that an exclusive spring fauna was not present and the organisms were directly correlated to microhabitat availability. A macroinvertebrate community structure study conducted by Rudisill and Bass (2005) in Roman Nose State Park springs yielded 21,268 individuals, representing 64 taxa, with most individuals belonging to the order Diptera.

The PRNP is located in southeastern Pontotoc County, Oklahoma (Figure 1). It consists of 2,900 acres of assorted cross-timber and prairie vegetation, and limestone outcrops are quite common. Several springs and seeps are also located in and near the preserve, each draining from the Arbuckle-Simpson Aquifer (J. A. Tucker, pers. comm.). Because a number of years had passed since Bass (2000) initially investigated some of these springs, an extensive survey that included additional springs was requested by the PRNP to compare the macroinvertebrate community composition and determine the overall environmental condition of its springs.

This current investigation involves the two springs previously studied by Bass (2000) and a nearby spring located on a privately owned parcel of land adjacent to and immediately north of PRNP. Almost 16 years had passed since the initial survey was conducted in 1995 (Bass 2000), so a year-long study was done to gather additional information. Purposes of this investigation were to 1) determine the macroinvertebrate community composition of the springs, 2) compare macroinvertebrate community composition between the springs, 3) compare composition these communities to previously collected data on these and other springs, and 4) determine the water quality of the Pontotoc Ridge springs.

Methods

Aquatic macroinvertebrates were collected and physicochemical conditions were measured on a seasonal basis from January 2011 to January 2012 (January 2011, April 2011, July 2011, October 2011, and January 20112). The three study springs, Cave Spring, Smith Spring, and Canyon Spring, are all classified as rheocrene springs; flowing springs that emerge into one or more stream channels (Springer and Stevens 2008). Cave Spring is also classified as a true cave spring, which is a spring whose emergence comes from entirely

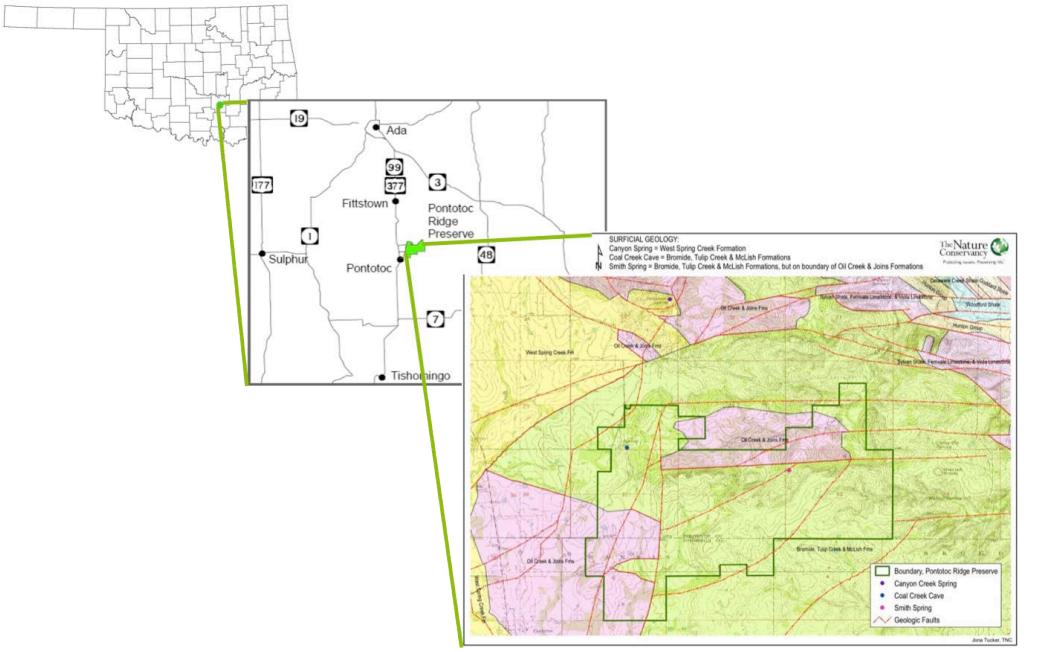

Figure 1. Pontotoc Ridge Nature Preserve, OK.

within a cave environment and is not directly connected to surface flow (Springer and Stevens 2008).

Two sampling sites (head and downstream) were established within each spring, where three Surber net samples were collected from each. Qualitative samples were also collected, using dip nets, to capture taxa potentially missed by the Surber net. All samples were washed through a number 60 (0.250mm) U.S. standard sieve bucket, and preserved with a 10% mixture of formalin and Rose Bengal dye. The preserved macroinvertebrates were returned to the laboratory to be sorted, identified, and counted. Identification of macroinvertebrates was determined using keys by Merritt et al. (2008), Pennak (1989), Epler (1995), and Wiederholm (1983).

Sorenson's index (Chao et al. 2006) of similarity was used to make comparisons between each collection period, combined collections for each spring, and comparisons made between the previous study conducted by Bass (2000) on Cave Spring and Smith Spring to the current study. Shannon's diversity index (Gotelli and Ellison 2004) was calculated for lower collection sites, upper collections sites, and combined collection sites for each spring for each collection period. Multi-Response Permutation Procedure (MRPP) was used to compare the species composition between the head and downstream areas and species composition between each spring. Calculations were conducted using the program 'R' (R Core Team 2013). Rarefaction curves were also generated to evaluate species richness between the head and downstream as well as richness seen between each spring (Gotelli & Entsminger 2000).

Physicochemical conditions such as temperature, dissolved oxygen, and pH were measured at both collecting sites within the spring, while alkalinity was measured only at the head. A water sample collected from the head of each spring was used to determine turbidity, conductivity, and concentrations of ammonia, nitrites, nitrates, and orthophosphates (Hach 2005) in the laboratory. Results from the analysis of physicochemical conditions in the water would indicate if its quality would be sufficient to support a diverse biota.

Results

Macroinvertebrates

A total of 127,048 individuals, representing 114 taxa, were collected over the course of the study (Table 1). Non-hexapods were the most abundant macroinvertebrate group collected with a total of 87,675 individuals, represented by 21 taxa (19.13%) and comprising 69% of macroinvertebrates collected. The amphipod, *Hyalella azteca* complex, was the most numerous non-hexapod with a total of 76,529 individuals, representing 60.24% of all individuals. Although non-hexapods represented 69% of all individuals, hexapods were represented by 93 taxa, which was 80.86% of taxa diversity.

The highest hexapod diversity was seen within the dipterans, comprised of 44 taxa (47.31% of hexapods) and having a total of 31,518 individuals, representing 80.05% of hexapods and 24.81% of all macroinvertebrates collected throughout the course of the study.

The highest number of individuals collected was from Canyon Spring, with a total of 84,399, representing 66.38% of all individuals collected, and a total of 38 taxa were identified. Canyon Spring was dominated by the amphipod *Hyalella azteca* complex with a total of 76,515 individuals collected over the entire study. Only 5,695 individuals were hexapods, the most abundant being trichopterans, with an individual count of 3,168, followed by dipterans, having a total of 2,178 individuals.

Smith Spring had a total of 38,837 individuals, representing 30.57% of all individuals collected throughout the course of the study. This spring was dominated by hexapods, with a total of 32,144 individuals, and was also the most species rich of the three springs with a total of 92 taxa collected. Dipterans were the most numerous group, with a total of 31,518 individuals and 37 taxa. The remaining hexapod groups comprised only 11.94% of individuals. Oligochaetes were the most numerous non-hexapod group collected with a total of 2,413 individuals, followed by nematodes and platyhelminths, each comprising around 20%

Table 1. Percent composition of total individuals for each taxon found in qualitative collections.

Taxon	Cave		Smith		Canyon		All	
	No.	Percent	No.	Percent	No.	Percent	No.	Percent
Turbellaria								
Dugesia sp.	26	0.671	1432	3.687	1718	2.037	3176	2.500
Nematoda								
Unknown Nematoda	136	3.511	1387	3.571	45	0.053	1568	1.234
Oligochaeta								
Lumbriculus sp.	13	0.336	265	0.682	83	0.098	361	0.284
Limnodrilus sp.	179	4.622	2148	5.531	235	0.279	2562	2.017
Gastropoda								
Gyraulus sp.	0	0	0	0	0	0	0	0
Physa sp.	99	2.556	9	0.023	0	0.0000	108	0.085
Pelecypoda								
Unknown Pelecypoda	1	0.026	639	1.645	11	0.013	651	0.512
Sphaeriidae	0	0	25	0.064	4	0.005	29	0.023
Sphaerium sp.	0	0	114	0.294	20	0.024	134	0.105
Copepoda								
Cyclopoida	81	2.091	572	1.473	3	0.004	656	0.516
Ergasilus sp.	0	0	1	0.003	0	0	1	0.001
Harpacticoidia	1780	45.959	74	0.191	0	0	1854	1.459
Isopoda								
Unknown Isopoda	0	0	2	0.005	0	0.000	2	0.002
Caecidotea sp.	2	0.052	13	0.033	10	0.012	25	0.020
Amphipoda								
Hyallela azteca	5	0.129	9	0.023	76515	90.726	76529	60.236
Decopoda								
Unknown Decapoda	7	0.181	0	0	0	0	7	0
Astacidae	1	0.026	0	0	0	0	1	0
Cambaridae	0	0	1	0.003	0	0.000	1	0.001
Cambarinae	8	0.207	0	0	0	0	8	0
Acarina								
Sperchonpsis verrucosa	0	0	1	0.003	0	0.000	1	0.001
Collembola								
Corynothrix sp.	0	0	14	0.036	0	0.000	14	0.011
Isotomidae	0	0	2	0.005	0	0.000	2	0.002
Spinactalets sp.	0	0	8	0.021	0	0.000	8	0.006

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Ephemeroptera								
Unknown Ephemeroptera	1	0.026	277	0.713	2	0.002	280	0.220
Baetidae	0	0	0	0	3	0	3	0
Baetis sp.	8	0.207	272	0.700	30	0.036	310	0.244
Stenonema femoratum	0	0	0	0	1	0	1	0
Leptohyphidae	0	0	553	1.424	86	0.102	639	0.503
Tricorythodes sp.	0	0	10	0.026	28	0.033	38	0.030
Paraleptophlebia sp.	21	0.542	0	0	0	0	21	0
Odonata								
Unknown Odonata	0	0	90	0.232	13	0.015	103	0.081
Anisoptera	0	0	1	0.003	0	0.000	1	0.001
Aeshnidae								
Tricanthagyna sp.	0	0	0	0	0	0	0	0
Cordulegastridae								
Cordulegaster sp.	0	0	1	0.003	0	0.000	1	0.001
Neurocordulia sp.	0	0	0	0	2	0	2	0
Zygoptera	0	0	127	0.327	0	0.000	127	0.100
Calopteryx sp.	1	0.026	3	0.008	0	0.000	4	0.003
Hetaerina sp.	0	0	4	0.010	2	0.002	6	0.005
Coenagrionidae	0	0	2	0.005	0	0.000	2	0.002
Argia sp.	0	0	743	1.913	176	0.209	919	0.723
Archilestes sp.	0	0	1	0.003	0	0.000	1	0.001
Lestes sp.	0	0	6	0.015	0	0.000	6	0.005
Plecoptera								
Pteronarcella sp.	2	0.052	0	0	0	0	2	0
Pteronarcys sp.	1	0.026	0	0	0	0	1	0
Hemiptera								
Corixidae	0	0	1	0.003	0	0.000	1	0.001
Glaenocorisa sp.	0	0	1	0.003	0	0.000	1	0.001
Graptocorixa sp.	0	0	1	0.003	0	0.000	1	0.001
Trepobates sp.	0	0	3	0.008	0	0.000	3	0.002
Rhagovelia sp.	0	0	0	0	1	0	1	0
Tricoptera								
Helicopsyche sp.	2	0.052	701	1.805	3074	3.645	3777	2.973
Hydropsychidae	53	1.368	343	0.883	50	0.059	446	0.351
Hydroptila sp.	0	0	74	0.191	0	0	74	0.058
Ochrotrichia sp.	374	9.657	127	0.327	40	0.047	541	0.426
Nectopsyche sp.	0	0	0	0	3	0	3	0

Polycentropes sp.	1	0.026	4	0.010	0	0.000	5	0.004
Dolophilodes sp.	0	0	0	0	1	0	1	0
Coleoptera								
Dytiscidae	12	0.310	0	0	1	0	13	0
Agabates sp.	0	0	1	0.003	1	0.001	2	0.002
Agabus sp.	7	0.181	7	0.018	0	0.000	14	0.011
Hydrotrupes sp.	0	0	1	0.003	0	0.000	1	0.001
Hygrotus sp.	18	0.465	4	0.010	0	0.000	22	0.017
Laccophilinae	0	0	1	0.003	0	0.000	1	0.001
Helicus sp.	0	0	0	0	0	0	0	0
Ordobrevia sp.	0	0	438	1.128	3	0.004	441	0.347
Peltodytes sp.	0	0	14	0.036	0	0	14	0.011
Tropisternus sp.	0	0	3	0.008	0	0	3	0.002
Diptera								
Atherix sp.	0	0	15	0.039	3	0.004	18	0.014
Empidiae	0	0	6	0.015	0	0.000	6	0.005
Hemerodromia sp.	0	0	70	0.180	0	0.000	70	0.055
Caloparyphus sp.	0	0	3	0.008	18	0.021	21	0.017
Euparyphus sp.	0	0	0	0	9	0	9	0
Myxosargus sp.	0	0	2	0.005	0	0.000	2	0.002
Silvus sp.	0	0	0	0	1	0	1	0
Chrysops sp.	0	0	2	0.005	0	0.000	2	0.002
Tabanus sp.	0	0	0	0	2	0	2	0
Culicoides sp.	4	0.103	168	0.433	189	0.224	361	0.284
Probezzia sp.	17	0.439	272	0.700	4	0.005	293	0.231
Dasyhelea sp.	0	0	1	0.003	0	0.000	1	0.001
Eucorethra sp.	0	0	1	0.003	0	0.000	1	0.001
Ablabesmyia sp.	1	0.026	0	0	0	0	1	0
Cardiocladius sp.	0	0	0	0	1	0	1	0
Chironomus sp.	23	0.594	3	0.008	0	0.000	26	0.020
Corynoneura sp.	695	17.945	532	1.370	504	0.598	1731	1.362
Cricotopus sp.	2	0.052	3	0.008	0	0.000	5	0.004
Cryptochironomus sp.	0	0	46	0.118	19	0.023	65	0.051
Dicrotendipes sp.	74	1.911	11	0.028	30	0.036	115	0.091
Einfeldia sp.	4	0.103	4	0.010	0	0.000	8	0.006
Eukiefferiella sp.	0	0	3	0.008	95	0.113	98	0.077
Heleniella sp.	0	0	215	0.554	0	0.000	215	0.169
Larsia sp.	61	1.575	498	1.282	12	0.014	571	0.449

Microtendipes sp.	47	1.214	6	0.015	0	0	53	0.042
Orthocladius sp.	9	0.232	101	0.260	349	0.414	459	0.361
Parametriocnemus sp.	26	0.671	1335	3.437	76	0.09	1437	1.131
Paraspectra sp.	8	0.207	0	0	0	0	8	0
Paratanytarsus sp.	5	0.129	58	0.149	8	0.009	71	0.056
Paratendipes sp.	0	0	279	0.718	4	0.005	283	0.223
Paratrichocladius sp.	0	0	2	0.005	88	0.104	90	0.071
Phaenopsectra sp.	28	0.723	91	0.234	0	0.000	119	0.094
Polypedilum sp.	0	0	12	0.031	2	0.002	14	0.011
Procladius sp.	0	0	4	0.010	0	0.000	4	0.003
Rheotanytarsus sp.	0	0	5	0.013	28	0.033	33	0.026
Stenochironomus sp.	0	0	21	0.054	0	0.000	21	0.017
Sublettea sp.	0	0	0	0	1	0	1	0
Tanytarsus sp.	25	0.645	24496	63.074	21	0.025	24542	19.317
Thienemannimyia sp.	0	0	13	0.033	6	0.007	19	0.015
Tvetenia sp.	5	0.129	1	0.003	702	0.832	708	0.557
Dixa sp.	0	0	4	0.010	6	0.007	10	0.008
Pericoma sp.	0	0	7	0.018	0	0.000	7	0.006
Protoplasa fitchii	0	0	3	0.008	0	0.000	3	0.002
Tipula sp.	0	0	13	0.033	0	0.000	13	0.010
Totals	3873		38836		84339		127048	

of the non-hexapods collected within Smith Spring. Pelecypods and copepods together comprised 21.31% of individuals, the remaining groups (amphipods, decapods, gastropods, isopods, and acarinids) comprised <1% of individuals collected from Smith Spring.

Cave Spring had both the lowest number of individuals and the fewest taxa of the springs investigated, with only 3,873 individuals and 44 taxa. Non-hexapods were the most abundant group seen with a total of 2,338 individuals, but in terms of diversity, the hexapods were more prominent, having a total of 31 taxa collected. Copepods were the most abundant non-hexapod collected in this spring over the course of the study, with a total of 1,861 individuals, followed by the oligochaetes having a total of 192 individuals. Of the hexapod groups collected, the dipterans were the most diverse group with a total of 16 taxa, and 1,034 individuals, collected. Trichopterans were represented by four taxa and 430 individuals and coleopterans were represented by four taxa and 37 individuals. The remaining hexapod groups (Ephemeroptera, Odonata, and Plecoptera) constituted less than 3% of the remaining individuals collected from Cave Spring.

The April 2011 collection yielded the most individuals, 34,368, as well as the highest diversity. October 2011 was the next most abundant and species rich with a total of 28,299 individuals and 65 taxa, followed by July 2011 (27,509 individuals and 63 taxa), January 2012 (20,814 individuals and 62 taxa), and January 2011 (16,059 individuals and 33 taxa).Cave Spring had the lowest species richness seen throughout the study, while Smith Spring had the highest species richness. The species richness values seen in Cave and Smith Springs were higher, with two exceptions, in the upper collection sites; this is dif-

ferent than species richness recorded in Canyon Spring, which had higher values in the lower collection sites.

The highest species similarity observed (0.822) was in Canyon Spring between the months of April and July 2011 whereas the lowest species similarity value (0.125) was recorded in Cave Spring between the months of January and April 2011. The species similarity for Cave Spring between was highly impacted by drought during much of the year in 2011. Similarity indices between springs showed that Cave Spring and Canyon Spring had the lowest similarity (0.105), seen in the lower collection site, during January 2011. Smith Spring and Canyon Spring had the highest similarity (0.615), observed in the lower collection site, during July 2011.

Species diversity in each spring was calculated for the lower, upper, and combined collection sites. Diversity values ranged from a high of 2.671, in Cave Spring during April 2011, to a low of 0.101 in Canyon Spring during January 2011. Canyon Spring had the lowest overall means, never averaging above 1.00. Cave Spring had the highest overall means, averaging 1.785, while Smith Spring had overall means of 1.571.

A significant difference in community composition was observed between the head and downstream regions (mrpp, p=0.005) as well as between Cave Spring, Canyon Spring, and Smith Spring (mrpp, p=0.001).

Comparisons of taxa similarity were made between Cave Spring and Smith Spring from the 1995 (Bass 2000) survey to Cave Spring and Smith Spring from the current survey. Cave Spring had a similarity value of 0.286 and Smith Spring had a similarity value of 0.333 between the two investigations.

Physiochemical Conditions

The overall water quality of each spring system, with a few exceptions, falls well within the standards that support and allow for aquatic life (Table 2). Water temperatures were fairly constant for two of the three springs (Smith Spring and Canyon Spring) sampled in the study, ranging from 17.2C to 20.4C. Cave Spring's temperature measurements varied more, with readings from 13.5C to 19.5C.

The dissolved oxygen concentration ranged from a minimum of 1.4 mg/L (Canyon Spring in October 2011) to a maximum of 7.6 mg/L (Cave Spring in January 2012). D.O. concentrations were, with one exception, higher at the downstream sites. The percent dissolved oxygen saturation ranged from 26% at Cave Spring (upstream) during April 2011 to 75% at Cave Spring (downstream) during January 2012.

Free carbon dioxide values varied throughout the springs, ranging from 0.0 mg/L to 38mg/L (Table 2). The pH varied minimally throughout the collection period, with a range of 7.1 (Canyon Spring in April 2011) to 8.0 (Cave Spring in January 2012). Values were typically lower at the head and higher downstream, with three exceptions. Alkalinity values ranged from 248 mg/L (Smith Spring in January 2012) to 334 mg/L (Canyon Spring in July and October 2011).

Turbidity readings varied little. All 13 readings were <0.02 JTU, with four of the 13 readings recorded as zero JTU (Appendix 1G). The lowest readings were seen in Cave Spring, during January and April 2011, while Canyon Spring had readings almost always near 100%T, a turbidity value of zero. Conductivity readings varied greatly, ranging from 328 umhos/cm (Smith Spring in January 2012) to 906 umhos/cm (Canyon Spring in July 2011).

Nutrient values were generally low. Ammonia readings varied little, ranging from 0.093 mg/L (Canyon Spring in January 2011) to 0.177mg/L (Smith Spring in January 2012). Nitrate readings ranged from a low of 0.252mg/L (Cave Spring in January 2011) to a high of 0.779mg/L (Smith Spring in January 2012), with one sample being recorded as under measuring range. Orthophosphates varied greatly throughout the collection period. Of the thirteen readings, four were under measuring range and three were recorded as negative numbers. Of the remaining six values, Cave Spring had the lowest value at 0.109 mg/L during January 2011 and Smith Spring had the

Table 2. Physiochemical ranges of Cave Spring, Smith Spring, and Canyon Spring January 2011 - January 2012.

Site	Cave Spring		Smith Spring		Canyon Spring	
	Upper	Lower	Upper	Lower	Upper	Lower
Water Temperature (°C)	16.4-17.5	4.6-7.6	17.3-17.9	17.2-20.4	18.6-19.3	18.3-19.9
Dissolved Oxygen (DO) (mg/l)	2.6-6.5	47-75	2.7-6.0	3.8-6.3	1.4-1.6	4.5-4.9
Percent DO Saturation	26-67	7.3-8.0	28-62	41-64	14-17	45-57
pH	7.3-7.7		7.3-7.9	7.3-7.7	7.1-7.5	7.4-7.6
Free Carbon Dioxide (mg/l)	<10-37		<10-29		16-38	
Alkalinity (mg/l)	289-330		248-300		309-334	
Turbidity (JTU)	<0.02		<0.02		<0.02	
Conductivity (µmhos/cm)	400-539		328-455		568-906	
Ammonia (mg/l)	0.097-0.161		0.094-0.177		0.093-0.168	
Nitrites (mg/l)	UMR		UMR		UMR	
Nitrates (mg/l)	0.252-0.747		0.280-0.779		0.364-0.558	
Orthophosphates (mg/l)	<0.110		<0.205		<0.165	

highest value of 0.204 mg/L during October 2011.

Discussion

Throughout the course of the study, a total of 127,048 individuals, representing 114 taxa were collected (Table 1). One macroinvertebrate in particular was dominant throughout the study, the amphipod, *Hyalella azteca* complex. This taxon represented 60.24% of all individuals collected during the study. The non-hexapods were the most numerous macroinvertebrates found, comprising 69% of individuals collected, whereas the hexapods represented 31% of individuals collected. The hexapods were more numerous and diverse in terms of taxa, with a total of 93, most of which are represented by members of the order Diptera. Similar findings are seen in other studies that investigate spring macroinvertebrate community composition (Rudisill and Bass 2005; Ilmonen *et al.* 2009; Gaskin and Bass 2000; Bass 2000).

Cave Spring was the least species rich of the three springs, having an overall average of 21 taxa found throughout the study. This lower number is partly due to the drought that caused Cave Spring to cease flow and desiccate during much of the study period. In addition, the substrate of Cave Spring contained fewer microhabitats existing within the spring. Canyon Spring had the next highest species richness value with an overall average of 32.4 taxa, while Smith Spring had the highest overall species richness with an overall overage of 44.8 taxa. Although Smith Spring and Canyon Spring are fairly similar, the landscape immediately surrounding each spring, as well as the chemical composition of the water, may have contributed to the species richness values seen throughout the study.

Temperature, resource and microhabitat availability, low dissolved oxygen levels, and high phosphates levels influenced overall species richness, diversity, and various similarity values calculated. Temperature effects can be clearly seen in Cave Spring, which desiccated during the months of July and October 2011. Although a negative effect of temperature was seen in Cave Spring due to temperature, a positive relationship between temperature and vegetation was seen in Canyon Spring and

Smith Spring. This growth of surrounding terrestrial vegetation during the spring months allowed for more resources and microhabitats, and increased macroinvertebrate densities. There was also a slight rise in the number of individuals and taxa during October 2011; this is thought to be due to the introduction of decomposing vegetation, allowing for a variety of new, or different, microhabitats and food resources to exist within the springs. The success of *Hyalella azteca* complex may be in part due to their ability to survive in environments with such low dissolved oxygen levels (Nebeker et al. 1992), such as those recorded in Canyon Spring.

Comparisons between the survey conducted by Bass (2000) and the current survey indicate a fairly high similarity value, 0.419, between the lower collections sites of Smith Spring. This would suggest that even after such a long time span between collections, the spring habitat and surrounding area has undergone little change, allowing the macroinvertebrate communities to also remain somewhat stable over time. However, a much lower similarity value, 0.286, was found between the collections taken from Cave Spring. This spring was dry for several months, including two of the collection periods during the present investigation. According to J. Tucker (pers. comm.), this happens quite often throughout the year. This reoccurring desiccation of Cave Spring may influence the macroinvertebrate fauna community structure, and over a 16-year period this pattern may have had a large impact. The similarity index of 0.333 between Smith Spring surveys is only slightly higher. A 16-year period between surveys is a large amount of time to pass and other factors will have also had an influence on the macroinvertebrate compositions. Variations in similarity observed within and between each spring community throughout the current study may be primarily attributed to life cycle patterns as well as vegetation within and around each spring that provide various microhabitats and food resources.

Conclusions

The Pontotoc Ridge Nature Preserve is a very important ecosystem in southern Oklahoma. It serves both as a site for various types of research and as an educational resource to the public. The continual study of spring systems within Oklahoma is vital and the springs found within and around the Pontotoc Ridge Nature Preserve are considered as nearly pristine, based on the findings of this investigation. To keep the springs in this area and other areas throughout the state in this nearly pristine condition continued research is important. This research allows for identification and inventory of the macroinvertebrate community and water quality analysis indicating potential groundwater pollution.

Acknowledgements

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Records for Fox Squirrels in the Oklahoma Panhandle

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Abstract: In recent years, fox squirrels (*Sciurus niger*) have been documented from several locations in the Oklahoma panhandle, far west of their normal range. Interviews with local residents revealed that these small populations were introduced and are dwindling.

According to Caire *et al. (1989,* Mammals of Oklahoma, Univ. Oklahoma, Norman, p. 189) introduced fox squirrels *(Sciurus niger)* "are occasionally seen in towns and tree-lined areas of the Panhandle" despite the decided lack of suitable habitat in that open, virtually treeless region of shortgrasses and high plains. Although no panhandle county records are indicated on their state map, records are shown for nearby Harper and Woodward counties.

On 3 July, 2014, I questioned Jimmy Woodard, veteran bird finder from Oklahoma City, about possible fox squirrel sightings in Guymon, Texas County, which lies at the center of the panhandle just south of the Beaver (North Canadian) River. During the past 20-odd years, Woodard has annually searched for birds in the well-wooded Guymon City Park. However, he could not recall having ever seen squirrels there.

During the past few years, I have interviewed several panhandle residents concerning fox squirrels. On 28 September, 2008, I spoke with Esther Israel in Keyes, a small town in northwest Cimarron County at the west end of the panhandle, about the provenance of three squirrels that John S. Shackford and I were watching in trees in her well-shaded back yard. Here, the constant availability of food and water, as well as protection from predators and the elements, had rendered this spot a well-known haven for birds. Esther informed me that these squirrels had been brought in from Elkhart, Kansas, 20 miles to the northeast, four years earlier (2004). The greatest number she had counted here was 18, but these had dwindled to three.

Later that day, I talked with Mike Johnson, who lives seven miles south and 2 ½ west of Elkhart, Kansas, but in Texas County, Oklahoma. When I mentioned the squirrels in Keyes, he told me that the year he gave up his job in Elkhart, a hunter had introduced fox squirrels there. This was in 1986, but he could confirm no other details.

In Boise City, central Cimarron County, Shackford and I discovered two fox squirrels among trees in the city park on 24 May, 2013. On 10 May of the following year, we returned. An interview with groundskeeper Mark Shannon revealed that a local man, Gunther Brant, had released squirrels here in 1991 or 1992, but Shannon knew neither how many nor their provenance. Now only three or four remain.

The question then becomes: just how far does this species range westward naturally? Judging from the above, it would appear that fox squirrels introduced to inhospitable regions to which they are only marginally adapted will eventually succumb to environmental rigors such as the lack of dependable food plants (e. g., pecans, walnuts, oaks), nest sites, adequate vegetative cover in winter, the intense heat and drought of summer, or some combination of these. A fact worth noting is that house cats thrive in urban settings.

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New Records of Chiggers and Ticks from an Oklahoma Amphibian and Reptile

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Little is known about ectoparasites of Oklahoma herpetofauna. Larval chigger mites, *Hannemania dunni* Sambon and *Eutrombicula alfreddugesi* (Oudemans) have been reported from amphibians (Loomis 1956; Anthony et al. 1994) and various lizards (Crossley 1960) in Oklahoma, respectively. In addition, the blacklegged tick (*Ixodes scapularis* Say) has been reported from five-lined skinks, *Plestiodon fasciatus* and ground skinks, *Scincella lateralis* from McCurtain County (McAllister et al. 2013a, 2014). Here we report additional records for these ectoparasites from one species of amphibian and one species of reptile from Oklahoma.

Between March 2013 and October 2014, 37 juvenile and adult (mean ± 1SD snout-vent length [SVL] = 60.0 ± 12.8, range = 29-75 mm) dwarf American toads, *Anaxyurus americanus charlesmithi*, and 10 juvenile and adult (55.3 ± 5.8, 48-67 mm SVL) prairie lizards, *Sceloporus consobrinus* were collected by hand from wooded areas in Beavers Bend State Park (34.13527ºN, 94.687796ºW), Hochatown (34.17124ºN, 94.751863ºW), and Lukfata (34.005396ºN, 94.759438ºW), McCurtain County. Ectoparasites were collected, preserved in 70% ethanol, and processed and identified using appropriate guides (Brennan and Goff 1977; Loomis and Wrenn 1984; Durden and Keirans 1996). *Hannemania* chiggers were carefully removed from encapsulations on hosts using dissecting scissors and fine forceps and stored in vials of 70% ethanol until they could be cleared in lactophenol and slide-mounted in Hoyer's medium (Walters and Krantz 2009). Voucher ectoparasites are deposited in the General Ectoparasite Collection in the Department of Biology at Georgia Southern University (accession nos. L3641, 3643, 3689-90, 3695). Host vouchers are deposited in the Arkansas State University Museum, Herpetological Collection (ASUMZ), State University, Arkansas.

Two of 29 (7%) *A. a. charlesmithi* (male 51 mm, female 82 mm SVL) collected in September 2013 were found to be infested with two and six larval *H. dunni*, respectively. These chiggers were found within the dermis of these hosts (Fig. 1). One male *S. consobrinus* (53 mm SVL) collected in June 2014 was infested with 52 larval *E. alfreddugesi* (Fig. 2); two additional lizards (male 50 mm SVL, female 48 mm SVL) collected in September and October 2014 were infested with fewer *E. alfreddugesi*. Two of 8 (25%) *S. consobrinus* (female 48 mm, male 55 mm SVL) collected in May 2014

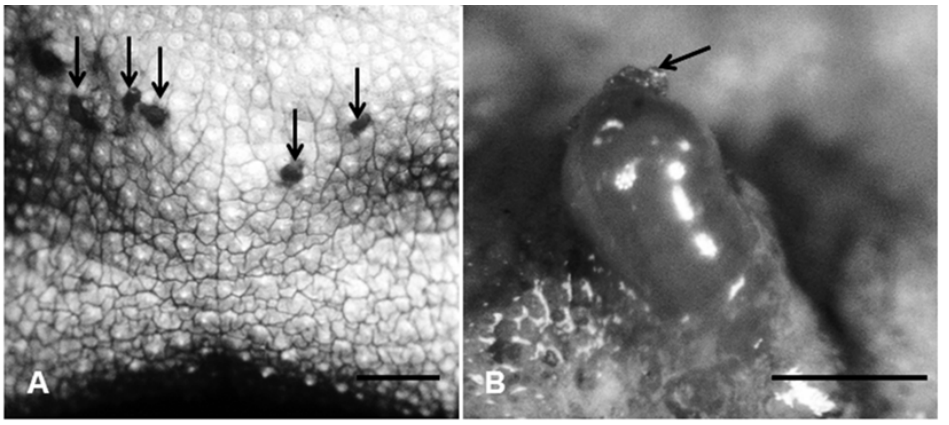

Figure 1. ***Hannemania dunni*** **chiggers from** ***Anaxyrus americanus charlesmithi*****. A. Encapsulated chiggers (arrows) in dermis of venter. Scale bar = 5 mm. B. Closer view of engorged chigger teased from encapsulation showing legs (arrow). Scale bar = 2 mm.**

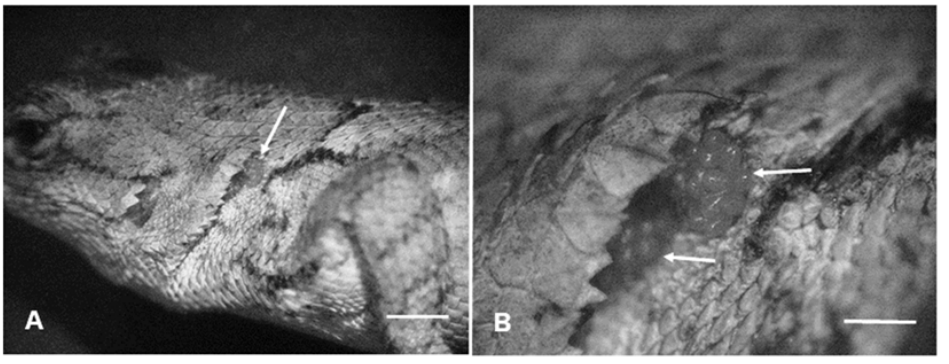

Figure 2. ***Eutrombicula alfreddugesi*** **chiggers on** ***Sceloporus consobrinus*****. A. Lateral view showing chiggers on neck region (arrow). Scale bar = 5 mm. B. Close-up showing two groups of chiggers (arrows). Scale bar = 2.5 mm.**

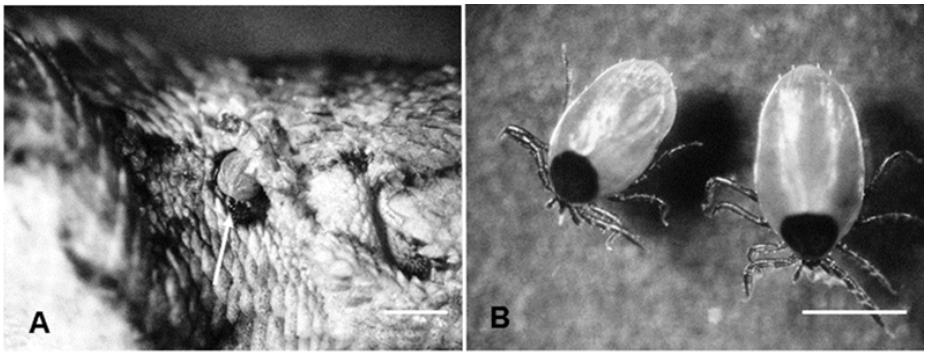

Figure 3. ***Ixodes scapularis*** **from** ***Sceloporus consobrinus*****. A. View of engorged tick (arrow) near ear opening. Scale bar = 2 mm. B. Two nymphal ticks removed from host. Scale bar = 2 mm.**

harbored two nymphs each of *I. scapularis* (Fig. 3).

Arthropoda: Acarina: Leeuwenhoekiidae

***Hannemania dunni* Sambon, 1928**

This chigger has been reported from a variety of amphibians, including both anurans and caudates (Walters et al. 2011). The geographic range includes Alabama, Arkansas, Georgia, Kansas, North Carolina, Oklahoma, Texas, Virginia and West Virginia (Walters et al. 2011; McAllister et al. 2013b). Loomis (1956) previously reported *H. dunni* from Ouachita dusky salamander, *Desmognathus brimleyorum*, Woodhouse's toad, *Anaxyrus woodhousii*, Blanchard's cricket frog, *Acris blanchardi* and southern leopard frog, *Lithobates sphenocephalus utricularius* from Le Flore and Woods counties, Oklahoma; we add a new host for *H. dunni* and an additional record from Oklahoma. This chigger has been reported to adversely affect the nasolabial groove chemosensory structure in plethodontid salamanders but the effect on *A. a. charlesmithi* is not known at this time.

Trombiculidae

***Eutrombicula alfreddugesi* (Oudemans, 1910)**

This is a widely-ranging chigger that parasitizes a large variety of vertebrates including amphibians and (primarily) reptiles (both lizards and snakes) (Walters et al. 2011). It has been previously reported on herpetofauna from Alabama, Arkansas, California, Colorado, Delaware, Florida, Georgia, Illinois, Indiana, Kansas, Maine, Michigan, Minnesota, North Carolina, Nebraska, New York, Ohio, Oklahoma, South Carolina, South Dakota, Tennessee and Texas (Walters et al. 2011). In much of eastern and central North America, *E. alfreddugesi* is the primary pest chigger that parasitizes humans, often causing red, pruritic skin lesions at attachment sites (Loomis and Wrenn 1984).

Arthropoda: Acari: Ixodidae

***Ixodes scapularis* Say, 1821**

Several species of lizards within the families Anguidae, Dactyloidae, Phrynosomatidae, Scincidae and Teiidae are known to be hosts of larval and nymphal stages of *I. scapularis* (for summary see McAllister et al. 2013a). It has been reported previously on various lizards from Alabama, Florida, Georgia, Mississippi, Missouri, New York, North Carolina, Oklahoma, South Carolina, Tennessee and Texas (see McAllister et al. 2013a). This tick has significant medical and veterinary importance as a zoonotic vector of the causative agent of Lyme Disease (= Lyme borreliosis) (Nicholson et al. 2009). We report *I. scapularis* for the third time on a lizard from the state.

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New Geographic Distribution Records for Centipedes (Chilopoda: Scolopendromorpha) from Oklahoma

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Since the seminal publication on scolopendromorph centipedes by Shelley (2002), our knowledge of the centipede fauna of Oklahoma has increased significantly (McAllister et al. 2003, 2004, 2006). However, there remain several distributional gaps for many of these centipedes in the state. Here, we continue to help fill some of those gaps with six new county records for four species within three families. Additionally, in an effort to aid in future research, we provide an updated distribution for all Oklahoma scolopendromorphs.

Between October 2009 and March 2013, we followed techniques of McAllister et al. (2003) in collecting centipedes from sites in six counties of the state, primarily from trails in State Parks, including those in Atoka, Blaine, Choctaw, Osage, Pushmataha, and Woodward. We also visited Black Mesa State Park in Cimarron County and Quartz Mountain State Park in Greer County in February 2011, but found no centipedes. Specimens were placed in vials containing 70% ethanol and, following preliminary identification, representative samples were shipped to R. M. Shelley at the North Carolina State Museum of Natural Sciences (NCSM), Raleigh, for verification of identifications and deposition of select vouchers. Other voucher specimens were deposited in the Sam Noble Oklahoma Museum of Natural History (SNOMNH), Norman. Collectors of specimens are the coauthors who are designated below by their initials.

Annotated List of Taxa

Scolopendromorpha: Scolopendridae

Scolopendra heros Girard – *Woodward Co.*, Boiling Springs State Park off Park Road 52 (36.455402ºN, 99.291403ºW), 11 Feb. 2011, CTM (SNOMNH). Although expected statewide, this large centipede had been reported previously from 22 counties of the state (Shelley 2002; McAllister et al. 2003) (Fig. 1) and several counties to the east in adjoining Arkansas (McAllister et al. 2010). *Scolopendra heros* is the largest North American centipede with lengths exceeding 200 mm (Sandefur 1998; Walls 2000).

Scolopocryptopidae

Scolopocryptops rubiginosis L. Koch – *Osage Co.*, Osage Hills State Park (36.735789ºN, 96.185397°W), 9 Oct. 2009, CTM, HWR (NCSM). Previous records for this centipede (Shelley 2002; McAllister et al. 2003, 2006) include 11 counties of the state (Fig. 2). We provide the northernmost record in Oklahoma which is well within the distribution of *S. rubiginosus* in the central USA (see Shelley 2002, fig. 108; McAllister et al. 2003, fig. 1, 2006, fig. 1).

Scolopocryptops sexspinosus (Say) – *Atoka Co.*, McGee Creek State Park off McGee Creek Dam Road (34.327498ºN, 95.914813ºW), 8 Mar. 2013, CTM, MBC

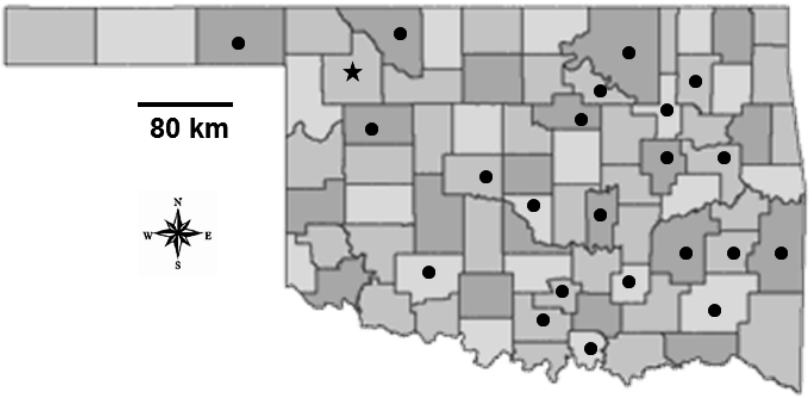

Figure 1. Records of *Scolopendra heros* in Oklahoma. Dots = previous records; star = new record.

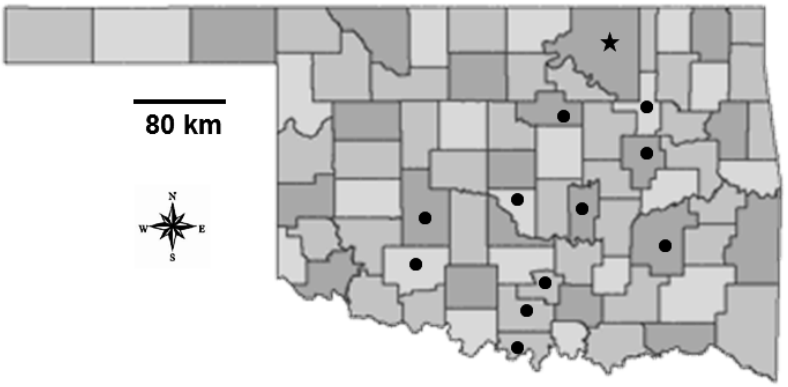

Figure 2. Records of *Scolopocryptops rubiginosus* in Oklahoma. Dots = previous records; stars = new records.

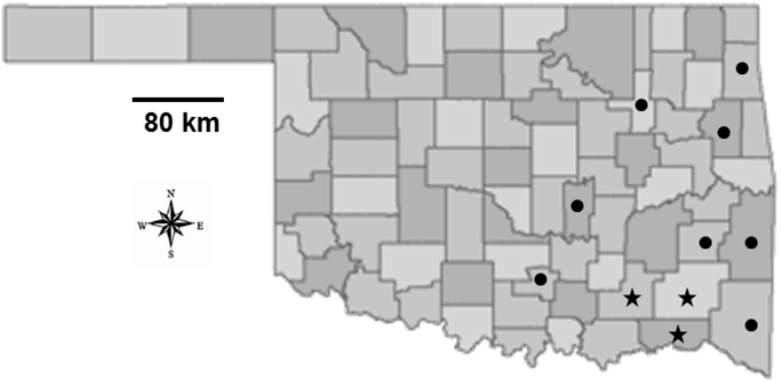

Figure 3. Records of *Scolopocryptops sexspinosus* in Oklahoma. Dots = previous records; stars = new records.

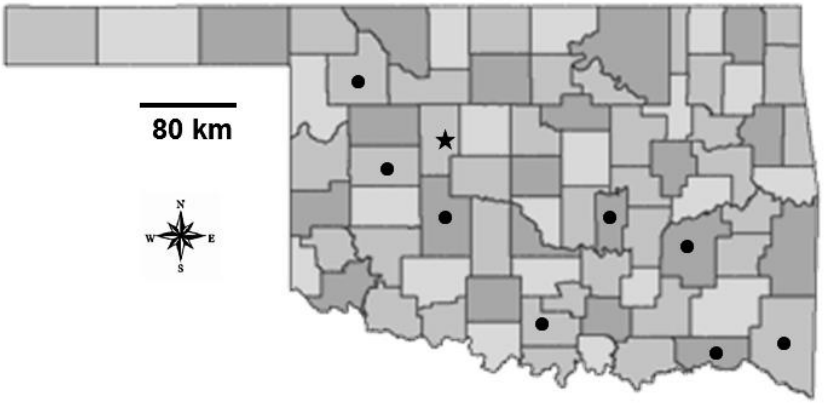

Figure 4. Records of *Theatops posticus* in Oklahoma. Dots = previous records; stars = new records.

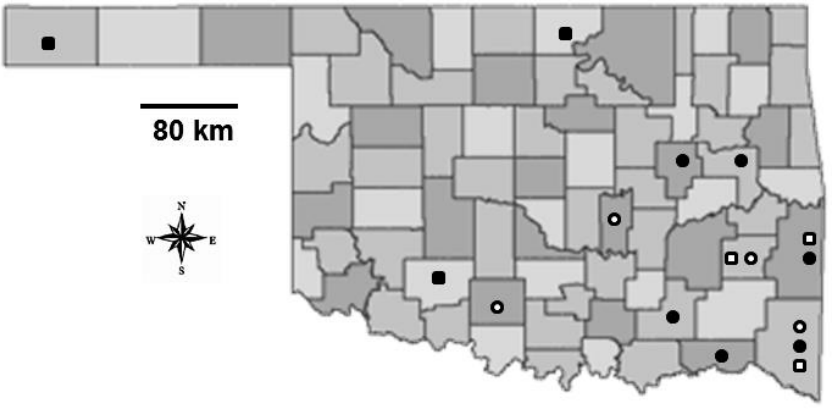

Figure 5. Records of *Theatops spinicaudus* (solid dots), *Scolopendra viridis* (solid squares), *Hemiscolopendra marginata* (open squares), and *Cryptops leucopodus* (open dots) in Oklahoma.

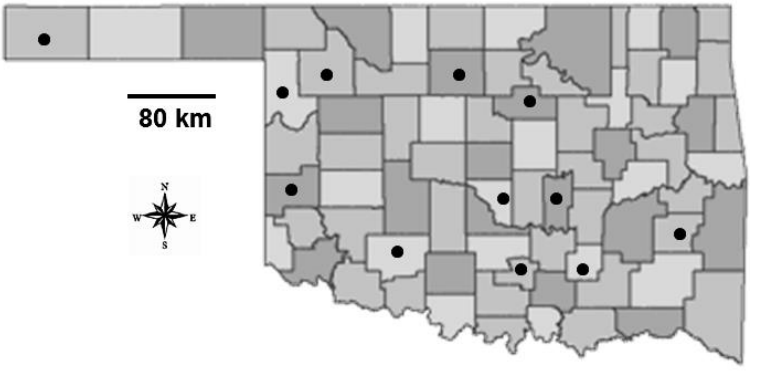

Figure 6. Records of *Scolopendra polymorpha* in Oklahoma. Dots = previous records.

(SNOMNH). *Choctaw Co.*, Ft. Towson Historic Site, off co. rd. E2060 (34.026165ºN, 95.259259ºW), 11 Nov. 2011, CTM (SNOMNH). *Pushmataha Co.*, Indian Nation Turnpike, mile marker 34 (34.485056ºN, 95.73412ºW), 20 Feb. 2011, CTM, HWR (NCSM). This scolopendromorph had been reported previously from only eight counties of the state and is expected in the eastern half of Oklahoma (Shelley 2002; McAllister et al. 2003, 2006) (Fig. 3). We provide three new county records for the southeastern part of the state.

Cryptopidae

Theatops posticus (Say) – *Blaine Co.*, Roman Nose State Park off Park Road 60 (35.935669ºN, 98.431486ºW), 18 Feb. 2011, CTM, HWR (SNOMNH). This centipede has a spotty distribution in the state with eight previous records (Shelley, 1997, 2002; McAllister et al. 2004) spanning from the western portion (Woodward County) to the far southeastern corner of the state (McCurtain County) (Fig. 4).

Interestingly, four other scolopendromorphs of Oklahoma have fewer than seven county records documented (see Shelley 2002; McAllister et al. 2003, 2006), including *Theatops spinicaudus* (Wood) with six, *Scolopendra viridis* Say with three, *Hemiscolopendra marginata* (Say) with three, and *Cryptops leucopodus* (Rafinesque) with four (Fig. 5). One other, *Scolopendra polymorpha* Wood is expected statewide except for the eastern portion of the state and has 12 counties (Fig. 6) documented for its Oklahoma distribution (Shelley 2000; McAllister et al. 2003). With additional collecting, especially in the western and southwestern parts of the state, we expect new county records to be added to our growing knowledge of the scolopendromorph centipede fauna of Oklahoma.

Acknowledgments

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Reproductive Notes on the Western Painted Crayfish, *Orconectes* (*Buannulifictus*) *palmeri longimanus* (Decapoda: Cambaridae), from Southeastern Oklahoma

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The western painted crayfish, *Orconectes* (*Buannulifictus*) *palmeri longimanus* (Faxon) is a medium-sized crustacean found in all western tributaries of the Mississippi River from the Arkansas River to the Gulf, and the Gulf drainage streams of Arkansas, Kansas, Louisiana, Mississippi, Oklahoma and Texas (Metcalf and Distler 1963; Hobbs 1974; Pflieger 1996). Nationwide, populations of *O. p. longimanus* are considered currently stable, CS (Taylor et al. 2007). In Oklahoma, this crayfish is ranked S5 (currently stable) by NatureServe (2014). Little is known about the reproductive biology of *O. p. longimanus* in Oklahoma and elsewhere. Here, we document some noteworthy natural history information on ovigerous *O. p. longimanus* in the state that includes, for the first time, counts of eggs, size of young, and an unusual timing of reproduction.

Between June 2011 and July 2014, 107 *O. p. longimanus* (50 females) were collected from various sites in McCurtain County, including Boktuklo Creek (n = 5), Eagle Fork Creek (n = 7), Glover River (n = 8), Lukfata Creek (n = 21), Mt. Fork River (n = 18), Mud Creek (n = 14), Salt Creek (n = 10), Yanubbee Creek (n = 6) and Yashau Creek (n = 18). When females were found to ovigerous or "in berry" (Fig. 1) they were measured for body length (BL) and placed in 37 l aquaria with air stones and conditioned tap water. Once young hatched, they were counted, measured and preserved in 70% ethanol. Voucher specimens of crayfish were deposited in the Henderson State University Collection, Arkadelphia, Arkansas.

Two adult female *O. p. longimanus* (80, 85 mm BL) collected on 16 January and 4 May 2014 from the Mt. Fork River, Beavers Bend State Park (34.138315°N, 94.766173°W) produced 60 and 49 young, respectively. These young (Fig. 2) hatched ca. 2 wk later and measured 8-12 (mean 10) mm BL. Interestingly, the January ovigerous date is the earliest known date for *O. p. longimanus*. Water temperature in this cold, spring-fed stream was 7ºC.

In the first report on Oklahoma crayfishes, Creaser and Ortenburger (1933) did not mention anything about reproduction in *O. p. longimanus*. In the most recent survey of state crayfishes of Oklahoma, Morehouse and Tobler (2013) did not report ovigerous females nor did Reimer (1967) in an earlier report on crayfishes of the state. However, Jones and Bergey (2007) noted that *O. p. longimanus* becomes reproductively active in September and October and continued to display glair through January; the only females in berry were observed in March, an indication they mentioned of may be capable of producing young late in the year. In his classic study of the crayfishes of the Ozarks of Arkansas, Oklahoma, and Missouri, Williams (1954) unfortunately did not comment on the biology or reproduction of *O. palmeri*

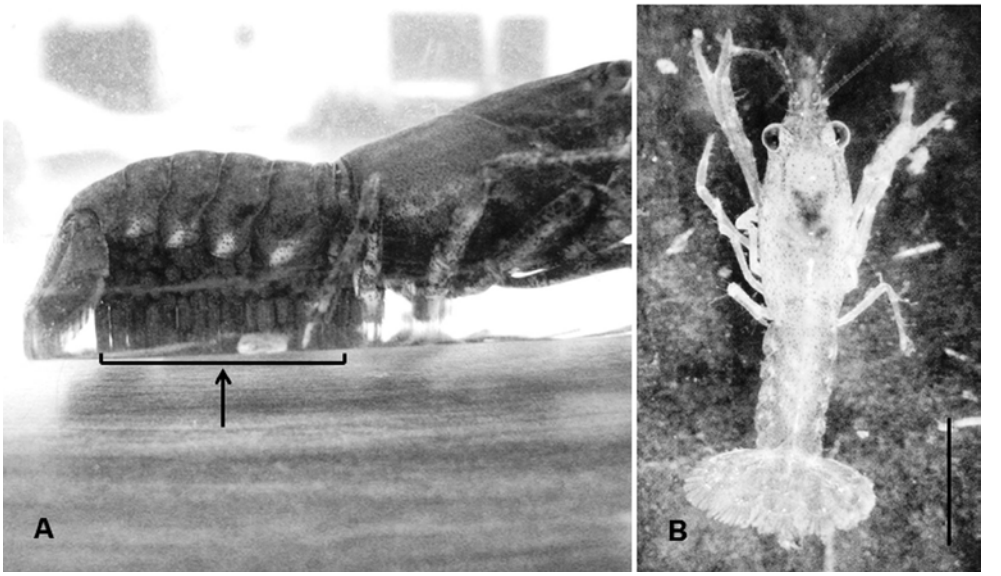

Figure 1. Reproduction in *Orconectes palmeri longimanus* from southeastern Oklahoma. A. Ovigerous female showing "berries" (arrow in bracket). B. New hatchling. Scale bar = 2.5 mm.

longimanus. In Arkansas, Reimer (1963) found *O. p. longimanus* females with eggs in April (3) and May (1). He opined that eggs were likely laid in April. In personal collections of one of us (HWR) from Arkansas, he collected ovigerous females of *O. p. longimanus* from the Caddo River on 23 March 1994 and the upper Ouachita River on 15 April 1996; however, no count of eggs was made. In Missouri, Pflieger (1996) reported gray-speckled crayfish (*O. palmeri*) females produce eggs in late March and April. Taylor and Schuster (2004), in their treatise on Kentucky crayfishes, reported no ovigerous females have been collected from Kentucky. Walls (2009) commented on the habitat and distribution of *O. p. longimanus* in Louisiana, but did not mention anything related to biology or reproduction of this form.

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Myxozoan and Helminth Parasites of the Dwarf American Toad, *Anaxyrus americanus charlesmithi* (Anura: Bufonidae), from Arkansas and Oklahoma

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Abstract: We examined 69 dwarf American toads, *Anaxyrus americanus charlesmithi*, from McCurtain County, Oklahoma (n = 37) and Miller, Nevada and Union counties, Arkansas (n = 32) for myxozoan and helminth parasites. The following endoparasites were found: a myxozoan, *Cystodiscus* sp., a trematode, *Clinostomum marginatum*, two tapeworms, *Cylindrotaenia americana* (Oklahoma only) and *Distoichometra bufonis*, five nematodes, acuariid larvae, *Cosmocercoides variabilis*, *Oswaldocruzia pipiens*, larval *Physaloptera* sp. (Arkansas only), and *Rhabdias americanus* (Arkansas only), and acanthocephalans (Oklahoma only). We document six new host and four new geographic distribution records for these select parasites.

Introduction

The dwarf American toad, *Anaxyrus americanus charlesmithi*, is a small anuran that ranges from southwestern Indiana and southern Illinois south through central Missouri, western Kentucky and Tennessee, and all of Arkansas, to eastern Oklahoma and northeastern Texas (Conant and Collins 1998). It occurs in various habitats, from suburban back yards to mountain wildernesses, where it breeds in temporary pools or ditches or shallow portions of streams. Compared to the eastern American toad, *A. a. americanus* (see Muzzall and Andrus 2014, and refs therein), little is known about its helminth parasites. The following papers report fragmentary information on various helminths of *A. a. charlesmithi* as follows: *Mesocoelium monas* (McAllister et al. 2008), *Cosmocercoides variabilis* (McAllister and Bursey 2012a) and tetrathyridia of *Mesocestoides* sp. (McAllister et al. 2014c) from *A. a. charlesmithi* from Arkansas, and *Clinostomum marginatum* from dwarf American toads from Oklahoma (Cross and Hranitz 2000). In addition, Langford and Janovy (2013) reported *Rhabdias americanus* from *A. a. charlesmithi* from Missouri. Although McAllister and Bursey (2012b) recently provided information on helminth parasites of various herpetofauna from southeastern Oklahoma, we are not aware of any additional reports of helminths from this toad nor has a complete survey of its endoparasites been carried out to date. Here, from a survey on specimens from Arkansas and Oklahoma, we report six new host and

four new distributional records for a myxozoan and helminths of *A. a. charlesmithi*.

Methods

Between August 2012 and October 2014, 69 juvenile and adult *A. a. charlesmithi* (mean ± 1SD snout-vent length [SVL] = 51.3 ± 14.8, range = 28-82 mm) were collected by hand, including 37 from Oklahoma (McCurtain County) at Beavers Bend State Park (*n* = 2) (34.13527ºN, 94.687796ºW), Hochatown (*n* = 34) (34.171155ºN, 94.751834ºW), and Lukfata (*n* = 1) (34.005396ºN, 94.759438ºW), and 32 from Arkansas in Miller at Nix Creek (*n* = 2) (33.433478ºN, 94.027763ºW), Nevada at White Oak Lake (*n* = 1) (33.688228ºN, 93.110322ºW) and Union at Calion Lake (33.330527ºN, 92.528422ºW), El Dorado (33.209011ºN, 92.590186ºW) and Junction City (*n* = 29) (33.01971ºN, 92.7333ºW) counties. Methods for necropsy and examination by light microscopy and processing have been previously described for myxozoans (McAllister and Trauth 2005) and helminths (McAllister and Bursey 2005). For SEM, myxozoan trophozoites were dehydrated in a graded series of increasing ethanol solutions (50-100%), followed by several fluid exchanges in 100% ethanol. An Autosamdri-815 critical point drier (Tousimis Research Corporation, Rockville, MD) was used (31 ºC, 1072 psi, ventilation rate ~100 psi/min) to remove excess ethanol. Samples were then mounted on 25.4 mm aluminum pin stub specimen mounts and coated with gold using a Cressington 108 sputter coater (Cressington Scientific Instruments Ltd, Watford, UK). Samples were analyzed both qualitatively and quantitatively with a Vega TS 5136XM digital scanning electron microscope (Tescan USA Inc., Cranberry Township, PA) at 19.5 kV.

Parasites were deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland or the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, Nebraska. Host voucher specimens are deposited in the Arkansas State University Herpetological Collection (ASUMZ), State University, Arkansas, or the Henderson State University Herpetological Collection (HSU), Arkadelphia, Arkansas.

Results

Forty-eight of 69 (70%) of the *A. a. charlesmithi*, including 27 (84%) from Arkansas and 21 (57%) from Oklahoma harbored Protista and nine helminths as follows: a myxozoan, a trematode, two tapeworms, five nematodes and an acanthocephalan (Table 1). Nine (24%) of the *A. a. charlesmithi* from Arkansas and nine (28%) from Oklahoma were concurrently infected with myxozoans and one or two helminths or with two or three helminths. The mean number of helminths found in Arkansas toads was 1.3 ± 0.5 and in Oklahoma toads 1.3 ± 0.7. An annotated list of the myxozoans and helminths found and the host data follows.

Protista: Myxosporea: Myxidiidae

Cystodiscus sp. Lutz, 1889 (Fig. 1)

Trophozoites and free spores (HWML photovoucher 75105) of a *Cystodiscus* sp. (syn. *Myxidium*) identified by ribosomal DNA sequencing (C. Whipps, *pers. comm*.) was found in the gall bladder of six toads (47.8 ± 9.6, 36-62 mm SVL) from Union County, Arkansas, and six *A. a. charlesmithi* (53.5 ± 12.3, 36-70 mm SVL) from Hochatown, McCurtain County, Oklahoma. *Cystodiscus serotinus* (=*Myxidium serotinum*) (Kudo and Sprague 1940) Hartigan, Fiala, Dyková, Rose, Phalen, and Šlapeta, 2012 has previously been reported from one of five (20%) *A. a. charlesmithi* from Arkansas (McAllister and Trauth 1995); however, we are not aware of any amphibian myxozoan reported previously from Oklahoma. Other bufonid hosts of *C. serotinus* include the green toad (*Anaxyrus debilis*), Texas toad (*Anaxyrus speciosus*), Woodhouse's toad (*Anaxyrus woodhousii*) and Coastal Plain toad (*Incilius nebulifer*) from Texas (McAllister et al. 1989; McAllister and Trauth 1995) and southern toad (*Anaxyrus terrestris*) from Florida (Kudo 1943).

Table 1. Myxozoa and helminths found during this study in *Anaxyrus americanus charlesmithi* from Arkansas and Oklahoma.

Helminth	State	Prevalence*	Intensity†
Myxosporea			
Cystodiscus sp.	Arkansas	6/32 (19%)	-
	Oklahoma‖	6/37 (16%)	-
Trematoda			
Clinostomum marginatum	Arkansas	1/32 (3%)	1
	Oklahoma	2/37 (5%)	5, 32
Cestoidea			
Cylindrotaenia americana‡	Oklahoma	8/37 (22%)	7.5 ± 10.1, 1-28
Distoichometra bufonis‡	Arkansas‖	2/32 (6%)	5
	Oklahoma	3/37 (8%)	1-5
Nematoda			
Acuariid larvae‡	Arkansas	3/32 (9%)	1-3
	Oklahoma‖	1/37 (3%)	3
Cosmocercoides variabilis	Arkansas	6/32 (19%)	4.7 ± 5.3 (1-15)
	Oklahoma	4/37 (15%)	1, 1, 1, 1
Oswaldocruzia pipiens‡	Arkansas	17/32 (53%)	2.6 ± 1.9 (1-7)
	Oklahoma	8/37 (22%)	2.3 ± 2.3 (1-7)
Physaloptera sp. larvae‡	Arkansas	2/32 (6%)	1, 1
Rhabdias americanus	Arkansas‖	1/32 (3%)	1
Acanthocephala			
Unknown species‡	Oklahoma	2/37 (5%)	1

*Number infected/number examined = %.

†mean ± 1SD, range (where applicable).

‡New host record.

‖New distributional record.

Platyhelminthes: Trematoda: Digenea: Clinostomidae

Clinostomum marginatum Rudolphi, 1819 (Fig. 2)

We found metacercaria ("yellow grubs") of this digenean (USNPC 107669) in the musculature and viscera of two toads (72 and 82 mm SVL) from the Hochatown site and in one toad (52 mm SVL) from El Dorado. This digenean has been previously reported from 17 of 69 (25%) *A. a. charlesmithi* from Oklahoma (Cross and Hranitz 2000). It has also been reported in a wide variety of amphibians in North America that primarily live, or breed in, lentic habitats (see McAllister et al. 2010). More recently, *C. marginatum* has been reported in *Eurycea* spp. salamanders (Bonett et al. 2011) and Pirate Perches, *Aphredoderus sayanus* (McAllister and Bursey 2013) from Oklahoma, and madtoms, *Noturus* spp. from Arkansas (McAllister et al. 2014b).

Cestoidea: Cyclophyllidea: Cylindrotaeniidae

***Cylindrotaenia americana* Jewell, 1916 (Fig. 3)**

This tapeworm (HWML 75056) was found in the small intestine of eight toads (44.5 ± 8.9, 34-55 mm SVL) from the Hochatown site. One of these *A. a. charlesmithi* (50 mm SVL) collected on 12 August 2014 had a massive infection of *C. americana* that completely filled its intestinal tract from the duodenum to near its rectum (Fig. 3). McAllister et al. (2013b) recently summarized the hosts and Western Hemisphere localities of *C. americana* and several previously reported bufonid hosts from North America have been documented with this cestode, including *A. a. americanus* from Iowa (Ulmer and James 1976). It has also been reported from amphibians in Oklahoma (Trowbridge and Hefley 1934) and Arkansas (McAllister et al. 1993, 2013b). We document a new host for *C. americana*.

Nematotaeniidae

***Distoichometra bufonis* Dickey, 1921 (= *Distoichometra kozloffi* Douglas, 1958)**

Specimens of *D. bufonis* (HWML 64657) were taken from the small intestine of five toads, three (52, 54, 71 mm SVL) from Hochatown and two (49, 60 mm SVL) from El Dorado. This cestode has been previously reported from Oklahoma in Great Plains toads, *Anaxyrus cognatus* (Kuntz 1941) and from Ohio in *A. a. americanus* (Odlaug 1954). It has also been reported from other anurans of the genera *Anaxyrus*, *Pseudacris*, *Rana*, *Scaphiopus*, *Smilisca* and *Spea* in Arizona, California, Georgia, Nebraska, New Mexico, North Carolina, Ohio, Oregon, and Utah, and *Rhinella* in Mexico (Koller and Gaudin 1977; Hardin and Janovy 1988; Goldberg and Bursey 1991; Goldberg et al. 2001; and others). This is the first time *D. bufonis* has been reported from *A. a. charlesmithi* and from Arkansas.

Nematoda: Rhabditida: Rhabdiasidae

***Rhabdias americanus* Baker, 1978**

This nematode (retained in author's collection) was found in the lung of a single toad (40 mm SVL) from El Dorado. The species has been previously reported from *A. a. americanus* (type host) from Canada and the eastern United States (see Baker 1987), Nebraska (Langford and Janovy 2013), Michigan (Muzzall and Andrus 2014) and Wisconsin (Bolek and Coggins 2000, 2003;Yoder and Coggins 2007), and from *A. a. charlesmithi* from Missouri (Langford and Janovy 2013). Other bufonid hosts include *A. alvarius*, *A. cognatus*, *A. debilis*, *A. hemiophrys*, *A. microscaphus*, *A. retiformis*, and *A. woodhousii*. In addition, Kuzmin (2013) recently provided a review of the Rhabdiasidae from the Holarctic. In the life cycle, infective larvae penetrate the host skin and eventually migrate to the body cavity where subadults invade the lungs, mature and produce eggs (Anderson 2000). This is the first time, to our knowledge, that *R. americanus* has been reported from Arkansas.

Ascaridida: Cosmocercidae

***Cosmocercoides variabilis* (Harwood, 1930) Travassos, 1931**

This nematode (HWML 64658) was the third most commonly found parasite in *A. a. charlesmithi*, occurring in the rectum of six toads (52 ± 18.7, 34-75 mm SVL) from Calion Lake and El Dorado and four toads (52, 56, 72, 82 mm SVL) from Hochatown. McAllister and Bursey (2012a) previously reported *C. variabilis* from Arkansas *A. a. charlesmithi*; additional amphibians from the state have also been reported to harbor *C. variabilis* (see McAllister et al. 2013a). In Oklahoma, it has been reported in Sequoyah slimy salamanders, *Plethodon sequoyah* (McAllister and Bursey 2004a), American bullfrogs, *Lithobates catesbeianus* (Trowbridge and Hefley 1934) and Hurter's spadefoot, *Scaphiopus hurterii* (McAllister et al. 2005a). *Cosmocercoides variabilis* has been reported from numerous other North American amphibians, including *A. a. americanus*, from at least 24 U.S. states and four provinces of Canada, Baja California Norte, Mexico, Costa Rica, and Panama (see McAllister et al. 2013a).

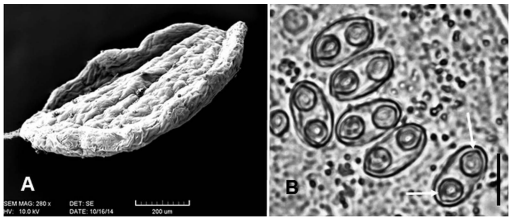

Figure 1. *Cystodiscus* sp. from *Anaxyrus americanus charlesmithi* from Oklahoma. A. Scanning electron micrograph on black background showing trophozoite. B. Light microscopy of spores showing two polar capsules (arrows) per myxospore; scale bar = 10 µm.

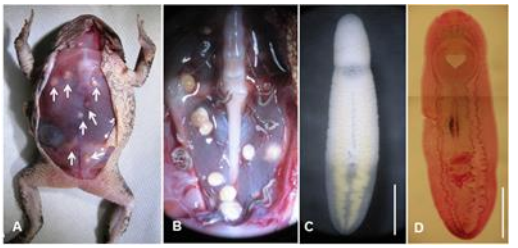

Figure 2. *Clinostomum marginatum* from *Anaxyrus americanus charlesmithi* from Oklahoma. A. View showing venter of frog with numerous encapsulated metacercaria in musculature (arrows). B. Closer view of encapsulated metacercaria deeper in abdominopelvic cavity. C. Unstained metacercaria teased from encapsulation. D. Stained metacercaria. Scale bars = 500 µm.

Figure 3. *Cylindrotaenia americana* from *Anaxyrus americanus charlesmithi* from Oklahoma. A. Massive infection of tapeworms in small intestine. Scale bar = 2 mm. B. Worms removed to Petri dish. Scale bar = 5 mm. C. Individual tapeworm with scolex (arrow) embedded in intestinal mucosa. Scale bar = 1 mm.

Strongylida: Molineidae

***Oswaldocruzia pipiens* Walton, 1929**

The most common endoparasite of *A. a. charlesmithi* was *O. pipiens* (HWML 64659) found in the small intestine of 17 toads (49.2 ± 17.3, 28-78 mm SVL) from Arkansas (Calion Lake, El Dorado, Junction City, Nix Creek and White Oak Lake) and eight toads (64.8 ± 11.9, 29-82 mm SVL) from Oklahoma (Hochatown). This nematode has been previously reported from *A. woodhousii*, *L. catesbeianus*, southern leopard frog, *Lithobates sphenocephalus utricularius* and *S. hurterii* from Oklahoma (Trowbridge and Hefley 1934; Kuntz 1941; Kuntz and Self 1944; McAllister et al. 2005a) and cave salamander, *Eurycea lucifuga*, bird-voiced treefrog, *Hyla avivoca*, pickerel frog, *Lithobates palustris* and wood frog, *Lithobates sylvaticus* from Arkansas (McAllister et al. 1993, 1995a, b; McAllister and Bursey 2004b). *Oswaldocruzia pipiens* is also a common helminth of *A. a. americanus* (Coggins and Sajdak 1982; Bolek and Coggins 2000, 2003; Yoder and Coggins 2007). In addition, it is obvious that there is no host specificity in *O. pipiens* as this strongylid has also been reported in various North American reptiles, including the ground skink, *Scincella lateralis* from Arkansas (see McAllister et al. 2014a). We document a new host for *O. pipiens*.

Spirurida: Physalopteridae

***Physaloptera* sp. Rudolphi, 1819 (third-stage larvae)**

This nematode (HWML 64661), which has a direct life cycle (Anderson 2000), was found as third-stage larvae in the stomach lumen of two toads, one from El Dorado (52 mm SVL) and one from White Oak Lake (72 mm SVL). Physalopterans have been reported in *A. a. americanus* from Ohio (Ashton and Rabalais 1978). McAllister et al. (2013a) recently reported this nematode from the Cajun chorus frog, *Pseudacris fouquettei* from Arkansas. The dwarf American toad is a new host of *Physaloptera* sp.

Acuarioidea: Acuariidae

Acuariid larvae

Larval acuariids (HWML 64661) were found encapsulated in stomach tissue in a single toad (37 mm SVL) from Hochatown and three toads (36, 40, 51 mm SVL) from El

Dorado. Acuariids typically mature in aquatic birds and require an arthropod intermediate host while anurans may serve as paratenic hosts (Anderson 2000). The occurrence of acuariids in amphibians and reptiles was summarized by Goldberg et al. (2007) and McAllister et al. (2013a) updated the host list. In addition, McAllister et al (2014a) recently reported acuariid larvae from *S. lateralis* from Arkansas and Oklahoma. We document a new host for acuariid larvae.

Acanthocephala

Unknown genus and species

Two female acanthocephalans (retained in author's collection) were found in the stomach and encapsulated on the serosal surface of the stomach of two toads (47, 52 mm SVL) from Hochatown. Unfortunately, because a male was not present, it is not possible to provide an identification. There are several reports of acanthocephalans in anurans, most being noted as unidentified cystacanths (Odlaug 1954) or *Centrorhynchus* sp. cystacanths (Brandt 1936; Campbell 1968; Hollis 1972). However, we report an acanthocephalan in *A. a. charlesmithi* for the first time

Discussion

In summary, we provide the first complete survey on myxozoans and helminths of *A. a. charlesmithi* from Arkansas and Oklahoma. Although its parasite fauna is depauperate, like most of those reported in North American anurans (see Aho 1990), we document six new host and four new distributional records. Also, when our data on *A. a. charlesmithi* and that of McAllister et al. (2014c) are compared to surveys of *A. a. americanus* from Michigan (Muzzall and Andrus 2014), Ohio (Odlaug 1954; Ashton and Rabalais 1978) and Wisconsin (Coggins and Sajdak 1982; Bolek and Coggins 2000, 2003; Yoder and Coggins 2007), six helminths (*D. bufonis*, *Mesocestoides* sp., *C. variabilis*, *O. pipiens*, *Physaloptera* sp., *R. americanus*) are shared by these subspecies. Additional surveys in other parts of its range where *A. a. charlesmithi* has not yet been examined (i.e., Kentucky, Tennessee, Texas) could potentially report additional new host and geographic records for its parasites.

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A Floristic Inventory of Six Tracts of the Ozark Plateau National Wildlife Refuge, Adair County, Oklahoma

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Abstract: Five hundred sixty taxa in 102 families were encountered in a five-year floristic inventory of the Eagle Pass, Sally Bull Hollow, Workman Mountain, Gittin Down Mountain, Varmint, and Liver tracts of the Ozark Plateau National Wildlife Refuge and the adjoining Ozark Plateau Wildlife Management Area, which encompass 1262.6 ha in Adair County, Oklahoma (USFWS, 2013a). One hundred thirty-three species were new records for Adair County (Hoagland et al. 2004). Native species constituted 91.2 % of the species and the largest families were Asteraceae, Fabaceae and Poaceae, together making up 33.1% of the total taxa. Six known invasive species—*Lespedeza cuneata, Lonicera japonica, Sorghum halepense, Microstegium vimineum, Albizia julibrissin,* and *Nasturtium officinale*—were encountered, but at the time of the surveys they did not appear to be flourishing nor establishing large colonies or stands. Species federally or state listed as endangered or threatened were not found. Thirty-two species of concern and tracked by the Oklahoma Natural Heritage Inventory (OHNI 2014) were encountered. Fourteen types of habitats, 15 associations, and one alliance were observed.

Introduction

Initially known as The Oklahoma Bat Caves National Wildlife Refuge, the Ozark Plateau National Wildlife Refuge (OPNWR) was established in April, 1986 to protect several cave-dwelling species including the federally listed endangered *Myotis grisescens* (gray bat), *Myotis sodalis* (Indiana bat), and *Corynorhinus townsendii ingens* (Ozark big-eared bat). In addition, these caves also are critical habitat for the federally listed threatened *Amblyopsis rosae* (Ozark cave fish) and *Noturus placidus* (Neosho madtom) and the Oklahoma listed endangered *Cambarus tartarus* (Oklahoma cave crayfish) (USFWS (United States Fish and Wildlife Service) 2002; ODWC (Oklahoma Department of Wildlife Conservation) 2014). Restriction of these species to these areas/caves is believed to be the result of deforestation, pollution, and other anthropogenic disturbances in their natural ranges (USFWS, 2002; O'Shea and Brogan, 2003; Hensley, 2004). Caves on the refuge have been gated to eliminate or minimize further human disturbance.

In Oklahoma, the refuge currently comprises 1700 ha in nine tracts in Adair, Cherokee, Delaware, and Ottawa Counties, and efforts to further expand its boundaries are ongoing. There exists a critical need for a thorough knowledge of the plants and vegetation present in order to facilitate effective management. Recognition that protection of the refuge's cave-dwelling species also requires protection of the habitat surrounding the caves has led the United States Fish and Wildlife Service (USFWS) to employ an ecosystem approach, which includes protecting the area's hydrology and maintaining populations of native plant species in situ (Christensen et al. 1996; Grumbine 1994; USFWS 2001, 2002). With common management goals, personnel of multiple cooperating agencies—USFWS, ODWC, The Nature Conservancy, The Land Legacy, ONHI, United States Forest Service, The Cherokee Nation, The Arkansas Game and Fish Commission—conservation and caving organizations, and private land owners coordinate the supervision of the tracts. This ecosystem approach to cave management was instituted when the refuge was renamed OPNWR in 1995.

Unfortunately, Oklahoma's Ozark Region (Ozark Highlands and Boston Mountains Level III Ecoregions; Woods et al., 2005) is floristically under surveyed. Illustrative of this lack of knowledge is that the only general surveys of the region are a master's thesis and doctoral dissertation completed by Charles Wallis, who collected 328 species in Adair County, although not within the current refuge boundaries (1953, 1959). In 2008, Hoagland and Buthod reported (2008a, b) the results of their surveys of the 6070 ha J.T. Nickel Family Nature and Wildlife Preserve in Cherokee County and a small site on the east shore of Grand Lake of Cherokees in Ottawa County. When publishing his classification of the vegetation of Oklahoma, Hoagland (2000) noted the need for further floristic studies of the Ozark Plateau because it is one of the two most botanically rich parts of the state.

Inventory of the vascular plants of the OPNWR began in 2001. Charriss York was commissioned by the USFWS to compile an inventory of the refuge's Sally Bull Hollow Tract in Adair County (Hayes, 2003). Her fieldwork is the beginning of the survey efforts described in this report. Following her work in Sally Bull Hollow, adjoining parcels of land known as the Eagle Pass and Workman Mountain tracts were acquired by the Oklahoma Department of Wildlife Conservation (ODWC) and managed cooperatively with the refuge. These three tracts were surveyed by Gard (2009) beginning in 2006. At the same time, nearby but disjunct parcels designated the Varmint, Liver and Gittin Down Mountain tracts were surveyed by Lowry (2010). The primary objectives of the work reported here were three: (1) to compile a list of the vascular plant species present in each tract; (2) to document, using GPS coordinates, the geographical locations of any species federally listed as endangered or threatened, or tracked as "of concern" by ONHI (2014); and (3) to prepare two sets of voucher specimens documenting the species present for use by refuge personnel and for deposition in the Oklahoma State University Herbarium (OKLA). Field work was conducted in the growing seasons of 2001 and 2002 in Sally Bull Hollow, and in all six tracts from 2006 through 2008.

ECOGEOGRAPHY OF THE OPNWR AREA

Geology & Soils—The six tracts of the OPNWR surveyed in this study are located southeast and southwest of Stilwell, Oklahoma in southern Adair County (Figure 1). They lie between latitude 35°45'N and 35°41'N, and longitude 94°44'W and 94°29'W and are situated in the Boston Mountains Level III Ecoregion at the southwestern edge of the area commonly known as the Oklahoma Ozarks (Woods et al., 2005). The Boston Mountains area is the highest and most rugged section of the region (Soil Conservation Service 1965; Unklesbay and Vineyard 1992). Topographically, it comprises ridges with steep escarpment faces and saddles separated by narrow valleys known locally as hollows. Elevation ranges from 265 to 456 m. Karst features such as caves and sinkholes are common. The area

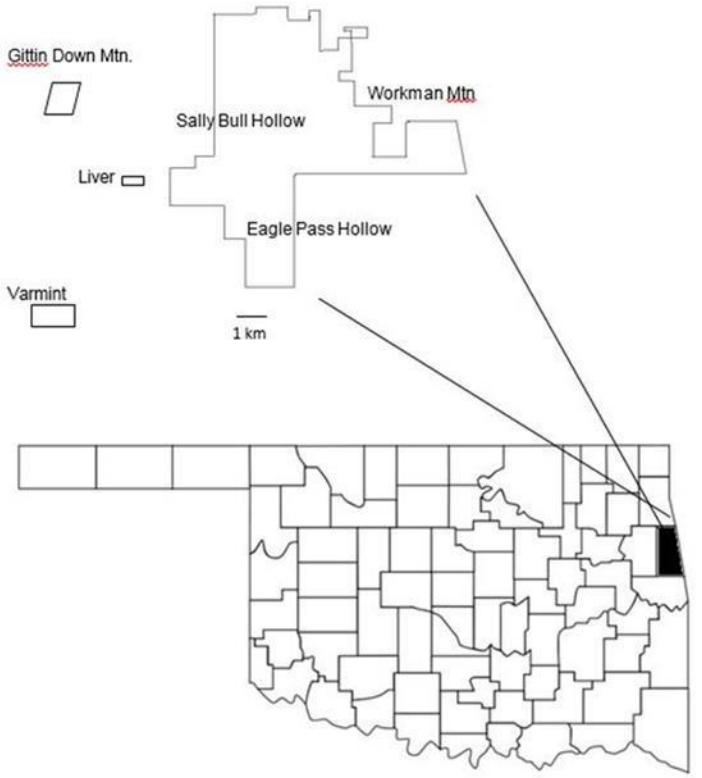

Figure 1. The six tracts of the Ozark Plateau National Wildlife Refuge approximately 7 km southeast and 3–4 km southwest of Stilwell, Adair County, Oklahoma.

bedrock is made up of dolomite, limestone, shale, and sandstone that have been weathered away by seasonal torrential rains developing into fast-flowing spring and creek systems (Goodman 1977). These meandering creeks and streams have carved steep ravines through the ridges. The major drainages are parallel to the area's fault lines which, in general, have a northeast orientation (Soil Conservation Service 1965). The sources of this stream water are seeps, which are common on the sloping hillsides and escarpments, flow from cave mouths, and intermittent or continual springs in the creek beds (Goodman 1977).

The parent rock strata of the region are limestone, sandstone, and marine shale of the Pennsylvanian and Mississippian ages (Branson and Johnson 1972). Atoka sandstone of the Pennsylvanian and some Cotter dolomite alluvium of the early Ordovician generally form the surface rocks (Soil Conservation Service 1965). These rocks are typically overlain by terrace and alluvial deposits of Quaternary age. Weathering of the limestone imparts the characteristic variability of the region—sinkholes, ridges, crevices, caves, and troughs (Goodman 1977). Caves are formed from weathering processes between the Hale layer in the Lower Morrow series of the Pennsylvanian system, and the Pitkin layer in the Upper Chesterian series of the Mississippian System (Russell 1971). The Hale layer often comprises the primary parent material of the upland forests while simultaneously acting as a ceiling above all cave entrances and hallways. Roots are visible along the ceiling of certain corridors of the caves.

The Soil Conservation Service (1965) cites four series—Hector, Linker, Etowah, and Greendale— and five associations—Linker Fine Sandy Loams, Hector-Linker Fine Sandy

Loams, Hector Complex, Bodine Silt Loams, and Etowah-Greendale—to be present in various parts of the refuge. The Hector soils are shallow, acidic, sandy or stony lithosols, and are by far the major soil type in the refuge and characteristic of the ridgelines. The Linker soils are on nearly level to moderately sloping mountaintops and are deep, fine sandy loams with few sandstone fragments in the surface layers and found only associated with the Hector soils. Etowah-Greendale soils are found in the creek beds associated with talus from cherty limestone and contain large amounts of gravel.

Climate—Adair County's climate is classified as Subtropical Humid (Trewartha 1968) and the Oklahoma Climatological Survey (2008) described it as warm-temperate with cool winters and hot humid summers. Average annual precipitation ranges from about 127 cm to nearly 137 cm throughout the county. May and June are the wettest months, on average. Nearly every winter has at least 2.5 cm of snowfall, with 67% of the years having 25 cm or more. Temperatures average near 15°C, with a slight increase from north to south. Temperatures range from an average daytime high of 33°C in July to an average low of -3°C in January. The growing season averages 192 days, but plants that can withstand short periods of colder temperatures often may have an additional four to eight weeks of growth. Significant daily temperature discrepancies within the refuge are due to the varied topography. Winds from the south to southeast are quite dominant, averaging nearly 12 km/h. Relative humidity, on average, ranges from 44% to 95% during the day.

Vegetation—The natural vegetation of the refuge and Boston Mountains ecoregion is primarily oak-hickory deciduous forest (Bruner 1931; Duck and Fletcher, 1943; Woods et al. 2005). Fifteen of the forest associations of Hoagland (2000) are present in addition to the alliances and associations correlated with the valley streams and anthropogenic disturbance. The area has a history of use by humans, albeit there is no formal written history of the changes in the county. Perhaps most notable was the resettlement of the Cherokee Indians from the southeast United States in the 1830s, followed by an influx of European settlers. Scattered farms and rural homes were established and the broader valleys were cleared to create pastures. Cattle were grazed on the slopes, hunters created access trails and campsites, and trees were harvested for timber. At the present time, local residents hunt, ride horses and ATVs along the trails, and cut firewood. Likewise, there is no formal account of the fire history of the area occupied by the refuge. Recollections of local residents such as Clayton Russell, Claude Liver, Neva Kirk, and Nancy Sawney, and refuge manager Steve Hensley, indicate that fires occur sporadically, cover areas of varying sizes, and burn until they die out naturally or are extinguished by local firefighters. Fire is certainly still a part of the ecogeography of the area, and our observations of burned areas revealed that the fires generally burned at low intensities and were patchy in nature. The understory layers of detritus and duff were consumed whereas the canopy and the subcanopy layers were essentially undamaged. Overall, fire seems to be a sporadic and natural part of the ecosystem in the refuge, doing little damage to trees and clearing away much of the preceding year's litter.

CHARACTERISTICS OF THE SIX TRACTS

The tracts known as Sally Bull Hollow, Eagle Pass and Workman Mountain are contiguous and form a unit of 922.6 ha approximately 7 km via county roads southeast of Stillwell, Oklahoma. The Gittin Down Mountain, Liver, and Varmint tracts are all separate properties 3 to 4 km southwest of Stillwell.

Sally Bull Hollow (SBH)—This tract comprises two meandering ridges oriented northeast to southwest with a narrow valley (the hollow) between them. The tops of the ridges are approximately 200 m above the valley floor, along which a small, spring-fed, intermittent stream flows. Numerous escarpments are present, as are numerous entrances to the extensive network of caves that infiltrates bedrock of the tract.

Anthropogenic disturbance is relatively slight, the major alterations include a dirt road that traverses the tract, hunter campsites, and occasional sites of firewood cutting. The area near the middle of the hollow burned in the fall of 2006.

Eagle Pass Tract (EP)—Included within this tract are both north- and south-facing escarpments formed by an east–west meandering ridge system bordering branches of spring-fed creeks in the valley known as Eagle Pass. A good deal of the tract is hilly, but there are some flat, upland areas sufficiently large enough to permit growth of xeric woodlands with patchy open canopies. The area is currently 90% forested, but was logged to an unknown extent in the past. Numerous cave entrances are found in the limestone ridges. The tract contains some highly disturbed areas in the form of debris and trash dumps and ATV paths winding across the upland areas. Disturbance also occurs adjacent to paved county road D0900RD that dissects the tract, as well as former logging sites in both the upland and lowland areas. Signs of recent fire were not apparent.

Workman Mountain Tract (WMT)—This tract is somewhat similar to the Eagle Pass Tract in that the terrain is quite steep, but it also features some gentler slopes as well. The major topographic feature is the north–south oriented Workman Mountain, with east–west ridges and valleys forming the flanking slopes. The headwaters of Indian Creek is a prominent feature in the tract. In the tract's upland areas, disturbance due to free-range livestock grazing has occurred. A dirt county road D4771RD dissects the tract. Signs of recent fire were not apparent.

Gittin Down Mountain Tract (GDM)—This 230.6 ha tract sits on the top and east-facing slope of a wide ridge oriented more or less north–south. Relief is approximately 100 m and below the irregularly undulating ridgeline, the east-face consists of gentle to steep rocky slopes and intermittent vertical rock faces. A small stream arising from seeps and springs on the slope flows out of the tract in the southeast corner. This corner also exhibited the greatest diversity in habitats due to the irregular topography of the slope. The vegetation is dense upland forest with the exception of the of tract's northwest corner which is savannah presumably due to past clearing. The ridge top and upper portions of the east-facing slope burned in the early spring of 2007, the second year of the collecting reported here.

Liver Tract (LT)—This 85 ha tract surrounds the source of Greasy Creek, which originates in the saddle between the two tallest of a north–south oriented series of hills collectively known as Welch Mountain. The majority of the tract comprises undulating, shallow, north- and south-facing slopes with occasional low escarpments that descend from the relatively level tops of the hills. Elevation ranges between 320 and 400 m on the tract. Near its source in the eastern part of the tract, the broader creek floodplain is bordered by more open forests on the slopes. Westward, the creek floodplain narrows and is bordered by dense forests as it flows into the broad valley on the west side of the tract. Signs of recent fire were not apparent.

Varmint Tract (VT)—This 24 ha tract includes a small stretch of land—primarily on the south side of Tributary No. 22 of Sallisaw Creek, which flows to the southeast. The creek is the north edge of a steep, north-facing, boulder-laden slope with a distinct bluff line approximately 10 m high. This slope descends from a relatively level upland ridge. Elevation within the tract ranges from 340 – 364 m. A dense forest covers the slope. Signs of recent fire were not apparent.

Methods

Floristic surveys began in 2001 with York's collecting in Sally Bull Hollow. She continued in 2002 and summarized her observations in a master's thesis (Hayes 2003). Gard and Lowry collected in all six tracts from 2006 through the 2008 growing season; their data likewise incorporated into theses (Gard 2009; Lowry 2010). Surveys were conducted throughout the growing season—early March through late October. Trips were made at three- to four-week intervals. Surveying involved traversing each tract repeatedly on foot using topographic maps (USGS 1:24,000), compass bearings, and global positioning system

(GPS) units in order to encounter as many different habitats and species as possible. Using compass bearings and topographic maps, transects were established to grid each tract so that it could be searched completely. Depending upon the topography and uniformity of vegetation, the distances between parallel transects varied from 100 to 400 m. Typically, the surveys involved following the north-south or east-west meandering ridges found in each tract and at periodic intervals, exploring the slopes by going downhill at right angles to the ridges, moving laterally and then returning uphill to the ridge tops. Topographic maps were continuously examined and areas with unusual features were targeted for specific exploration. In this way, all of the tracts were systematically traversed. In addition, an attempt was made to find as many different ecological habitats as possible (Palmer et al. 1995, 2002; Palmer 2007). The purpose of this intentional search was to encounter as many species as possible. Species listed as S1, S2, or S3 by ONHI (2014) were of particular interest due to their vulnerable status. As the tracts were explored, species were identified and recorded as present. Information about their abundance, habit, features of their habitat, and associated species were recorded. Relative abundance of a species was assessed using the five category system of Palmer et al. (1995) — abundant: dominant or codominant in one or more common habitats; frequent: easily seen in one or more common habitats but not dominant in any habitat; occasional: widely scattered but not difficult to find; infrequent: difficult to find with few individuals or colonies but found in several locations; and rare: very difficult to find and limited to one or very few locations or uncommon habitats.

Two or more voucher specimens of each species encountered were collected: one to be deposited in the Oklahoma State University Herbarium (OKLA) and one to be laminated for refuge personnel. An attempt was made to collect specimens in flower and/or fruit. Standard collecting and herbarium techniques were used in the preparation of the specimens and labels (Radford et al. 1974). When plants of species listed as S1, S2, or S3 by ONHI were encountered, they were not collected; rather their presence and GPS coordinates recorded for subsequent transmittal to USFWS personnel. Some of the abundant, easily recognized dominant species, e.g., *Fraxinus pennsylvanica*, present in all tracts were not vouchered each time they were encountered, but rather their presence simply recorded.

Specimens were examined and compared to specimens deposited in OKLA and unknowns were identified by using state and regional floras including Waterfall (1973), Tyrl et al. (2001-2010), Correll and Johnston (1970), and Diggs et al (1999). Taxa were not identified beyond the species level unless it was necessary to determine a taxon's rarity on the ONHI list. As the surveys progressed, lists of the species in each tract were compiled. The PLANTS *Database* (USDA NRCS 2009) served as the nomenclatural reference for the scientific and common names used and the designation of each species' nativity.

In this publication the species lists for the six tracts are combined in a single list. In addition, the family, genus, and species names have been updated as necessary to reflect those in current use by the Angiosperm Phylogeny Group (APG3; Stevens, 2001 onward) and the Integrated Taxonomic Information System (ITIS 2014) on-line database.

Results and Discussion

Floristic Overview—Five hundred sixty species in 102 families were documented. A complete species list can be accessed online at the journal website:

http://ojs.library.okstate.edu/osu/ Specific information about the species occurring in each tract, their abundance, and the habitats they occupy is available in Hayes (2003), Gard (2009), and Lowry (2010). These 560 species constitute 22% of Oklahoma's vascular flora of 2540 species (Tyrl et al., 2010). In terms of families, the Asteraceae, Fabaceae and the Poaceae dominate and together constitute about 33.1% of the flora of the six tracts. The largest genera were *Carex* (14 species), *Quercus* (10), *Dichanthelium* (8), *Desmodium (8),Viola* (7), *Plantago* (7), and *Lespedeza* (7). In general, differences in distribution of species were related to habitat

type and not correlated with the six tracts. As noted above, information about the flora and vegetation of the Oklahoma Ozarks is sparse and thus comparisons to other floristic studies limited. Hoagland and Buthod reported (2008a, b) 597 taxa to be present on the 6070 ha J.T. Nickel Family Nature and Wildlife Preserve in Cherokee County and 318 taxa present at a 532 ha site on the east-shore of Grand Lake of the Cherokees in Ottawa County.

New Records For Adair County—One hundred thirty-three species were new records for Adair County (ONHI 2014, Hoagland et al. 2004, Appendix). As noted previously, Wallis (1959) collected 328 species in Adair County, but did not collect within the present boundaries of the refuge.

Introduced Species—Of the 560 species encountered, 8.8% were introduced taxa in comparison with 14% for the state's flora as a whole (Tyrl et al 2010) and the 12.1% and 10.4% reported by Hoagland and Buthod (2008a, b) for the Nickel Preserve and Grand Lake sites respectively. Many were familiar weedy introductions from Eurasia, such as *Daucus carota* and *Bromus japonicus.* As would be expected, these introduced species were not found in abundance throughout each tract, but rather in the disturbed areas along trails, in man-made clearings, and at the road margins. Among these introduced species are six particularly invasive species: *Lespedeza cuneata, Lonicera japonica, Sorghum halepense, Microstegium vimineum, Albizia julibrissin,* and *Nasturtium officinale.* However, at the time of the surveys they did not appear to be flourishing or establishing large colonies or stands and were confined to the conspicuously disturbed soils of the banks and gravel bars of streams and roadsides. Other introductions such as *Rosa multiflora, Perilla frutescens, Dactylis glomerata, Potentilla recta,* and *Ranunculus sardous* were encountered throughout the tracts as scattered plants or small populations and typically not in the highly disturbed habitats occupied by the other introduced species.

Endangered, Threatened, and Species of Concern—Thirty-two species being tracked by the ONHI (2014) designated S1 or S2 were discovered in the six tracts (Table 1). The ONHI inventory ranks a species' rarity at state (S) levels and Naturserve (2014) rates the global (G) levels on a scale of 1–5. A ranking of 1 designates a plant as being critically imperiled (5 or fewer sites of occurrence or very few remaining individuals or acreage); 2 if it is imperiled (6–20 occurrences or few individuals or acreage remaining); 3 if it is rare and local in its range (or found locally in a restricted range with 21–100 sites); 4 if apparently secure, but may be quite rare in parts of its range, especially at the edges; and 5 if demonstrably secure, however it may be quite rare at the distributional limits of its range.

Plant Communities and Habitats—Based on our field observations and the classification of Hoagland (2000), 14 types of habitats occur in the six tracts of the OPNWR: (1) mesic slopes, (2) disturbed areas, (3) moist soils, (4) seeps, (5) cobble bars, (6) gravelly streams, (7) marshes and/or ponds, (8) uplands, (9) ravines, (10) lowlands, (11) woodlands, (12) riparian corridors, (13) xeric slopes, and (14) shallow rocky soils. The vegetation of the region is oak-hickory deciduous forest (Bruner, 1931; Duck and Fletcher, 1943; Woods et al., 2005). Using the classification of Hoagland (2000), 15 associations and one alliance occur in the six tracts. To facilitate use by refuge personnel, the 12 forest associations of Hoagland were combined into two more inclusive categories—xeric forest and mesic forest. Features of these two categories and those of five other vegetation types described by Hoagland are summarized in the following paragraphs.

Xeric Forests [XF]—This category comprises plants indicative of the *Quercus stellata-Quercus marilandica-Carya texana-Vaccinium arboreum, Quercus stellata-Quercus marilandica-Carya texana, Quercus stellata-Quercus shumardii-Carya cordiformis, Quercus stellata-Ulmus alata* forest associations. These xeric forests predominate on south-facing and exposed slopes, and intergrade with adjacent woodlands and prairies. Typical understory species include: *Andropogon gerardii, Carex*

Table 1. Thirty-two taxa encountered in this survey designated of concern and tracked by the Oklahoma Natural Heritage Inventory (2014). Global and state rankings follow each taxon. Where T-rankings are given, T refers to the global status of the subspecific taxon indicated; by expressing the rank as a range, e.g. G4G5, the rank lies somewhere between these two values; H-rankings mean all sites are historical; ? following a rank indicates the level of certainty lies between that rank and the next level of ranking; Q-rankings denote there are taxonomic questions associated with the taxon indicated.

Species of Concern	Common name	Family	Status
Maianthemum racemosum ssp. *racemosum*	feathery false lily of the valley	Asparagaceae	S3 G5T5
Asplenium rhizophyllum	walking fern	Aspleniaceae	S3 G5
Ionactis linariifolia	flaxleaf aster	Asteraceae	S1 G5
Impatiens pallida	pale jewelweed	Balsaminaceae	S2 G5
Boechera dentata	Short's rockcress	Brassicaceae	S1 G5
Uvularia grandiflora	large-flowered bellwort	Colchiaceae	S1 G3
Silene regia	royal catchfly	Caryophyllaceae	S2 G3
Tradescantia ozarkana	Ozark's spiderwort	Commelinaceae	S2 G3GHQ
Carex cephalophora	oval-leaf sedge	Cyperaceae	S2 G5
Dryopteris filix-mas	male fern	Dryopteridaceae	SH G5
Monotropa uniflora	Indianpipe	Ericaceae	S1 G5
Desmodium pauciflorum	fewflower ticktrefoil	Fabaceae	S1 G5
Castanea ozarkensis	Ozark chinquapin	Fagaceae	S2 G5T3
Hamamelis vernalis	Ozark witchhazel	Hamamelidaceae	S3 G4?
Blephilia ciliata	downy woodmint	Lamiaceae	SH G5
Tilia americana	American basswood	Malvaceae	S3 G5T5
Corallorhiza wisteriana	spring coralroot	Orchidaceae	S1 G5
Triphora trianthophora	nodding pagonia	Orchidaceae	S1 G3G4
Agalinis tenuifolia	slenderleaf false foxglove	Orobanchaceae	S1 G3
Dicentra cucullaria	Dutchman's breeches	Papaveraceae	S2 G5
Brachyelytrum erectum	bearded shorthusk	Poaceae	S3 G5
Diarrhena americana	American beakgrain	Poaceae	S1 G4?
Elymus hystrix var. *hystrix*	bottlebrush grass	Poaceae	S2 G5T5
Cheilanthes alabamensis	Alabama lipfern	Pteridaceae	S1 G4G5
Clematis virginiana	devil's darning needles	Ranunculaceae	SH G4G5
Rosa woodsii	Wood's rose	Rosaceae	S1 G5
Rubus allegheniensis	Allegheny blackberry	Rosaceae	S3 G5
Galium arkansansum	Arkansas bedstraw	Rubiaceae	S2 G5
Houstonia ouachitana	Ouachita bluet	Rubiaceae	S2 G3
Urtica chamaedryoides	weak nettle	Urticaceae	S3 G4G5
Urtica dioica ssp. *gracilis*	stinging nettle	Urticaceae	SH G5T5
Vitis mustangensis	mustang grape	Vitaceae	S2 G4?
Total 32 taxa		26 families	

albicans, Danthonia spicata, Helianthus hirsutus, Rhus spp., *Schizachyrium scoparium, Sporobolus asper, Symphoricarpos orbiculatus, Tephrosia virginiana,* and *Vaccinium arboreum*.

Mesic Forests [MF]—This category includes plants of the *Acer saccharum-Quercus alba-Carya alba, Acer saccharum-Quercus rubra-Carya cordiformis, Quercus alba-Carya alba-Tilia americana, Quercus falcata-Carya alba, Quercus muehlenbergii-Acer saccharum, Quercus muehlenbergii-Quercus shumardii, Quercus rubra-Quercus shumardii,* and *Fraxinus pennsylvanica-Ulmus americana* forest associations. Mesic forests predominate on north-facing slopes, protected slopes, and in bottomland areas. Associated species include: *Adiantum pedatum, Geranium maculatum, Hypericum hypericoides, Juglans nigra, Monarda* spp., *Myosotis verna, Nyssa sylvatica, Ostrya virginiana, Pedicularis canadensis, Sassafras albidum, Silene virginica,* and *Woodsia obtusa*.

***Acer saccharinum-Acer negundo* Forest Association [ASAN]—**This association dominates stream margins and the slopes immediately adjacent to them. Associated

species include: *Betula nigra, Castanea ozarkensis, Lindera benzoin, Melica nitens, Polygonum* spp., *Quercus rubra, Toxicodendron radicans,* and *Vitis mustangensis.*

***Betula nigra-Platanus occidentalis* Forest Association [BNPO]—**This association co-dominates with the ASAN association on cobble bars and in moist soils associated with seeps and streams. Typical species include: *Acer negundo, Chasmanthium latifolium, Parthenocissus quinquefolia, Phytolacca americana, Tridens flavus,* and *Hamamelis vernalis.*

***Quercus stellata-Quercus marilandica-Schizachyrium scoparium* Woodland Association [QSQM]—**Upland areas on ridge tops are often covered with this association. It characteristically has a more open canopy than the surrounding forests. The trees are scrubby in some cases. Associated species include: *Andropogon gerardii, Antennaria parlinii, Baptisia bracteata* var. *leucophaea, Carya texana, Crataegus crus-galli, Cornus drummondii, Juniperus virginiana, Prunus mexicana, Viburnum rufidulum, Rhus glabra, Schizachyrium scoparium,* and *Symphoricarpos orbiculatus.*

***Nasturtium officinale* Herbaceous Alliance [NOHA]—**predominately in gravely streams and cobble bars, or free floating in streams, this alliance is dominated by *Nasturtium*. Associated taxa include: *Carex* spp., *Eleocharis* spp., *Juncus* spp., and *Scirpus* spp.

Disturbed Areas [DIST]—This association was located along roadsides and ATV paths that cut through the refuge, or in areas of intensive human alteration such campsites, logged areas, or refuse dump sites. Common plants encountered were: *Verbascum thapsus, Achillea millefolium, Coreopsis tinctoria, Hordeum pusillum, Microstegium vimineum, Salix caroliniana, Rhus copallinum, Lespedeza cuneata,* and *Trifolium* spp.

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Haemogamasus harperi Keegan (Arthropoda: Acari: Laelapidae): New to the Mite Fauna of Oklahoma

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Little is known about the parasitic and phoretic mite (Acari) fauna of vertebrates of Oklahoma. In their summation on mites of North American wild mammals north of Mexico, Whitaker et al. (2007) list only seven species from mammals of Oklahoma. Of these, one was reported from rodents (Elsen and Whitaker 1985) and the remainder from bats (Reisen et al. 1976; OConnor and Reisen 1978). More recently, McAllister et al. (2013) reported a louse from a fox squirrel, *Sciurus niger*, in McCurtain County, Oklahoma. Therefore, nothing is known of mites from any of the five insectivores (Caire et al. 1989) from the state. Here we report, for the first time, a species of parasitic mite from the eastern mole, *Scalopus aquaticus* from Oklahoma.

On 7 August 2014, an adult male *S. aquaticus* was collected alive by hand from its burrow under a rock on the campus of Eastern Oklahoma State College in Wilburton (Latimer County) off North Hill Street (34.915679°N, 95.327487°W). The mole died within 48 hr and its pelage was searched for ectoparasites following previous methods (Connior et al. 2014). Three mites were collected and placed in vials containing 70% ethanol and shipped to the junior author for identification. Mites were cleared in lactophenol and slide-mounted in Hoyer's medium (Walters and Krantz 2009). Voucher specimens of mites are deposited in the General Ectoparasite Collection in the Department of Biology at Georgia Southern University (accession no. L3698). The voucher host is deposited in the Henderson State University (HSU) collection, Arkadelphia, Arkansas.

Two parasitic mites (1 female, 1 nymph) identified as *Haemogamasus harperi* Keegan, 1951 were recovered from the mole. Keegan (1951) originally described *H. harperi* from the least shrew, *Cryptotis parva* from Georgia. This large mite has been previously reported from moles, shrews and voles from nine states, including nearby Arkansas, Louisiana and Texas (Keegan 1951; Whitaker and Wilson 1974; Whitaker et al. 2007; McAllister and Wilson 2012; Connior et al. 2014) (Fig. 1). Previous reports of this ectoparasite on *S. aquaticus* include Keegan (1951) in Georgia and Mississippi, Whitaker and Schmelz (1974) in Indiana, Wilson and Durden (2003) in Georgia, and McAllister and Wilson (2012) in Texas. It has not been, to our knowledge previously reported from any mammalian host in Oklahoma. We therefore document a new state record for *H. harperi* in Oklahoma. A third mite specimen recovered from the mole was an adult female *Tyrophagus putescentiae* (Schrank), a widespread, non-parasitic mold-consuming acarid mite that is associated with stored products, plants and, sometimes, mammal nests (OConnor 2009).

Oklahoma supports an exceptionally rich mammalian fauna (Caire et al. 1989) distributed over 12 ecoregions of the state (http://www.forestry.ok.gov/ecoregions-of-oklahoma). Yet, compared to surrounding states, little is known about the ectoparasite fauna of Oklahoma's mammals. With additional study, the geographic distribution and host associations of this fauna will surely increase and the likelihood of discovering new species is a further possibility.

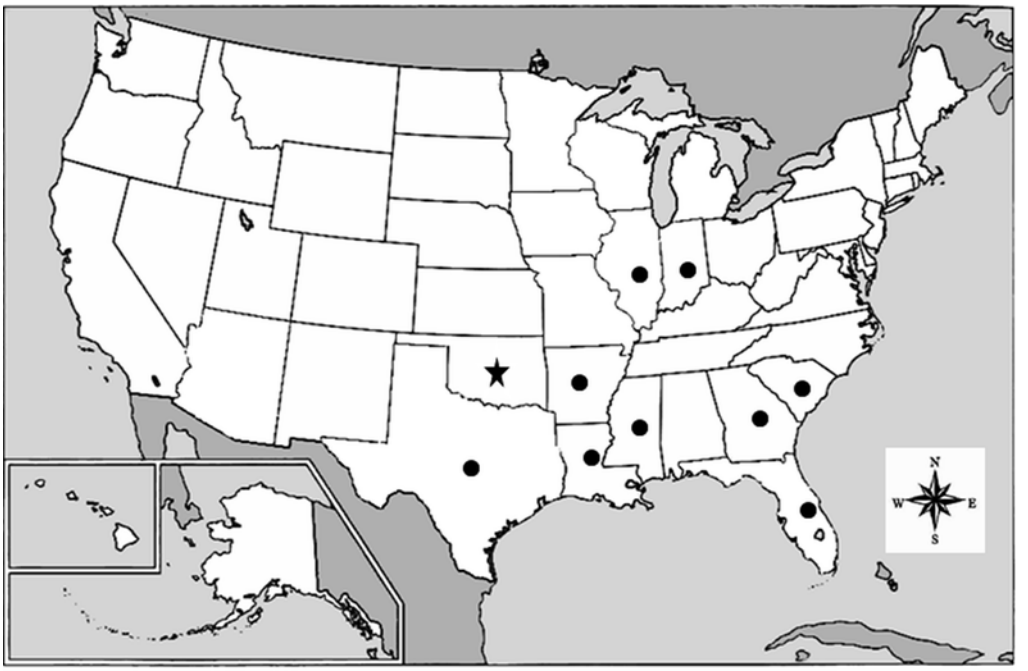

Figure 1. Records of *Haemogamasus harperi* from 10 states. Dots = previous records; star = new state record.

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Isospora scinci (Apicomplexa: Eimeriidae) from Five-Lined Skinks, *Plestiodon fasciatus* (Sauria: Scincidae): Additional Records from Arkansas and First Report from Oklahoma

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A moderate amount of information is available on coccidian parasites (Apicomplexa) of snakes of Oklahoma (McAllister et al. 1995, 2011, 2013), and, although another reptile from the state, the ground skink (*Scincella lateralis*) has been thoroughly surveyed (McAllister et al. 1994, 2014), nothing, to our knowledge, has been published on coccidia of any lizard in Oklahoma. Yet, Oklahoma supports 19 species and subspecies of lizards in its diverse habitats (Sievert and Sievert 2011). Here we report, for the first time, a species of coccidian from the five-lined skink, *Plestiodon fasciatus* (L.) from Oklahoma and add information on this coccidian from additional isolates obtained from *P. fasciatus* from Arkansas.

Between February 2010 and August 2014, 37 juvenile and adult (mean ± 1SD snout-vent length [SVL] = 57.6 ± 10.5, range 40-74 mm) *P. fasciatus* were collected by hand from McCurtain County, Oklahoma (n = 14) and Bradley (n = 1), Calhoun (n = 2), Faulkner (n = 1), Greene (n = 1), Hempstead (n = 1), Marion (n = 2), Searcy (n = 1), and Union (n = 14) counties, Arkansas. Fresh fecal samples were collected from the rectum of each individual for examination of coccidia and placed in individual vials containing 2.5% (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$). They were examined by light microscopy after flotation in Sheather's sugar solution (specific gravity = 1.30). Measurements were taken on 5–30 sporulated oocysts from three lizards using a calibrated ocular micrometer and reported in micrometres (µm) with means followed by the ranges in parentheses; photographs were taken using Nomarski interference-contrast optics. Oocysts were up to ~257 days old when measured and photographed. Descriptions of oocysts and sporocysts follow guidelines of Wilber et al. (1998) as follows: oocyst length (L) and width (W), their ranges and ratios (L/W), micropyle (M), oocyst residuum (OR), polar granule(s) (PG), sporocyst length (L) and width (W), their ranges and ratio (L/W), sporocyst (SP), Stieda body (SB), substieda body (SSB), parastieda body (PSB), sporocyst residuum (SR). Photovouchers of the parasites are deposited in the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, Nebraska. Host vouchers are deposited in the

Table 1. Select measurements for oocysts and sporocysts of *Isospora scinci* from *Plestiodon fasciatus*.

Isolate (no. oocysts measured)	Oocysts (L × W) µm (mean L/W ratio)	Sporocysts (L × W) µm (mean L/W ratio)	Reference
Van Buren Co., AR (*n* = 25)	26.5 × 24.3 (22–31 × 18–27) (1.1)	14.9 × 10.4 (12–16 × 9–12) (1.4)	Upton et al. (1991)*
Bradley Co., AR (*n* = 20)	25.3 × 22.9 (23–27 × 22–24) (1.1)	13.3 × 8.6 (12–14 × 8–10) (1.5)	This report
Marion Co., AR (*n* = 5)	25.1 × 23.3 (22–27 × 21–25) (1.1)	14.4 × 8.9 (13–16 × 8–10) (1.6)	This report
McCurtain Co., OK (*n* = 30)	26.0 × 22.9 (24–31 × 21–25) (1.1)	13.8 × 9.2 (11–16 × 8–10) (1.5)	This report

*Original description

Arkansas State University Museum of Zoology (ASUMZ) Herpetological Collection, State University, Arkansas. Lizard taxonomy follows the TIGR reptile database (Uetz and Hošek 2014).

Two (14%) *P. fasciatus* (two males, 71 and 65 mm SVL) collected on 4 July 2013 and 15 September 2014 from Hochatown, McCurtain County, Oklahoma (34.171321ºN, 94.751792ºW) was found to be passing sporulated oocysts (Fig. 1A) matching the description of *Isospora scinci* Upton, McAllister and Trauth, 1991 (Upton et al. 1991). Oocysts (*n* = 30) were ovoidal, (L × W) 26.2 × 22.8 (24–31 × 21–25) with a L/W ratio of 1.1 (1.0–1.3); a M, OR and PG were absent. The oocyst wall was smooth and bilayered, measuring 1.0 (inner 0.4, outer 0.6). SP were ovoidal with a distinct point on the end opposite the SB, 13.8 × 9.2 (11–16 × 8–10) with a L/W ratio of 1.5 (1.3–1.7). A SB with a distinct knob was present as well as a distinct SSB; PSB absent. The SR was composed of dispersed granules. When compared to the original description of *I. scinci* from *P. fasciatus* from Arkansas (Table 1), measurements were well within the ranges reported except for SP width, which was slightly smaller (9.2 [8–10] vs. 10.4 [9–12] µm) in our isolate.

Of the 23 five-lined skinks from Arkansas, two (9%) *P. fasciatus*, one (male, 74 mm SVL) collected on 1 May 2013 from 1 km N of Ouachita River at US 63, Bradley County (33.311446ºN, 92.34189ºW) and the other (female, 58 mm SVL) collected on 14 June 2013 from Mull off 448 St. Hwy 268E, Marion County (36.080216ºN, 92.597427ºW) were also found to be passing oocysts of *I. scinci* (Table 1, Figs. 1B, C). *Isospora scinci* was originally described from three of 13 (23%) *P. fasciatus* from Arkansas (Upton et al. 1991). In addition, McAllister et al. (1994) reported *I. scinci* from one of four (25%) broadhead skinks, *Plestiodon laticeps* from Arkansas. Prevalence of infection with this coccidian was reported to be moderate (23%) in Arkansas *P. fasciatus* (Upton et al. 1991; McAllister et al. 1994) while in the current study, it was much lower (9%) in our sample of *P. fasciatus* from the state. However, measurements of these two isolates from Arkansas *P. fasciatus* were within the ranges reported for *I. scinci* (Table 1).

In summary, we document *I. scinci* in Oklahoma for the first time. More importantly, this is the initial coccidian reported from any lizard of the state. Additional surveys are certainly warranted on other lizards which should increase our knowledge of the coccidian biodiversity of Oklahoma and the southwestern U.S.

Acknowledgments

The Oklahoma Department of Wildlife Conservation provided scientific collecting permits to C. T. McAllister (CTM) and the

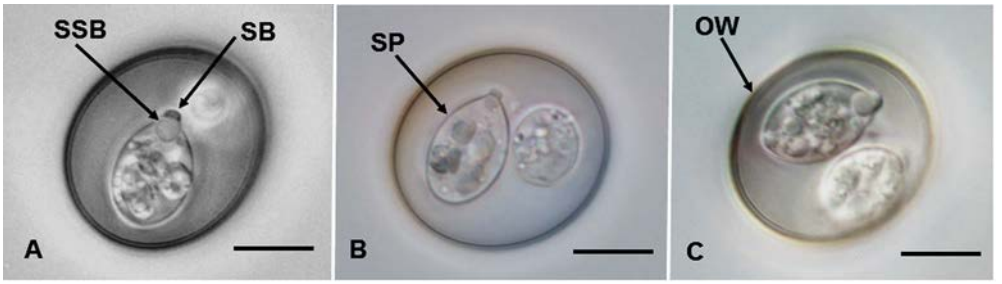

Figure 1. Three isolates of *Isospora scinci* from *Plestiodon fasciatus*. A. HWML 75059 from McCurtain County, Oklahoma. B. HWML 75060 from Bradley County, Arkansas. C. HWML 75061 from Marion County, Arkansas. Abbreviations: OW (oocyst wall); SP (sporocyst); SB (Stieda body); SSB (substieda body). Scale bars = 10 µm.

Arkansas Game and Fish Commission provided scientific collection permits to CTM and M. B. Connior. We thank Drs. S. E. Trauth (ASUMZ) and S. L. Gardner (HWML) for expert curatorial assistance. This project was, in part, supported by a grant from the National Institute of General Medical Sciences (8 P20 GM103432) from the National Institutes of Health to R. S. Seville. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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New County Records for the Eastern Mole, *Scalopus aquaticus* (Soricimorpha: Talpidae) and Woodland Vole, *Microtus pinetorum* (Rodentia: Cricetidae), in Oklahoma

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The eastern mole, *Scalopus aquaticus* (L.) is a robust insectivore that ranges from the northeastern United States west to the Midwest as far as southeastern Wyoming and south through Texas to northern Tamaulipas, Mexico; it is also found in eastern Canada (Yates and Schmidly 1978; Schmidly 1994; Reid 2006). In Oklahoma, *S. aquaticus* occurs over most of the state where suitable soils allow extensive burrowing (Caire et al. 1998); however, there are apparently no records for eight counties of the state (Fig. 1).

The woodland vole, *Microtus pinetorum* is a small mouse found throughout the eastern and midwestern U.S. and extreme southern Ontario, Canada, from southern Maine southwest to central Texas and southern Louisiana (Smolen 1981; Reid 2006). In Oklahoma, *M. pinetorum* is found predominantly in the eastern one-half of the state (Caire et al. 1998). Here, we document new county records for *S. aquaticus* and *M. pinetorum* in southeastern Oklahoma.

On 6 August 2014, a sub-adult male *S. aquaticus* (total length 125 mm, length of tail 21 mm, length of hind foot 20 mm) was collected alive by hand from underneath a rock in its unexposed burrow on the Eastern Oklahoma State Campus off North Hill Street in Wilburton, Latimer County (34.915673ºN, 95.327506ºW). There were no obvious runs (burrow system) atop the ground. Habitat was a mowed lawn dominated with an overstory of oaks (*Quercus* spp.) and pines (*Pinus* spp.). The voucher specimen (alcoholic) was deposited in the Henderson State University Mammal Collection (HSU), Arkadelphia, Arkansas as HSU 706.

Since the seminal publication of Caire et al. (1989), 10 additional county records for *S. aquaticus* have been documented as follows: Alfalfa (McDonald et al. 2006), Cimarron (Dalquest et al. 1990), Cotton (Stangl et al. 1992), Delaware (Braun and Revelez 2005), Jefferson (Stangl et al. 1992), Kiowa (Braun and Revelez 2005), Roger Mills (Braun and Revelez 2005), Texas (Roehrs et al. 2008), Tillman (McDonald et al. 2006), and Washita (Clark and Tumlison 1992). Prior to the present record in southeastern Oklahoma, there was a five-county hiatus in the range of *S. aquaticus* with noticeable distributional gaps in Atoka, Coal, Choctaw, Haskell and Latimer counties (Caire et al. 1989, p. 103) (Fig. 1). It is unknown why the eastern mole has not been previously reported from these counties since moist alluvial and sandy soils are present as well as Baird's pocket gophers, *Geomys breviceps* who also use these soils (see Connior et al. 2013). In addition, south of Choctaw County, Oklahoma, across the Red River in Texas, vouchers of eastern moles have not yet been collected in Fannin, Lamar and Red River counties (Schmidly 1994). We

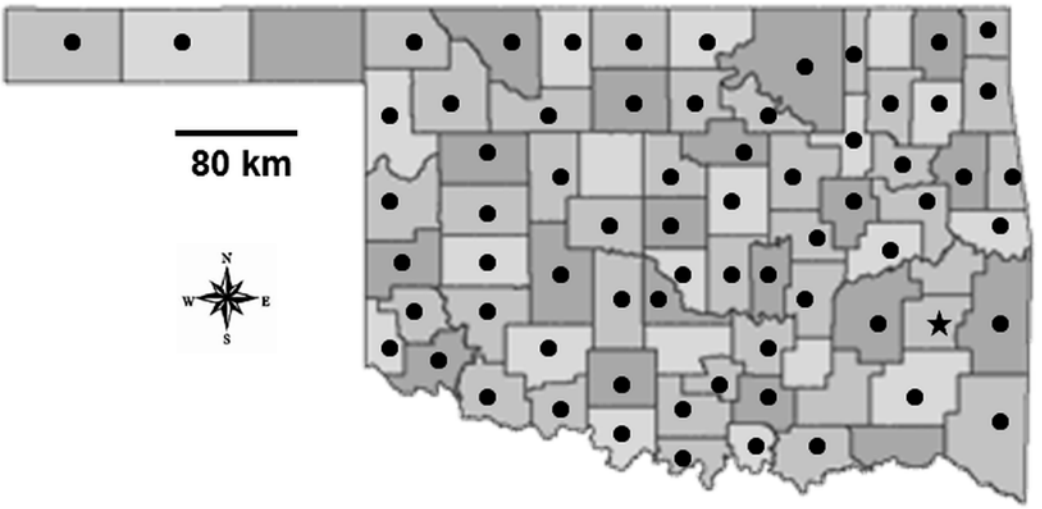

Figure 1. Records of *Scalopus aquaticus* in counties of Oklahoma. Dots = previous records; star = new record.

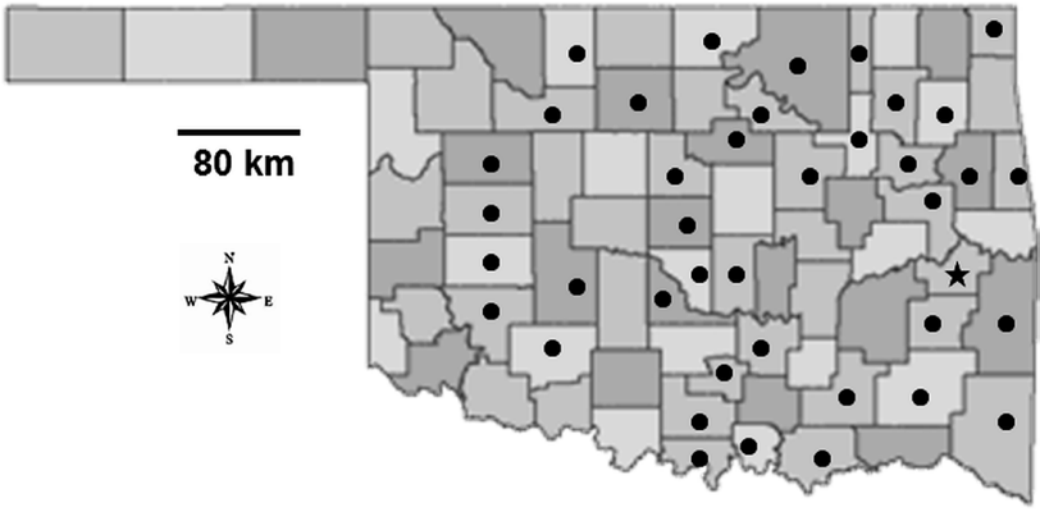

Figure 2. Records of *Microtus pinetorum* in counties of Oklahoma. Dots = previous records; star = new record.

recommend further attempts be made at collecting *S. aquaticus* in counties without vouchers in order to help explore this oddity.

An adult female *M. pinetorum* (total length 138 mm, length of tail 30 mm, length of hind foot 16 mm, length of ear 13 mm; weight 40.7 g), deposited in the Eastern Oklahoma State College Collection-Wilburton (EOSC 48), was collected by M. Nunn on 10 January 1989 from SE of Stigler Lake, Haskell County (35.235156ºN, 95.106121ºW). The habitat consisted of open fields with *Pinus* and *Quercus* spp.

Caire et al. (1989, p. 258) showed 27 counties of Oklahoma with previous records of *M. pinetorum* (Fig. 2). Numerous records have been documented since 1989, including those for Love and Murray (Stangl et al.1992), Washita (Clark and Tumlison 1992), Le Flore (Lutterschmidt et al. 1996), Major and Kiowa

(Braun and Revelez 2005), and Alfalfa, Custer, Dewey, Garfield, Kay, Mayes, and Ottawa (McDonald et al. 2006) counties. The current record (Fig. 2) helps fill a gap in records from southeastern Oklahoma and those more northward in Muskogee County.

Acknowledgments

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Record of North American Porcupine (*Erethizon dorsatum*) from Tulsa Co.

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Abstract: Despite a well-documented eastward expansion of North American porcupine (*Erethizon dorsatum*) along a broad front in Oklahoma, there previously have been no records from Tulsa Co. Here, we report details of a road-killed porcupine found near the Arkansas River in central Tulsa Co.

On the morning of Sept. 9, 2014, a dead North American porcupine (*Erethizon dorsatum*) was found along Avery Dr. in Tulsa Co., OK. It is likely that the animal was a road-kill from the previous night, as it was not present the previous afternoon. Photographs were taken and two quills were collected. Direct measurements were not possible at the time of the initial observation. However, the length of the animal could be estimated (55-60 cm) by normalizing the length of the animal to a 4 inch wide road edge line in photographs. This size range suggests that the animal was a young adult. Unfortunately the sex of the animal was not recorded. Returning for more detailed assessment of the animal was not possible because the carcass had been removed.

The portion of Avery Dr. where the animal was found is in the riparian zone along the Arkansas River with the average distance to the river in that area being approximately 50 m. Tyler and Joles (Tyler and Joles 1997) noted a preponderance of porcupine records from riparian areas and suggested that porcupines use riparian areas as dispersal routes. Thus, the location of the road-kill is consistent with known patterns of porcupine dispersal. Moreover, the time of year is consistent with increased activity in the fall (especially in September) by porcupines reported in other areas (Barthelmess and Brooks 2010), which likely reflects the onset of breeding or natal dispersal (Roze 2009).

The first Oklahoma record for porcupine, from Cimarron Co., was published in 1939 (Chase 1939). Over the next decade, porcupine numbers in that area increased rapidly. By 1949, they were considered to be a pest species: Glass and a party from Oklahoma A & M captured three in a two week period (Glass 1949). At that time, the porcupine was largely absent from the remainder of Oklahoma. In a 1997 review of porcupine reports from Oklahoma away from Cimarron Co., Tyler and Joles (1997) documented range expansion eastward as far as Pottawottamie Co, and a single record from Latimer Co. More recent reports have established the continued presence of porcupines in the western half of Oklahoma, and showed further eastward expansion: porcupine records now have been reported for several of the easternmost counties in Oklahoma (Tyler and Haynie 2001, Caire and Smith 2008). However, even the most recent

review (Caire and Smith 2008) did not include any records for Tulsa Co. This report fills that gap.

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Helminth Parasites of Select Cyprinid Fishes from the Red River Drainage of Southeastern Oklahoma

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Abstract: Between June and September 2014, 76 cyprinid fishes (seven taxa) collected from four sites in the Red River drainage of McCurtain County, Oklahoma, were examined for helminth parasites. Five endoparasites (four trematodes, one cestode) were found in 10 of 76 (13%) fish including: *Allocreadium lobatum* in Striped Shiners (*Luxilus chrysocephalus isolepis*), *Rhipidocotyle* sp. in Blacktail Shiners (*Cyprinella venusta*), *Postdiplostomum minimum* in a Highland Stoneroller (*Campostoma spadiceum*), *Clinostomum marginatum* in *L. c. isolepis*, and *Proteocephalus* sp. in Steelcolor Shiners (*Cyprinella whipplei*) and *L. c. isolepis*. In addition, the following were negative for helminths: Redfin Shiners (*Lythrurus umbratilus cyanocephalus*), Bigeye Shiners (*Notropis boops*) and Creek Chubs (*Semotilus atromaculatus*). Four new host and two new geographic distributional records are documented. There is a continued need to survey additional non-game fishes of the state for helminths as new host and distributional records are predicted as well as the possibility of discovery of new species.

Introduction

There are 176 species of fishes in Oklahoma (Miller and Robison 2004), yet little is known regarding helminth parasites of non-game species. The following papers report fragmentary information on various helminths of non-game fishes of Oklahoma: Self (1954) on Goldeyes; Self and Timmons (1955) on River Carpsuckers; Self and Campbell (1956) on buffalo fishes; Roberts (1957) on Carp; Calentine and Mackiewicz (1966), Mackiewicz (1964, 1968, 1969, 1970) and Williams and Ulmer (1971) on caryophyllaeid tapeworms of various catostomid fishes; Spall (1969) on parasites of fishes of Lake Carl Blackwell; Scalet (1971) on Orangebelly Darters; Oetinger and Buckner (1976) on Sunburst Darters; and McAllister and Bursey (2013) on Pirate Perches. Therefore, as recently noted by Scholz and Choudhury (2014), studies on freshwater fish parasites are mostly lacking with an obvious paucity of reports on helminth parasites of non-game fishes of Oklahoma. Here, we continue to augment that information by documenting new distributional and host records for select cyprinid fishes from four sites in the Red River drainage of McCurtain County, Oklahoma.

Methods

Between June and September 2014, 76 individual fishes (seven taxa) including 10 Highland Stonerollers (*Campostoma spadiceum*), 13 Blacktail Shiners (*Cyprinella venusta*), 20 Steelcolor Shiners (*Cyprinella whipplei*), 12 Striped Shiners (*Luxilus chrysocephalus isolepis*), seven Redfin Shiners (*Lythrurus umbratilus cyanocephalus*), 10 Bigeye Shiners (*Notropis boops*) and four Creek Chubs (*Semotilus atromaculatus*) were collected by dipnet or 3.7 m (1.6 mm mesh) seine from Yashau Creek at the US 70 bridge (33.98705°N, 94.74329°W), Yashau Creek at Memorial Street (34.011421ºN, 94.749924ºW), Beaver Creek, a tributary of the Mountain Fork River at Beavers Bend State Park (34.132033°N, 94.679418°W) and Yanubbee Creek N of Broken Bow off US 259 (34.062097ºN, 94.73965ºW). Fish were placed in aerated creek water, taken to the laboratory for necropsy within 24 hr and killed by prolonged immersion in a concentrated chloretone® (chlorobutanol) solution. The gills and gill filaments were not examined for monogenean trematodes. A mid-ventral incision was made to expose the viscera and the entire gastrointestinal tract and other organs were examined for helminths. Trematodes and cestodes were fixed in hot tap water without coverslip pressure, stained with acetocarmine, dehydrated in a graded ethanol series, and mounted in Canada balsam. Voucher specimens were deposited in the Harold W. Manter Laboratory of Parasitology (MWML), Lincoln, Nebraska. Host voucher specimens were deposited in the Henderson State University Herpetological Collection (HSU), Arkadelphia, Arkansas as HSU lots 3591-3593, 3595-3600.

Results and Discussion

Ten of 76 (13%) fish, including one (10%) *C. spadiceum*, two (15%) *C. venusta*, three (15%) *C. whipplei*, and four (33%) *L. chrysocephalus* harbored helminths; all infected fish came from Yashau Creek. The helminths found are presented below in annotated format.

Trematoda: Digenea: Allocreadiidae

***Allocreadium lobatum* Wallin, 1909 (Figs. 1-2)**

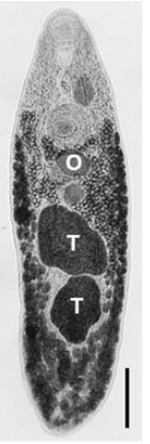

Figure 1. *Allocreadium lobatum* from *Luxilus chrysocephalus*. Note tandem testes (T) and ovary (O). Scale bar = 500 µm.

Two each *A. lobatum* were found in the small intestine of two adult (105, 140 mm TL) *L. chrysocephalus*. These digeneans measured (mean L × W µm): body, 4,147 × 1,113, oral sucker, 349 × 359, pharynx, 170 × 201, cirrus sac, 490 × 218, ventral sucker, 407 × 399, ovary, 289 × 209, seminal receptacle, 213 × 205, anterior testis, 612 × 601, posterior testis, 640 × 510, ova, 65 × 44. These measurements fall within ranges previously reported for *A. lobatum* (Willis 2002). This is the second time *A. lobatum* has been reported from *L. chrysocephalus* (Kentucky, Aliff 1977); however, we report this digenean from Oklahoma for the first time. In addition, *A. lobatum* has been reported from various fishes from Kentucky, Idaho, Indiana, llinois, Maine, Michigan, Nebraska, Ohio, North Dakota, West Virginia, Wisconsin and Wyoming (Hoffman 1999; Willis 2001, 2002; Barger 2006). In the life cycle of *A. lobatum*, the first intermediate host is a sphaeriid clam (*Pisidium* spp.) and the second, amphipods (*Cragionyx gracilis*, *Gammarus pseudolimnaeus*) and isopods (*Caecidotea communis*, *C. intermedius*) (DeGiusti 1962; Schell 1985; Camp 1989). In addition to documenting a new state record, we report the southernmost distribution for *A. lobatum* in North America (Fig. 2).

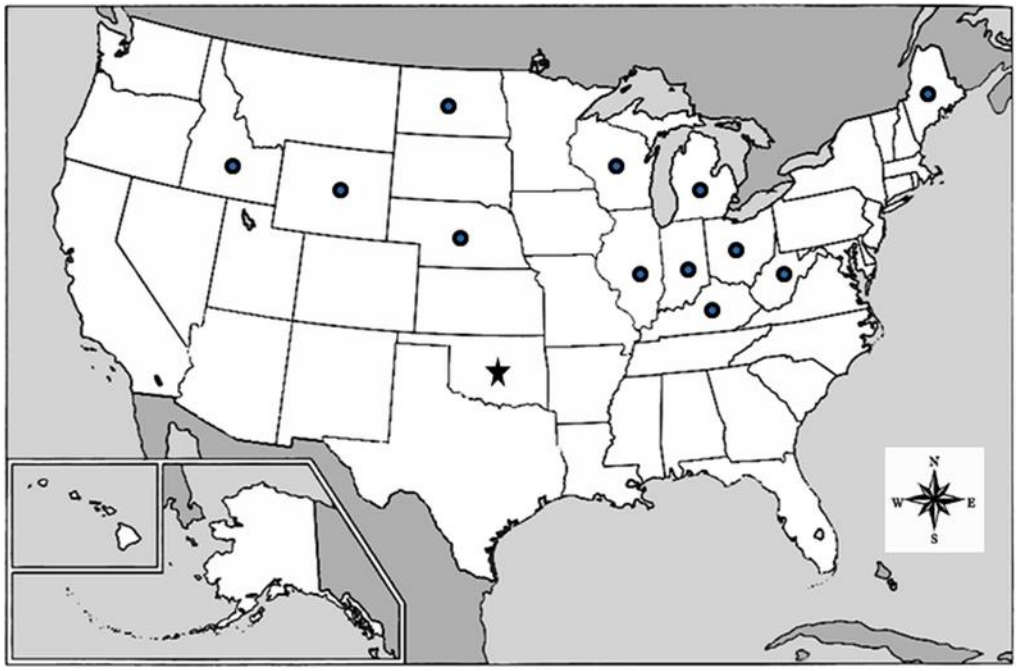

Figure 2. Records of *Allocreadium lobatum* in the United States. Dots = previous records; star = new record. There are also records from Canada (not shown).

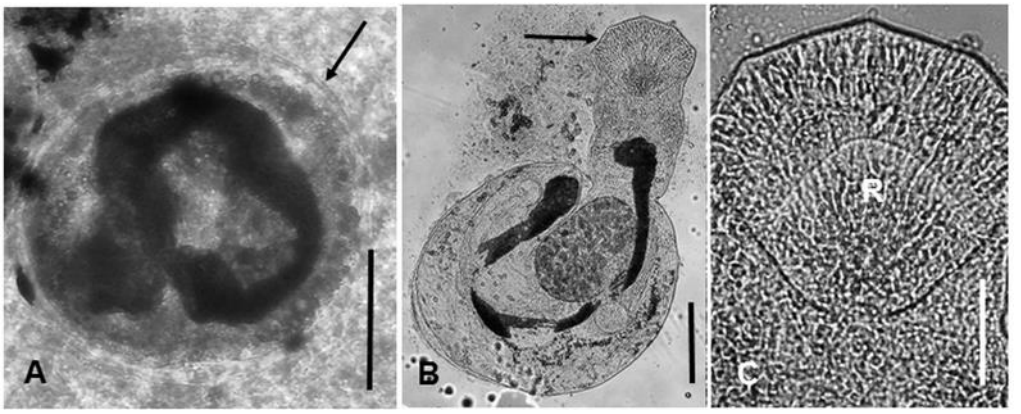

Figure 3. *Rhipidocotyle* sp. from *Cyprinella venusta*. A. Cyst (arrow) in liver containing metacercaria. Scale bar = 50 µm. B. Metacercaria removed from cyst; note rhynchus (arrow). Scale bar = 100 µm. C. Close-up of rhynchus (R). Scale bar = 50 µm.

Bucephalidae

***Rhipidocotyle* sp. (metacercaria) (Fig. 3)**

Two *C. venusta* (62, 66 mm TL) harbored encysted metacercaria of *Rhipidocotyle* sp. in their mesenteries and liver (Fig 3A). These metacercaria possessed the characteristic rhynchus with a pentagonal cap or hoodlike expansion and ventroposterior suctorial pit (Figs. 3B-C) (see Hoffman 1999, fig. 223). Three species of *Rhipidocotyle* are known from North American freshwater fishes (gars, pikes, suckers, centrarchids, moronids), including *R. papillosa* (Woodhead, 1929), *R. septpapillata* Krull, 1934, and *R. tridecapapillata* Curren and Overstreet, 2009 (Hoffman 1999; Curren and Overstreet 2009).

There are previous reports of *R. papillosa* and *R. septpapillata* from Smallmouth Bass (*Micropterus dolomieu*) from adjacent Arkansas (Becker et al. 1966; Kilambi and Becker 1977; Becker 1978). The life cycle includes cercaria in clams and metacercaria in fishes with the adult worm in the intestine and caeca of predatory fishes (Hoffman 1999). This is the first time this digenean has been reported from *C. venusta* and Oklahoma.

Strigeatida: Diplostomidae

***Postdiplostomum minimum* (MacCallum, 1921) Dubois, 1936**

Metacercariae of *P. minimum* (white grub) were found encapsulated in the mesenteries of a single adult *C. spadiceum* (122 mm TL). Our specimens are presumed to be *P. minimum* because many metacercariae cannot be identified to species using only features of metacercarial morphology. However, if metacercaria are fed to a definitive host then adult worms can be identified to species based on morphology. This digenean has been reported previously from related Central Stoneroller, *C. anomalum* (Hoffman 1999) and from Oklahoma (Spall 1969). It has also been reported from a variety of other fishes of different families (including nine cyprinids) from Alabama and Florida (Williams and Dyer 1992). This is the first time, to our knowledge, that this parasite has been reported from *C. spadiceum*.

Clinostomidae

***Clinostomum marginatum* Rudolphi, 1819**

Two metacercariae of *C. marginatum* (yellow grub) were recovered from the dermal tissue of an adult (138 mm TL) *L. chrysocephalus*. Although this digenean is a very common trematode that is cosmopolitan in distribution (Lane and Morris 2000), this is the first time it has been reported from *L. chrysocephalus*. In Oklahoma, the yellow grub has been reported previously from other non-game fishes, including Pirate Perches, *A. sayanus* (Hopkins 1933; McAllister and Bursey 2013), and Carp, *Cyprinus carpio* (Spall 1969).

Cestoidea: Proteocephalidea: Proteocephalidae

***Proteocephalus* sp. (Fig. 4)**

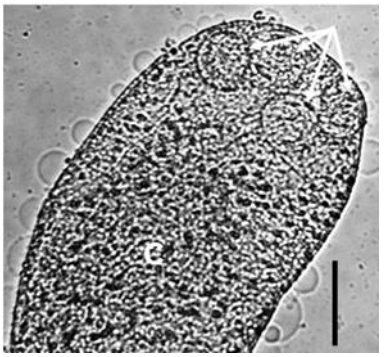

Figure 4. *Proteocephalus* sp. tapeworm from *Cyprinella whipplei* showing four unarmed suckers (arrows). Abbreviation: Calcareous corpuscles (C); Scale bar = 250 µm.

Two immature cestodes, *Proteocephalus* sp. were found in the small intestine of three (47, 49, 52 mm TL) *C. whipplei*. In addition, a single (8%) *L. chrysocephalus* (102 mm TL) harbored an extraintestinal immature *Proteocephalus* sp. tapeworm. Because these were immature (no mature or gravid proglottids present), specific identification was not possible. Interestingly, no cestodes have been previously reported from these hosts (Hoffman 1999). However, this genus of tapeworm has been commonly reported from various fishes, including several from Oklahoma (see Hoffman 1999).

In summary, examination of several cyprinid fishes revealed few helminth parasites. These results of low diversity of helminths are similar to those of Barger (2006) who examined over 600 *S. atromaculatus* from Nebraska and reported only four helminths (*A. lobatum*, *Proteocephalus* sp., a nematode (*Rhabdochona canadensis*) and an acanthocephalan (*Paulisentis missouriensis*) in this cyprinid host. Perhaps increasing the sample sizes and collecting at different sites that support suitable intermediate hosts

(aquatic molluscs) may increase our knowledge of the diversity and abundance of helminths in fishes of southeastern Oklahoma.

Acknowledgments

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Comparison of Antibiotic Susceptibility Patterns between *Serratia marcescens* Strain Isolated in 1920 versus 2008

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Abstract: *Serratia marcescens* is a non-pathogenic, saprophytic, Gram-negative bacterium classified as a member of *Enterobacteriaceae*. It has recently been classified as a nosocomial pathogen, causing infections such as urinary tract infections, lower and upper respiratory tract infections, and septicemia. These nosocomial infections have mostly been detected in intensive care units (ICUs). Though there is a standard treatment for *S. marcescens* infections, the bacterium has recently exhibited resistance to several antibiotic treatments. This study was conducted to determine the difference between two strains of *S. marcescens* (1920 and 2008 strains) using antibiotic resistance as the main criterion. In this study, antibiotic susceptibility tests using three classes of antibiotics (beta-lactams, aminoglycosides, and sulfonamides), genomic DNA isolation, restriction fragment length polymorphism (RFLP), and polymerase chain reaction (PCR) were used to evaluate the differences between the two strains. PCR was performed using primers for the integron classes 1, 2, and 3, and the methicillin-resistance gene *mecA*. Genomic DNA digestions were carried out using EcoRI-HF and Hind III. Both the strains were found to be resistant to the beta-lactams, but susceptible to the aminoglycoside and sulfonamides and both the strains lacked integron elements. However, RFLP of genomic DNA showed differences, suggesting possible variation within the genomic DNA sequences of 1920 versus 2008 strains.

Introduction

In recent years, some hospitalized patients and recent outpatients have acquired infections from hospitals and healthcare facilities. These infections have come to be known as nosocomial infections, with most of them occurring within intensive care units (Ivanova 2008). Most nosocomial infections are caused by antibiotic resistant bacteria, making them very difficult to treat. The emergence of antibiotic resistant bacteria causing nosocomial infections was marked by the wide and rapid spread of methicillin-resistant *Staphylococcus aureus* (MRSA) (Chen 2003) in hospitals of developed countries such as the United States and the United Kingdom. The emergence of MRSA marked the beginning of an exponential increase in the number of multi-drug-resistant bacteria; however, this increase has somewhat abated over the years (Ellner 1987). Nevertheless, due to the recent rampant use of antibiotics in both human medicine and animal husbandry, intense selective pressures have created resistance to several classes of antibiotics such as beta-lactams, sulfonamides, aminoglycosides, tetracyclines, and many others (Rowe-Magnus 2002). Such resistance-conferring elements can be transferred

between members of the same species or of different species. Antibiotic resistance-conferring elements transfer between bacteria mainly by vertical transfer of genetic material, via reproductive processes and by horizontal transfer of genetic material such as that seen in the transfer of the shiga toxin gene from *Shigella* to *Escherichia coli* (Rowe-Magnus and Mazel 2002). This transfer is usually achieved using the R plasmid as a major vector (Okuda 1984).

Serratia marcescens is a non-pathogenic, saprophytic, Gram-negative, and ubiquitous water organism classified as a member of *Enterobacteriaceae* (Sleigh 1983, Bagattini 2004,). Previously, most cultured *S. marcescens* exhibited a red or orange coloration that was caused by the production of a pigment known as prodigiosin (Sleigh 1983). However, *S. marcescens* colonies not exhibiting this distinct coloration have often been seen in bacteriology and hospital laboratories (Wilfert 1970); these strains are suspected to be pathogenic (Sleigh 1983). Because of the ubiquitous nature of bacteria, *S. marcescens* reservoirs can be located in diverse places including homes, offices, hospitals and other healthcare facilities. Pathogenic bacterial infections are usually treated with a single antibiotic or a combination of antibiotics that have different chemical properties and modes of action. There has been a global surge of antibiotic resistance organisms.

Antibiotic resistance-conferring elements can be transferred via mobile genetic elements known as integrons. These integrons have several general features such as the presence of the integrase gene *intI*, a gene cassette recombination site *attI*, and a promoter region P_{ant} for gene expression (Bennet 1999); all of these features can be used for identification. These integrons can not only used to transfer antibiotic resistance genes from one bacterium to another, but they can also be used to transfer genes that code for different characteristics such as toxins (Collis 1993). Such elements (both antibiotic resistance and other characteristics) are carried on gene cassettes inserted into the integrons. Gene cassettes, sequences of genes with a recombination site *attC* and no promoter (Waites 2000), cannot be expressed independently. Without insertion into an integron, these gene cassettes cannot be replicated (Bennet 1999) and thus cannot be propagated. Therefore, the needed insertion is done via site-specific recombination at the recombination site *attI* of the integron, mediated by an integrase protein (Collis 1993). Five different classes of integrons have been discovered: classes 1, 2, 3, 9, and an unnumbered class (Collis 2002), with classes 1, 2, and 3 being the most studied. These three classes of integrons are differentiated using specific markers unique to each class. Class 1 integrons, usually found on the transposon *Tn402* (Collis 2002) have an integrase gene *IntI*, a recombination site *attI*, a promoter region P_{ant} at its 5′ conserved region, and a variable 3′ conserved region (Waites 2000). In contrast, class 2 integrons have the integrase gene *Int1*at the 3′ conserved region (Waites 2000). Class 3 integrons were first identified in a *S. marcescens* strain isolated in Japan in 1993 (Arakawa et al., 1995). Although class3 integrons have a structure comparable to class 2, their recombination between the 59-be element and secondary sites occurs at lower frequencies. Moreover, in class 3 integrons the *Int13* can recognize and integrate at different *attC* sites (Collis 2002).

The aim of the study was to determine if the *Serratia marcescens* 1920's strain and 2008 strain have mobile DNA elements known as integrons and to assess their resistance to antibiotics such as penicillin, oxacillin, trimethoprim-sulfamethoxazole (TMP-SMZ), and kanamycin.

Methods

Organisms used. Three organisms were used in this study. ATCC 60 (designated I-20 ATCC 13880 (designated I-08) and ATCC 33592 (designated MRSA) were purchased from American Type Culture Collection (Manassas, VA). The I-20 strain was deposited by Army Medical School, Washington, DC in 1925, the ATCC 33592 strain was deposited by Schaefler S in 2006 and the ATCC 13880 strain (designated I-08) was deposited in 2008 by M Koccur (Verslyppea 2011). All cultures were maintained on nutrient agar slants and cultured in TSB at 25^0 C for 18 h.

Antibiotic Susceptibility Test. In preparation for the antibiotic susceptibility test, cultures of the samples were grown in Tryptic Soy Broth (TSB) and incubated on an Incubating Mini Shaker (VWR, Arlington Heights, IL) at 25°C for 18 hours. After incubation, the strains were standardized to obtain a 0.5 McFarland Standard (absorbance of 0.1-0.15 at 540 nm) using UV-Vis Spectrophotometer (Bio-Rad Laboratories, Hercules, CA). Standardized samples were incubated on Mueller-Hinton agar at 37°C for 18 hours with penicillin, oxacillin, trimethoprim-sulfamethoxazole, and kanamycin disks (BD-BBL, Franklin Lakes, NJ). Results were documented using GelDoc XR+ System (Bio-Rad Laboratories, Hercules, CA) and zones of inhibition were measured.

Polymerase Chain Reaction. Genomic DNA was extracted from pure cultures of all three strains using a modified protocol for Gram-positive and Gram-negative bacteria using the PureLink Genomic DNA Isolation Kit (Invitrogen, Carlsbad, CA.). Primer sets for the *mecA, Igr1, Igr2, Int13 and Sa16s* genes (see below for sequences) were used to amplify respective genes using the genomic DNA from each strain. The PCR cycling conditions for *mecA* primers were as follows: one step of denaturation at 94°C for 4 min, followed by 30 cycles of 94°C for 0.5 min, 53°C for 0.5 min, 72° for 1 min, and a final extension cycle at 72°C for 4 min. For the *Igr1* and *Igr2* primers, the reaction conditions were: one step of denaturation at 95°C for 4 min, followed by 30 cycles of 94°C for 0.75 min, 64°C for 0.75 min, and a final extension cycle at 72°C for 1 min. The *Igr1* and *Igr2* reactions conditions were used for the *Int13* primers with a modification of the annealing temperature to 50°C. For the *Sa16s* primers, the reaction conditions were as follows: one denaturation step at 95°C for 3 min, followed by 40 cycles of 95°C for 0.5 min, 50°C for 0.45 min, 72°C for 2 min, and a final extension cycle at 72°C for 7 min. Amplicons were resolved by electrophoresis on a 1.2% agarose-Tris-Acetate-EDTA gels at 60V for 6 hours. Visualization was done using EZ-Vision dye (Amreseco, Solon, OH) and documentation was carried out using the GelDoc XR+ System (Bio-Rad Laboratories, Hercules, CA).

Primer set sequences:

Primer	Sequence (5'to 3')
Igr1	GCT CTA GAC CGA AAC CTT GCG CTC, and GGA ATT CAT GAT ATA TCT CCC AAT TTG T
Igr2	GCT CTA GAT AAT GTG CAT CGT GCA AGC, and GCG TTA TCT AGT TCG ACA TAG TCT
Int13	AGT GGG TCG CGA ATG AGT G and TGT TCT TGT ATC GGC AGG TG
MecA	CTC AGG TAC TGC TAT CCA CC and CAC TTG GTA TAT CTT CAC C
Sa16	GAA AGC CAC GGC TAA CTA CG and CAT TTC ACC GCT ACA CAT GG

Restriction Fragment Length Polymorphism. Genomic DNA (2µg) from the I-20 and I-08 strains was digested using 20 Units EcoRI-HF and 20 Units HindIII-HF (New England Biolabs, Ipswich, MA). Single and double digestions were performed as per the manufacturer's instructions. Briefly, 2 µg of genomic DNA was digested using 20 U of restriction enzyme in a total volume of 50 µl at 37^{o} C for 12 hours. Digests were resolved on 1.2% agarose-Tris-Acetate-EDTA gels at 50-95V for 5 hours and 30 min, visualized using EZ-Vision (Amreseco, Solon, OH) and documented using the GelDoc XR+ System (Bio-Rad Laboratories, Hercules, CA).

DNA Sequencing. Genomic DNA was extracted from I-20 and I-08 strains using the PureLink Genomic DNA Isolation Kit (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. DNA sequencing was performed to ascertain the presence of integron elements. Sequencing was done at Oklahoma Medical Research Foundation core facility (Oklahoma City, Oklahoma).

Results

Antibiotic susceptibility test

The antibiotic susceptibility test showed resistance to both beta-lactams used (penicillin and oxacillin) in both strains (Table 1). In contrast, both strains were susceptible to the sulfonamide TMP-SMZ and the aminoglycoside kanamycin, albeit to varying degrees (Table 1and Figure 1).

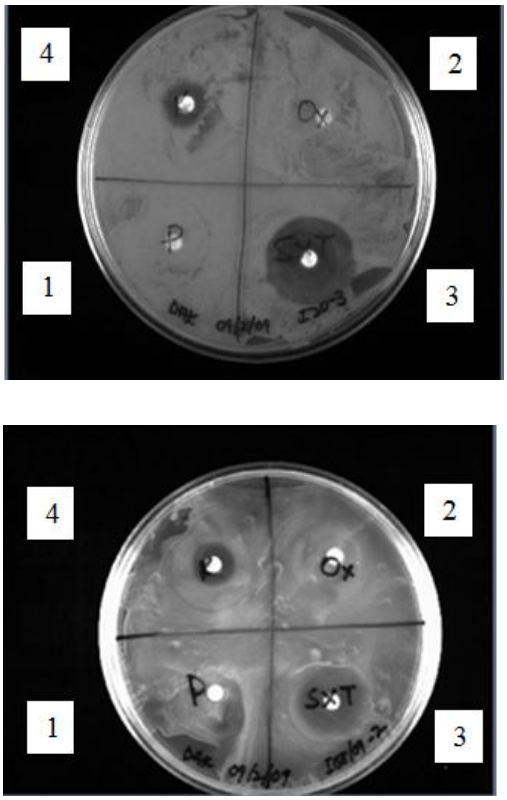

Figure 1. Plates showing zones of inhibition to penicillin (1), oxacillin (2), TMP-SMZ (3), and kanamycin(4) for the 1920 strain (I-20) (upper) and the 2008 strain (I-08) (lower).

PCR for integrin mobile elements

PCR was performed to determine the presence or absence of integrons, which have been shown to be responsible for the acquisition of resistance to antibiotics and other characteristics in *S. marcescens*. Neither strains showed evidence of containing the *mecA* gene (Figure 2, lanes 2 and 3), which is responsible for methicillin resistance in most bacteria, especially MRSA (De Lencastre1994). Amplification using the primer *Sa16s* served as an internal control for the PCR process (Figure 2; lanes 4, 5, and 6). The difference in intensity of the *Sa16s* bands might be due to loss of sample from the wells during the loading process for lanes 5 and 6. No amplicons were observed for either strains using *Igr1* (Figure 2, lanes 7 and 8), *Igr2* (Figure 2, lanes 9 and 10), and *IntI3* (Figure 2, lanes 11 and 12) for classes 1, 2, and 3 integron sequences respectively.

RFLP of genomic DNA

The strain I-08 showed no RFLP fragments in either single or double restriction digestions (Figure 3; lanes 2, 4, 6, and 8). Undigested genomic DNA was observed very close to the wells. However, the strains I-20 showed significant digestion in both the single (Figure 3, lanes 3 and 5) and double restriction digestions (Figure 3, lane 7). Few sizes were elucidated due to the close banding observed for the I-20 sample.

Observation of the genomic DNA of I-08 close to the wells, indicating high molecular weight DNA, prompted a resolution of the genomic DNA of both strains using 0.7% agarose-Tris-Acetate-EDTA gel electrophoresis. This showed a marked difference in the sizes of genomic DNA in both strains, though both samples were larger than 10 Kb (Figure 4)

DNA sequencing

DNA sequencing of genomic DNA from both strains was attempted but was unsuccessful possibly due the large stretches of repeating adenine sequences within the genomic DNA (internal poly-A tails).

Discussion

The *in vitro* bactericidal activity of antibiotics does not always correlate with their therapeutic effects in patients. However, their action on certain bacteria must be elucidated in order to advance to human testing. Kirby Bauer test was to qualitatively determine the efficacy of three different classes of antibiotics (beta-lactams, aminoglycosides, and sulfonamides) on *S. marcescens*. The results showing resistance to penicillin and oxacillin seem to indicate the presence of a beta-lactam resistance mechanism. Based on a

study conducted (Yang 1990), the main defense of *S. marcescens* against beta-lactam drugs is attributed to the production of the Richmond and Skye class I type of chromosomal beta-lactamase. Some strains of *S. marcescens* have also been known to produce plasmid-mediated beta-lactamases such as the TEM-1 enzyme, which confers resistance to penicillins and older cephalosporins (Yang 1990). These enzymes are responsible for the rupture of the beta-lactam rings found in drugs such as penicillin and oxacillin. These beta-lactam rings bind to the penicillin-binding proteins (PBPs) and inhibit the cross-linking of glycopeptide polymer units that form the cell wall mesh.

Another mechanism elucidated (Cozens 1986) suggests a phenotypically induced changes within the outer membrane reduces the drugs permeability across the outer and cytoplasmic membranes. One or both of these mechanisms might be responsible for the resistance to penicillin and oxacillin observed in both strains. This result is consistent with the findings that have shown that strains of *S. marcescens* have exhibited resistance to AZTREONAM, CEFOTAXIME, and even CEFTAZIDME (Bonnet 2000), all of which are advanced penicillins and cephalosporins. Since the ability to resist the action of beta-lactam rings can be conferred by exogenous genetic material (plasmids), it can be deduced that both strains have acquired genetic material that has conferred this ability. Alternatively, some studies have reported the acquisition of carbapenem (broad spectrum beta-lactam) resistance in some bacteria such as *Acinetobacter baumannii* after the loss of a 29-kD outer membrane protein (Mussi 2005) this might be a possibility in this study in light of genomic DNA size differences observed (Figure 4). The PCR results suggests that the antibiotic resistance is not via integrons since the three classes of integrons seem to be absent in both strains (Figure 2). Thus, it is appropriate to conclude that the production of beta-lactamase was conferred by some mechanism other than the insertion of resistance gene cassettes into integrons. Should integrons be considered for future studies, more specific primers should be used to target the first portion of the antibiotic resistance operon found in the other classes of integrons (class 9 and the unnumbered class), since all genes found in integrons coding for beta-lactamase have been found in the first position of the operon, with a single exception (Mussi 2005). Also, the 1920 strains showing resistance to penicillin suggests the ability of *S. marcescens* to obtain resistance elements from nature. Since the first therapeutic use of a penicillin drug was recorded in 1927, the 1920 strains must have obtained this resistance from contact with *Penicillum notatum* in its natural surroundings.

Aminoglycosides are naturally produced by some organisms such as *Streptomyces* spp and *Micromonospora* spp. These species have developed resistance to this class of antibiotics by methylation of the 16s rRNA found in prokaryotic 30s ribosomal subunits (Doi 2004). Another common mechanism for aminoglycoside resistance employed by different strains of *S. marcescens* is the modification of the drug by 'inactivating enzymes' that adenylate, acetylate, or phosphorylate the amino groups or hydroxyl group found in aminoglycosides (Hejazi and Falkiner 1997). Though research has revealed that most Gram-negative bacilli such as *E. coli* O157:H7 have been shown to be resistant to kanamycin (Zhao 2001), these mechanisms seem to be absent as both strains in this study were susceptible to the aminoglycoside kanamycin. It was also observed that both strains were susceptible to the sulfonamide drug TMP-SMZ (Table 1). This observation is in agreement with the current use of TMP-SMZ in the treatment of nosocomial urinary tract infections caused by *S. marcescens* (Okuda 1984) in ICUs. Resistance to sulfonamides such as TMP-SMZ is usually the result of chromosomal mutations in the dihydropteroate synthase (DHPS) gene or the introduction of the *sul1* into integrons and/or *sul2* genes into plasmids (Skold 2000). Susceptibility of the two strains to TMP-SMZ would indicate that they lack any of the aforementioned mechanisms of resistance to sulfonamides.

Table 1. Antibiotic susceptibility test results showing zones of inhibition for both strains using four different antibiotics.

	Antibiotics (mm)			
Sample Replicates	*Kanamycin (K-30)*	*Oxacillin (OX-1)*	*Penicillin (P-10)*	*TMP-SMZ (SXT1.25-23.75)*
I-08 – 1	18	0	0	25
I-08 – 2	18	0	0	30
I-08 – 3	28	0	0	28
I-08 – 4	17	0	0	0.0
I-08 – 5	19	0	0	*
I-20 – 1	21	0	0	35
I-20 – 2	20	0	0	34
I-20 – 3	19	0	0	36
I-20 – 4	21	0	0	35
I-20 – 5	20	0	0	35

Interpretive standards in mm in the order of Resistant (R), Intermediate (I) and Sensitive (S) for: OX:≤ 10, 11-2, ≥13; K:≤ 13, 14-14, ≥18; P: ≤28, -, ≥29; SXT: ≤10, 11-15, ≥16. * - indicates undeterminable zone of inhibition

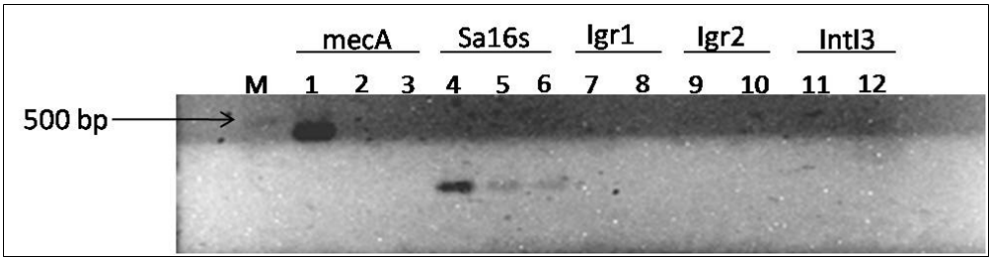

Figure 2. PCR amplification using *mecA*, *Sa16s*, *Igr1*, *Igr2*, and *IntI3* primers. The sizes of the molecular DNA marker shown on the left of gel. Lanes: M = molecular DNA marker EZ-Load (Bio-Rad Laboratories); 1 = MRSA with *mecA* primer, 2 = I-20 with *mecA* primer; 3 = I-08 with *mecA* primer, 4 = MRSA with *Sa16s* primer, 5 = I-20 with *Sa16s* primer, 6 = I-08 with *Sa16s* primer, 7 = I-20 with *Igr1* primer, 8 = I-08 with *Igr1* primer, 9 = I-20 with *Igr2* primer, 10 = I-08 with *Igr2* primer, 11 = I-20 with *IntI3* primer, 12 = I-08 with *Int13* primer.

Chetoui et al. have used pulse-field gel electrophoresis of macrorestriction fragments with biotyping, esterase and ribo typing performed typing of nosocomial strains of *Serratia marcescens*. Both strains were comparable with respect to antibiotic sensitivity. We have used RFLP in this study to find any differences and/or similarities between these two strains at the genomic level. The efficiency of RFLP in determining similarities and differences in DNA sequences of similar species was demonstrated in a study by Debast et al. where RFLP analysis was utilized to identify three genotypes of *S. marcescens* during a nosocomial outbreak in a hospital. In the present study, RFLP analysis indicated a pattern difference in the cleaved genomic DNA of the I-20 strains and the I-08 strains of *S. marcescens* (Figure 3). Treatment of the genomic DNA of both strains with Hind III and EcoRI-HF showed digestion of the DNA of the strains I-20 but did not do the same for the strain I-08. Based on the single band observed for the strains I-08, it was deduced that there were no cleavage sites for the two used restriction enzymes.

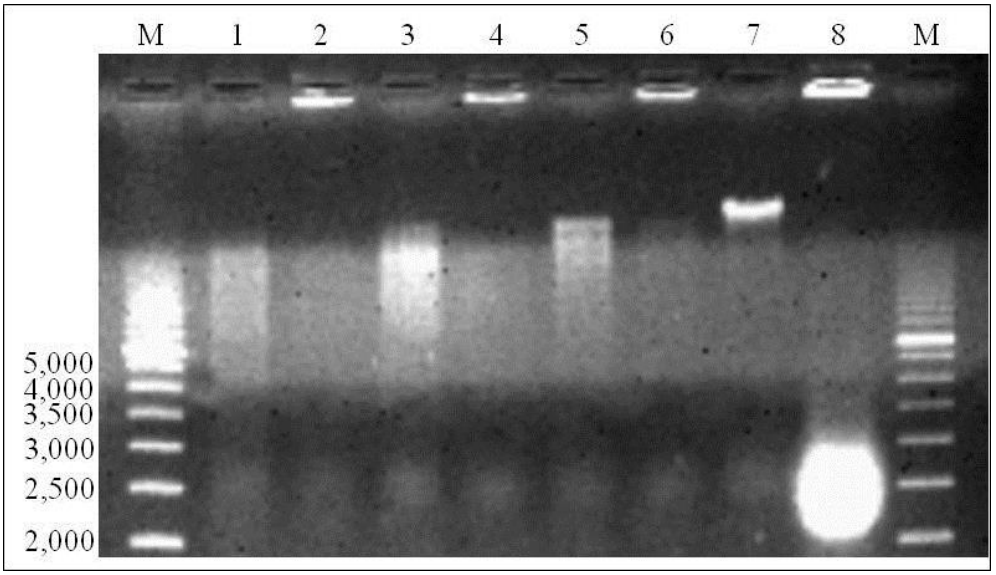

Figure 3. RFLP analysis of genomic DNA isolated from MRSA, I-20, and I-08. DNA was digested with Hind III and EcoRI-HF. The sizes of the molecular DNA marker are shown on the left of the gel. Lanes are as follows: M – molecular DNA marker EZ-Load (Bio-Rad Laboratories), 1 = I-20 without RE, 2 = I-08 without RE, 3 = I-20 with Hind III, 4 = I-08 with Hind III, 5 = I-20 with EcoRI-HF, 6 = I-08 with EcoRI-HF, 7 = I-20 with Hind III and EcoRI-HF, 8 = I-08 with Hind III and EcoRI-HF.

Since both strains are of the same species of organism, the absence of HindIII and EcoRI-HF cleavage sites in I-08 indicate a net insertion or deletion of genetic material at these sequences, eliminating the sites. Though either insertions or deletions are possible, the relatively large size of the genomic DNA of I-08 (Figure 4) would suggest a net insertion of genetic material over the 88-year period via class 9 or the unnumbered class integrons, plasmid insertion, or some other mechanism.

The results of the present study indicate that in addition to the acquisition of resistance elements due to selective pressures imposed by the use of antibiotics in modern medicine, *S. marcescens* can also obtain these elements from its natural environment. In the treatment of *S. marcescens* infections, however, the aminoglycoside kanamycin has been shown to be effective and holds potential as an alternative to the standard treatment using the sulfonamide TMP-SMZ.

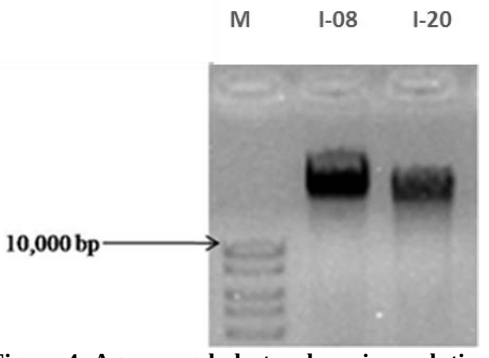

Figure 4. Agarose gel electrophoresis resolution of genomic DNA of the I-20 and the I-08 strain. The I-08 strain genomic DNA appears to show a difference in the size compared to the I-20 genomic DNA.

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Detection of Panton-Valentine Leukocidin (PVL) Genes within CA-MRSA Carriers of the Oral Roberts University Community

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Abstract: Over the years, the misuse of antibiotics has become a significant factor in the emergence of resistant bacteria. Methicillin-resistant *Staphylococcus aureus* (MRSA) is *staphylococci* that are resistant to Beta-lactam drugs. There are two types of MRSA infections: community-acquired MRSA (CA-MRSA) and hospital-acquired MRSA (HA-MRSA). They can be differentiated by the presence of the Panton-Valentine Leukocidin (PVL) gene in the CA-MRSA strain. Studies have shown that CA-MRSA is more virulent than HA-MRSA. The aim of this study is to determine the population of students at Oral Roberts University who are carriers of CA-MRSA. This was done by obtaining nasal swabs from 50 students and performing coagulase and antibiotic susceptibility tests to identify the samples that contained MRSA. Polymerase chain reaction (PCR) was then used to detect the presence of PVL genes in the positive samples using *lukS-PV*, *hlg*-1 and Sa16 primers. Results showed that 18.0% of the student nasal samples contained MRSA. Of those samples 6.0% were CA-MRSA.

Introduction

Staphylococcus aureus (*S. aureus*) is a common skin bacterium that only becomes a problem when it enters the skin through a cut, open wound, or breathing tube. These bacteria occur harmlessly in the nasal passages of roughly 30% of the U.S. population, and 20% of the human population are long-term carriers (Staywell et al. 2001). *Streptococcus aureus* causes several diseases from minor skin infections (boils, pimples, abscesses, scalded skin syndrome) to life-threatening diseases (pneumonia, meningitis, toxic shock syndrome). A recent study by Graffunder and Venezia (2002) has shown that *S. aureus* is one of the most common causes of nosocomial infections, often causing postsurgical wound infections. Over the years, *S. aureus* has developed multiple antibiotic resistance mechanisms.

The first methicillin resistant strain of *S. aureus* was isolated from a hospital in the UK in 1942 (Jevons et al.1961). By 1960, 80% of all *S. aureus* isolates were resistant to Beta-lactam antibiotics. These strains have gained methicillin resistance through the acquisition of the *mecA* gene (Deurenberg et al. 2006). The *mecA* gene is found on a genetic element known as the staphylococcal chromosomal cassette (SCC*mec*) and encodes low-affinity penicillin-binding protein (PBP-2a) (de Lencastre et al. 1994). This attribute causes the bacteria to be resistant to methicillin. Methicillin is a Beta-lactam antibiotic, structurally analogous to penicillin, oxacillin, and vancomycin. Under normal circumstances, the β-lactam nucleus binds to PBP-2a in the cell wall and inhibits the synthesis of peptidoglycan, thereby causing bacterial cell

death (Yocum et al. 1980). However, when the *mecA* gene is expressed, the β-lactam nucleus does not bind to PBP-2a; hence, the cell continues to synthesize peptidoglycan and is able to replicate and survive even in the presence of Beta-lactam antibiotics. *Staphylococcus aureus* that are resistant to methicillin are classified as Methicillin-resistant *Staphylococcus aureus* (MRSA).

MRSA constitutes 30% - 60% of *Staphylococcal* infections in the USA, Japan, and Europe (Davis et al. 2007). According to the Center for Disease Control, 0.8%-2% of the U.S. population is MRSA carriers; 94,000 people are infected each year, and 19,000 people die as a result (CDC Control and Prevention 2011). MRSA has consequently become a notorious life-threatening pathogen.

MRSA is commonly transmitted through physical contact. Skin-to-skin contact, cuts in the skin, sharing of personal hygiene items (towels, razors), and contact with contaminated items (door handles and athletic equipment) helps spreading this contagious disease. MRSA causes infections such as surgical wound infection, urinary tract infection, pneumonia, and skin infection. The most common signs of MRSA are cellulitis, abscess, folliculitis, and impetigo.

There are two strains of MRSA: hospital-acquired MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA). In recent years, HA-MRSA (85% of MRSA) has become more abundant in hospitals and other healthcare facilities, representing a growing public health problem in the United States (Groom et al. 2008). However, in 2010, encouraging results from a CDC study stated that the invasive MRSA infections in healthcare facilities declined by 28% from 2005 through 2008 (Kallen et al. 2010). On the other hand, it has been discovered that people who have not recently been in the hospital or have had no medical procedure such as dialysis or surgery within the past year are also vulnerable to MRSA infections. This type of MRSA is often known as community-acquired MRSA. Outbreaks of CA-MRSA have been seen among athletes, prisoners, military recruits, daycare attendees, school students, and other groups of people who live in a close quarters and share contaminated items.

Though both CA-MRSA and HA-MRSA cause similar infections, both strains differ in the virulence factors they express. Characteristically HA- MRSA s trains elicit staphylococcal enterotoxin A (SEA), while the CA-MRSA produces Panton Valentine Leukocidin (PVL), which is a toxin that belongs to the synergohymenotropic toxin family (Tristan et al. 2007, Dufour et al. 2002). It consists of LukS-PV, LukF-PV, and γ-hemolysin proteins. The association of PVL with five major pandemic clones of CA-MRSA led to the hypothesis that PVL is the key virulence factor for CA-MRSA (Labandeira-Rey et al. 2007, Shallcross et al. 2012, Wannet et al. 2005). The exact mechanism by which PVL toxins harm host cells is not clear. A study by Spann et al (2013) showed that PVL toxin binds to the complement receptors C5aR and C5L2, inhibiting the immune cell activation. Another study indicates that PVL binds to the ganglioside GM1 in cells and causes its cytotoxic effects (Nishiyama et al 2012). PVL has been shown to be a strong inducer of IL-1and inflammasome activation in primary human alveolar macrophages that in turn can trigger the release of chemotactic factors leading to massive neutrophil infiltration of the lung (Perret et al. 2012). Hence, PVL toxin is considered to be responsible for the high mortality rates associated with infection by CA-MRSA (Tristan et al. 2007). Gamma hemolysins (Hlg) are reported to lyse human and other mammalian erythrocytes (Kaneko et al. 2004), PMNs (Joubert et al. 2006, Malachowa et al. 2011), and to enhance the survival of *S. aureus* in human blood (Malachowa et al. 2011). Karauzum et al (2013) have recently shown that Hlg(B) can form heterologous oligomers with LukS-PV. A report has shown that a combination of Hlg(A) and LukF-PV is hemolytic towards rabbit red blood cells (Prevost et al. 1995). Intravitreal injection of rabbits with six different combinations of PVL and Hlg and comparing these combinations based on ability to induce inflammation and necrosis showed various degrees of symptoms with the following order of severity: Hlg(A) +

LukF-PV > Hlg(AB) ≥ LukS-PV + Hlg(B) ≥ PVL > Hlg(CB), suggesting that a variety of new toxins with distinct potencies can be generated by these cross combinations (Siqueira et al. 1997). The above study has clearly demonstrated that the individual virulence factors PVL and Hlg can play a role in clinical infections.

In this study, MRSA strains obtained from the Oral Roberts University student population were tested for the presence of Panton Valentine Leukocidin (*pvl*) and γ-hemolysin gene (*hlg*).

Methods

Study design and collection. Nasal swabs were obtained from fifty student volunteers who were recruited for this study. All samples were collected in the microbiology lab in a single day. Briefly, prepackaged sterile cotton swabs (Puritan Medical Products, Gilford, ME) were removed from the packing and soaked briefly 20 sec in sterile water. The moistened swab tip was then inserted into the volunteer student's right nostril and rotated gently for 5 seconds. The same procedure was done on the right nostril. The sample swabs were immediately placed into Tryptic Soy Broth (TSB) and incubated for 18 hours at 37 ºC. Each sample was then streaked onto Mannitol Salt Agar (MSA, Carolina Biological Supply Co, Burlington, NC) plates and incubated for 18 hours at 37 ºC to select for members of the genus *Staphylococcus*. Two isolated colonies of *Staphylococcus* were taken from each positive MSA plate, streaked onto a TSB slant, and incubated for 18 hours at 37 ºC.

Coagulase test. The Staphylo Monotec Test Kit (Sigma Aldrich, St. Louis, MO) was used to confirm *Staphylococcus aureus* identity. As per the manufacturer instructions, a drop of control reagent was placed on an analysis card, and a small amount of the sample (*Staphylococcus*) was mixed with the reagent. A test reagent was then added to the mixture, and mixed for less than 20 seconds to observe agglutination on the analysis card. A coagulase positive culture of *Staphylococcus aureus* (Presque Isle Cultures, Erie, PA, catalog number 4651) was used as a positive control.

Antibiotic resistance test. The use of methicillin has been discontinued. Oxacillin is in the same class of drugs as methicillin and was chosen as the agent of choice for testing *staphylococci* in the early 1990s. The acronym MRSA is still used to describe these isolates because of its historic role. Sensitivity to oxacillin (1ug), and vancomycin (30 ug) (Beckton Dickenson and Company, San Diego, CA) was performed by disk diffusion method. Coagulase-positive cultures were regrown in TSB broth for 18 hours at 37 ºC. Approximately 1 mL of culture was spread on Muller Hinton agar plate (Carolina Biological Supply Co, Burlington, NC) and allowed to dry at room temperature. The antibiotic discs were then placed on the agar plate and incubated for 18 hours at 37 ºC. The zone of inhibition was measured using a ruler to determine the sensitivity of the bacteria according to the manufacturer's instructions.

Genomic DNA extraction. The extraction of genomic DNA was performed on resistant strains using the PureLink Genomic DNA Kit (Life Technologies, Grand Island, NY). TSB cell culture (1 ml) was taken from each sample and placed in a 1.5-ml micro centrifuge tube and centrifuged. The supernatant was discarded and the cell pellet was suspended in 180 µl of lysozyme solution (20 mg/ml). The solution was incubated for 30 minutes at 37 ºC, treated with 20 µl Proteinase K and 200 µl PureLink genomic lysis/binding buffer. The solution was again incubated for 30 minutes at 55 ºC, and the DNA was extracted by running the lysate through a PureLink spin column. The bound DNA in the column was eluted with Millipore water.

PVL and γ-hemolysin gene analysis. Extracted genomic DNA was used as a template for amplification. Oligonucleotide primers of *pvl* and *hlg* genes were used to obtain amplification of *lukS-PV* and *hlg,* respectively. The primer set sequences for the genes were as follows:

For *lukS-PV:* 5' ATCATTAGGTAAAATGTCTGGACATGAT CCA 3' and

5' GCATCAASTGTATTGGATAGCAAAAGC 3'. The expected amplicon size is 533 base pairs.

For *hlg*: 5' GCCAATCCGTTATTAGAAAATGC 3' and 5' CCATAGACGTAGCAACGGAT 3'

The expected amplicon size is 937 base pairs.

The 16S rRNA sequence for *S. aureus*, Sa16, was used as an internal control. The primer set for Sa16 was: 5'GAAAGCCACGGCTAACTACG 3' and 5'CATTTCACCGCTACACATGG 3'. The expected amplicon size is 203 bp.

PCR primers were designed using the freeware Primer 3 (Rozen et al. 2000). In brief, the PCR protocol consisted of an initial denaturation at 94 ºC for 2 min; 30 cycles of 30 s denaturation at 94 ºC, 30 s of annealing at 55 ºC, and 1 min of extension at 72 ºC. The PCR products were resolved by electrophoresis through a 1.5% agarose gel.

Results

MSA plate: *Staphylococcus* strains were recovered from 29 out of 50 (58.0%) were staphylococcus positive as shown by the production of acid on the selective MSA agar (Fig 1)

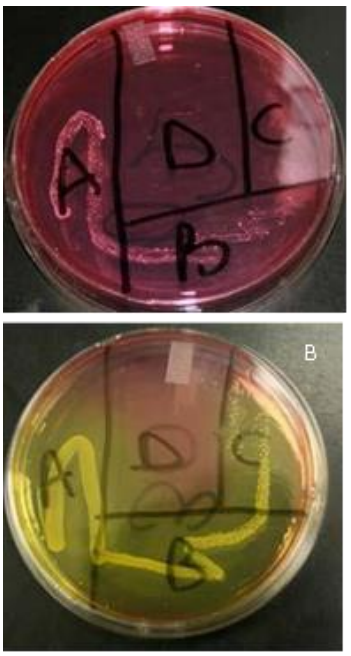

Figure 1. MSA plate after 18 hours at 37°C inoculated with nasal swabs. (A. Upper) MSA plate showing growth of non-*Staphylococci*; as indicated by the absence of yellow colonies. (B. Lower) MSA plate showing growth of *Staphylococci,* indicated by the presence of yellow colonies.

Coagulase test: Coagulase test is used to differentiate the potentially pathogenic species, *Staphylococcus aureus* from other nonpathogenic *Staphylococcus* isolates, which are usually coagulase-negative. Coagulase test was positive for the 13 out of 29 S. aureus culture (44.0%) samples tested (Fig. 2).

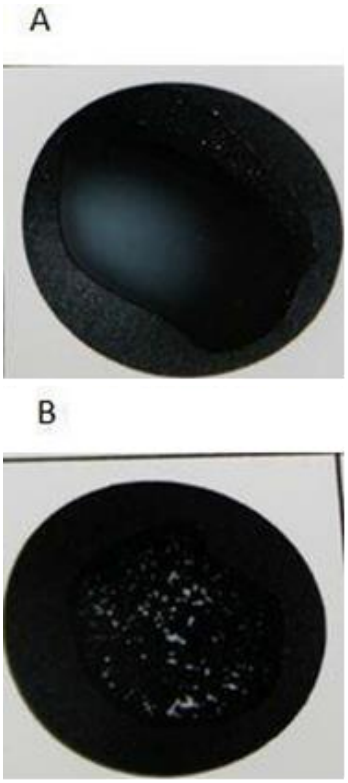

Figure 2. Coagulase test to identify pathogenic *Staphylococcus aureus.* (A) No agglutination observed on the analysis card, indicating a negative coagulase test (B) Positive coagulase test showing agglutination on the analysis card. Isolate is *Staphylococcus aureus* and likely pathogenic.

Antibiotic resistance: The analysis of oxacillin and vancomycin zone of inhibition was compared to the standard values provided by the manufacturer to determine the susceptibility or resistance to the antibiotics. Out of 50 samples, 9 samples (18%) were resistant to oxacillin and vancomycin antibiotics; these were considered to be MRSA. The use of methicillin has been discontinued. Oxacillin, which is in the same class of drugs as methicillin, is chosen as the agent of choice for testing *Staphylococci* (NCCLS). The acronym MRSA is still used to describe these isolates because of its historic role. The diameter of the zone of inhibition

measured from these samples was within the range that was considered to be resistant as per the manufacturer (Fig. 3).

Figure 3. Result of antibiotic susceptible test. (Upper left half) The sensitivity of the isolate to oxacillin with zone of inhibition of 19 mm. (Lower bottom half) The sensitivity of the isolate to vancomycin with zone of inhibition of 14 mm.

PVL(*pvl*) and γ-hemolysin (*hlg*) gene analysis: PCR for *pvl*, *hlg* and *Sa16* genes was carried out in separate tubes. The genomic DNA isolated from the bacterial strains was subjected to PCR to amplify genes for *pvl* and *hlg* genes. Of the 9 samples tested, 8 samples (88%) showed an amplicon size of ~ 937 bp as expected for the *hlg* gene; 2 out of 9 samples (22%) showed fragments for both *pvl* (amplicon size 533) and *hlg* genes, and 1 out of 9 samples (11%) showed fragment only for *pvl* gene (Fig. 4A). This indicated that 6.0% (*pvl* positive) of the samples are CA-MRSA. All PCR samples using the Sa16 primers as an internal control yielded a fragment of expected size, 203 bp, except for sample 38 (Fig. 4B)

Discussion

MRSA is no longer limited to the hospitalized, but also occurs among otherwise healthy communities. Such community-acquired MRSA is an emerging pathogen that primarily causes skin and soft tissue infections. A marked increase in CA-MRSA infection among people living in a closed area is demonstrated in the present study. Carriers of CA-MRSA in the Oral Roberts University student community were identified by detecting the presence of PVL genes among 50 isolates of bacteria randomly collected from students.

In this study, it was found that more than half of the samples (58.0%) contained *Staphylococcus*. These samples produced acid by-product that reacted with the phenol in the MSA plate to convert the red color of the plate to yellow. The frequency of *Staphylococcus* in our samples is consistent with the known prevalence of the bacteria in humans.

There are over 30 different types of *staphylococci*, but *Staphylococcus aureus* causes most staph infections. *Staphylococcus aureus* is part of the human flora, and its ability to clot plasma is the most widely accepted measure for identification. Coagulase test is often used to identify *Staphylococcus aureus* from other *Staphylococcus* species (Ryan 2004). From this study, it was determined that 26.0% of the student samples were positive for coagulase and were thus positive for *Staphylococcus aureus*. Based on previous research, 11-32% of healthy adults are carriers (Tolan 2011). Therefore, our results were consistent with previous estimates of the incidence of *Staphylococcus aureus* in the general population.

The increasing use of antibiotics is a factor in the occurrence of MRSA. Over time, *Staphylococcus aureus* has developed mechanisms that impart it resistance to multiple antibiotics. Oxacillin and vancomycin (β-lactams) were used during the study to determine percentage of samples that were MRSA. In this study we report that 18.0% (9/50) of the samples were resistant to the antibiotics. MRSA has been identified to be one of the leading causes of nosocomial infections. MRSA was originally isolated from patients in health care facilities; however, it has become increasingly common among healthy people who have not been living in the hospital. This change in disease distribution is illustrated in this study, which concludes that 6.0% of the students are carriers of community-acquired MRSA. CA-MRSA can be differentiated from HA-MRSA by the presence of *pvl* and/or *hgl*

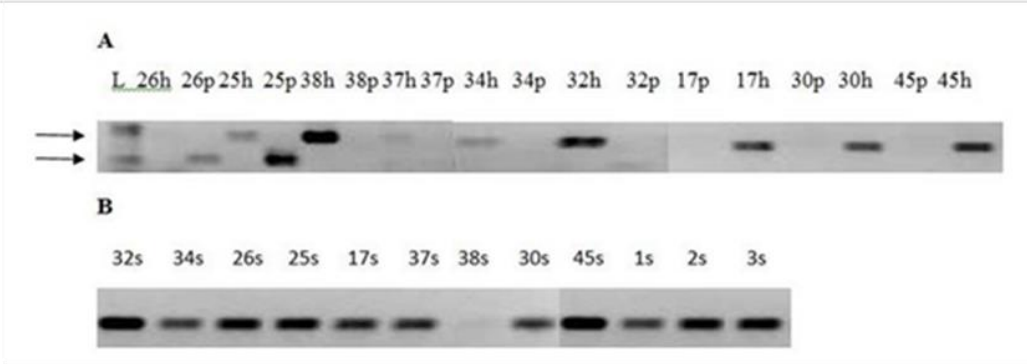

Figure 4. PCR for the identification of *hlg* and *pvl* genes. (A) Amplicon for *pvl* seen in three samples, 26, 25, and 32. All samples showed the presence of *hlg* amplicon, except 26. (h = *hlg*, p = *pvl* , L = DNA ladder: top arrow 1000 bp, bottom arrow 500bp,). (B) PCR results showing PCR products for Sa16 primers as an internal control

genes. Vandenesch et al (2003) described CA-MRSA clones in three continents that were tested positive for *pvl* gene specific sequences. The clones from Oceania showed the presence of both *pvl* and *hlg* sequences. During this research, PCR was used to amplify the PVL genes to validate the study by determining the prevalence of CA-MRSA within the population. The products observed in 6.0% of the samples indicated that those samples contained PVL-producing isolates. In a study of 123 uninfected children, 59.0% carried *Staphylococcus aureus*, and 2% were carriers of CA-MRSA (Sdougkos 2008). Our study corroborates this prevalence of CA-MRSA among healthy people.

Conclusion

MRSA is a major pathogen and threat to lives worldwide. Therefore, strategies must be taken to prevent this infection. Incision and Drainage (I&D) and Oral Antimicrobial Therapy are the two clinical approaches that are most useful in treating MRSA. As the name suggests, Incision and Drainage is a minor surgical procedure to release (drain) the pus under the skin (abscess) by using a sterile instrument such as a sharp needle to puncture the skin. In the case of Oral Antimicrobial Therapy, cultures of bacteria are taken from the infected person, and several antibiotics are used to test for the susceptibility of the bacteria. Every strain of bacteria is susceptible to a specific antibiotic; hence, it is important to identify the antibiotics before prescribing to the infected person. Even though these clinical approaches can be helpful, it is advisable to take strategies such as keeping personal items uncontaminated, covering open wounds, and washing hands in order to prevent the transmission of MRSA.

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Synthesis of *meso*-Substituted Porphyrin Metal Complexes Bearing Multiple Functional Groups

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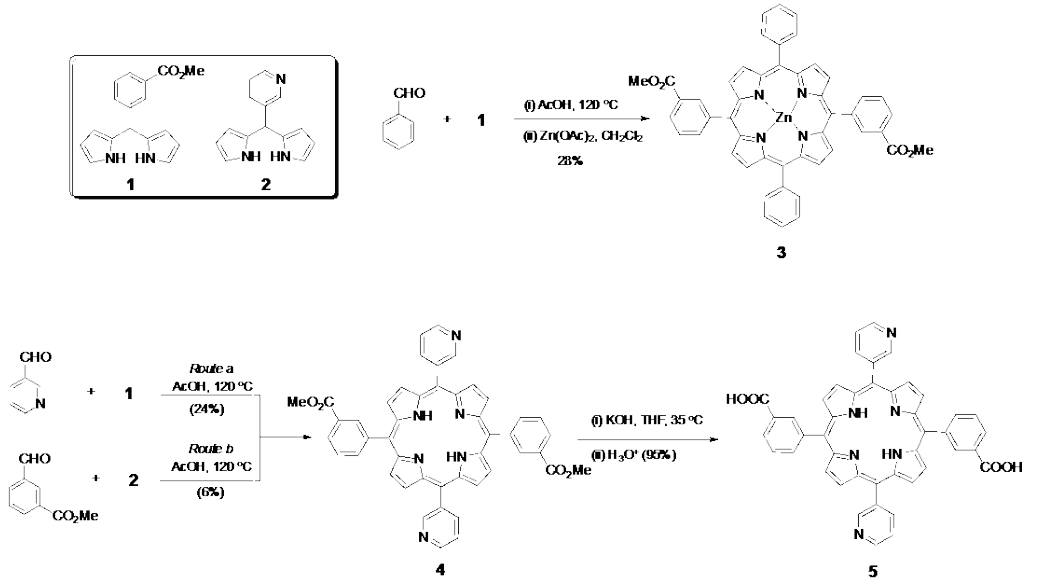
Abstract: The acid-catalyzed porphyrin syntheses of a set of rare and novel *meso*-substituted porphyrins possessing substitution at the *meta* and *para* positions have been completed. Such porphyrins have been prepared which possess at least one of the following peripheral functional groups namely, a free amine, a carboxylic acid, an ester, or a terminal alkyne group. Functionalized dipyrromethanes at the 3-position were condensed with the corresponding aldehydes under acidic conditions (AcOH, TFA or BF_3 ethereate) to afford the *meso*-substituted porphyrins. Characterization of products was accomplished by NMR and MALDI-TOF analyses.

Introduction

Results have been reported on the synthesis of a few *meso*-substituted porphyrins (Kadish et al., 2000); Holten et al., 2001) which can be widely utilized in the establishment of bio-organic model systems and molecular devices (Mak et al, 1998, 1999; Li et al., 1999; Mongin et al, 1999; Nakano et al. 1998). The design and the synthesis of porphyrins containing specific patterns of functionality still remain challenging despite the variety of available procedures (Cammidge et al, 2001; Dogutan et al., 2008). The major issue in the porphyrin synthesis is the isolation of the target molecules in very low yields owing to the scrambling processes. While a number of sophisticated experiments are available for the synthesis of porphyrins with less or no scrambling (Cammidge et al, 2001; Dogutan et al., 2008), porphyrin derivatives with certain functional groups remain unavailable. In the present work, we have synthesized a series of *meso*-substituted porphyrin derivatives possessing at least one of the following functional groups namely, a free amine, a carboxylic acid, an ester, and/or a terminal alkyne group. Such functional groups offer a variety of derivatives for potential molecular devices.



Scheme 1. Synthesis of ester- and acid-substituted porphyrins.

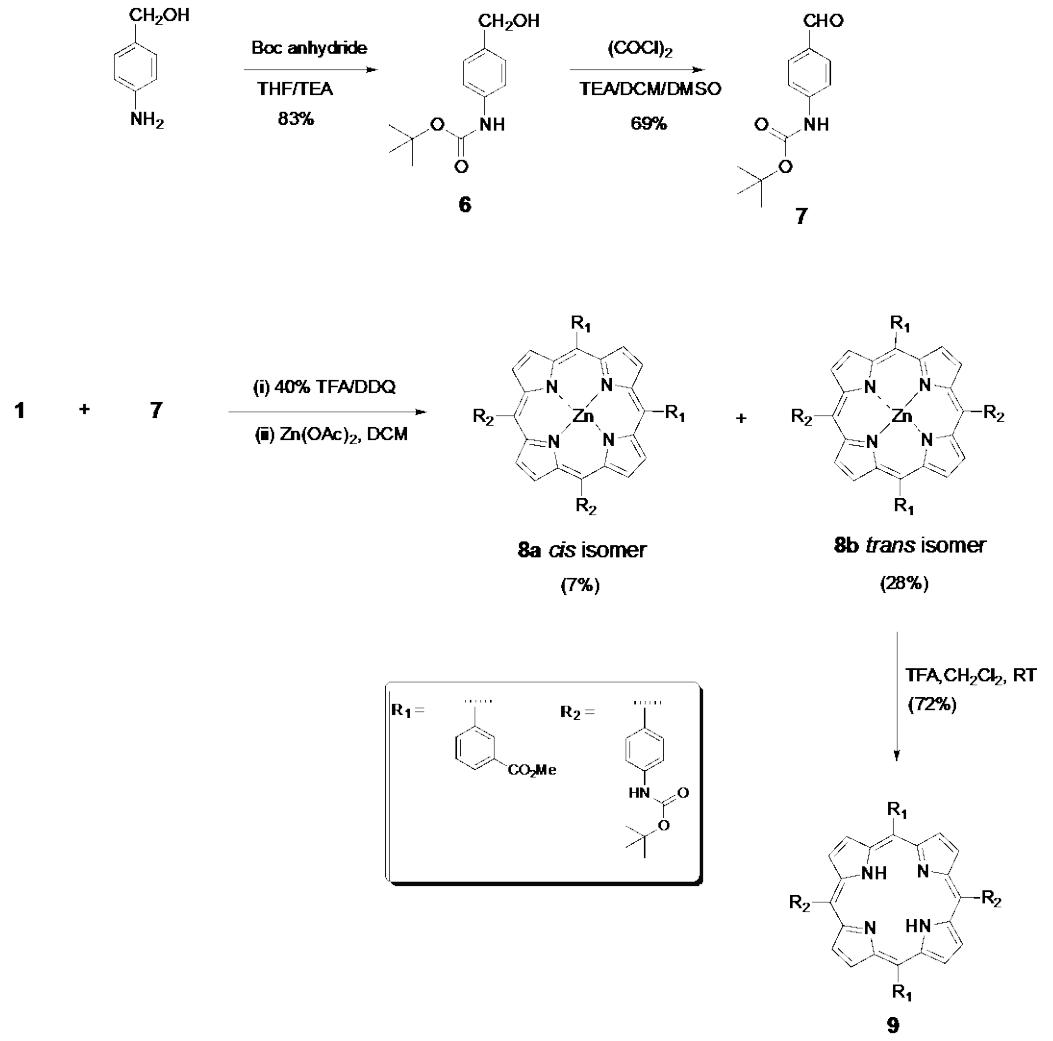
Results and Discussion

Synthesis

As a first step towards the synthesis of *meso*-substituted porphyrins, functionalized dipyrromethanes were prepared by the acid-catalyzed condensation of aldehydes with pyrrole. Dipyrromethanes **1** and **2** were obtained (Littler et al., 1999; Gryko et al., 2000), but the overall yields of the porphyrins are affected by a variety of factors, including the choice of acid catalyst and oxidant, the duration of the condensation period, the concentrations of acid, pyrrole, aldehyde, and the presence of water in the solvent. Dipyrromethanes **1** and **2** were reacted with the corresponding aldehydes in an acid to yield the target porphyrins. The synthesis of **3** was performed by reacting a mixture of dipyrromethane **1** and benzaldehyde in equimolar proportions with acetic acid (Scheme 1). For the synthesis of **4**, two different synthetic routes were investigated.

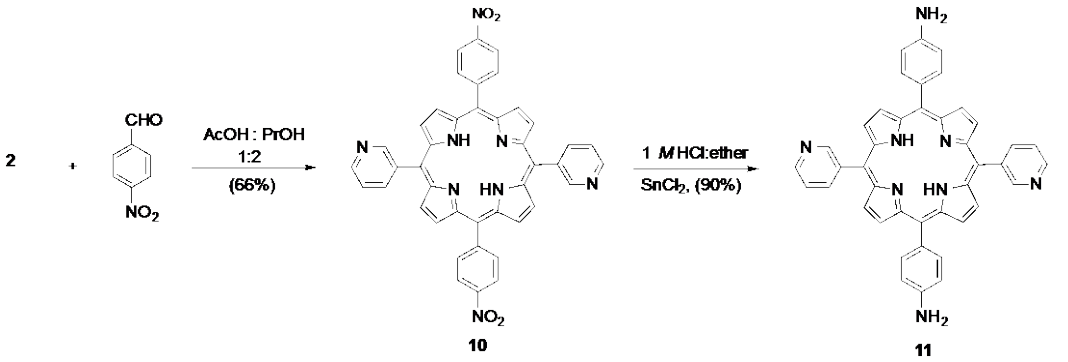
Reaction of a 1:1 mixture of nicotinaldehyde and dipyrromethane **1** with AcOH under reflux conditions afforded **4** (24%). In contrast, when dipyrromethane **1** was replaced by the pyridine-substituted dipyrromethane **2**, product **4** was achieved in relatively low yield (6%). Saponification (Muniappan et al., 2007) of **4** with 1 *M* KOH/methanol in THF at 35 ˚C, followed by acidification with 1 *M* HCl, led to the acid porphyrin **5** (95%)**.**

The synthesis (Scheme 2) of a diester-substituted porphyrin containing two free amine groups in a *trans*

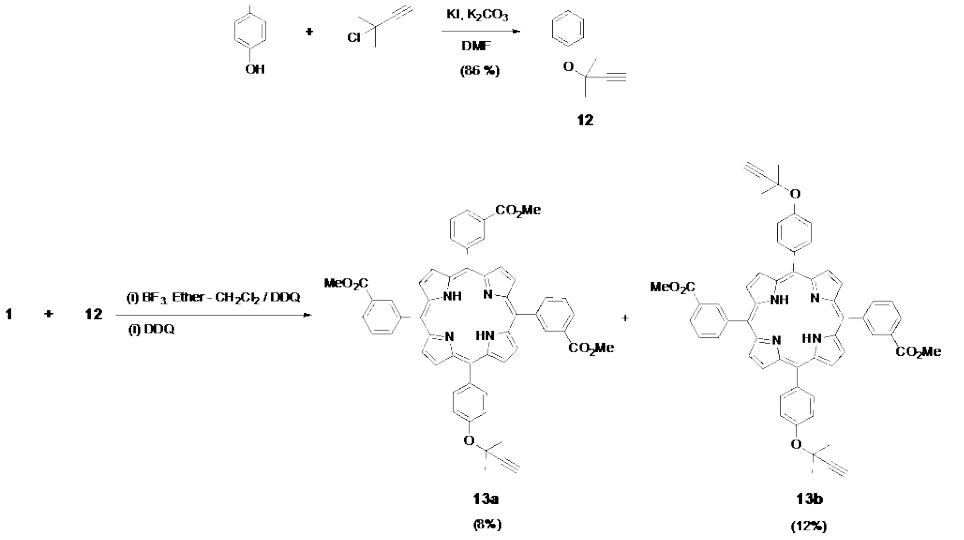


Scheme 2 Synthesis of diamineo diester porphyrins

arrangement is outlined in the scheme. Boc protection of *p*-aminobenzyl alcohol gave **6** (83%), and oxidation of compound **6** with oxalyl chloride, afforded aldehyde **7** (69%) (Rai et al., 1992). Acid-catalyzed condensation (Schmidt et al., 2006) of **7** with dipyrromethane **1**, using 40% TFA and DDQ as the oxidant and subsequent zinc metallation, gave a mixture of *cis*- and *trans*-substituted porphyrins **8a** and **8b** in the ratio 1:4. Isolation of the free *cis*- and *trans*-porphyrins did not prove feasible, and hence the compounds were converted to their zinc complexes which were separable by column chromatography. Reaction of **8b** with TFA resulted in removal of the Boc group and the metal to afford the diamino diester porphyrin **9**.



Scheme 3 Synthesis of diamino pyridyl substituted porphyrins.



Scheme 4. Synthesis of terminal alkyne substituted porphyrins.

Scheme 3 illustrates the synthesis of a *trans-meso*-diaminodipyridyl porphyrin. The starting material, *p*-nitrobenzaldehyde, was treated with dipyrromethane **1** in a 2:1 mixture of acetic acid and propionic acid and produced **10** (66%). Reduction of **10** with $SnCl_2.H_2O$ in a 1 *M* HCl/ether mixture at room temperaturn (Hatay et al., 2010) under dark conditions afforded **11** (90%)**.**

The aldehyde precursor **12**, required for the synthesis of the targeted terminal alkyne-substituted porphyrins, was prepared (Scheme 4) by O-alkylation of *p*-hydroxybenzaldehyde using 3-chloro-3-methylbutyne (Kyogoku et al., 1975). Condensation of **1** and **12** under mild conditions using BF_3 etherate in CH_2Cl_2 afforded a mixture of mono- and di-substituted porphyrins **13a** and **13b** which were separated by column chromatography.

In summary, by employing a variety of reaction conditions, convenient procedures were developed for the synthesis of several rare *meso*-substituted porphyrins containing specific functional groups. The compounds were characterized by ^{1}H NMR and MALDI-TOF spectroscopy. Such structures are potential candidates for light harvesting systems and energy storage via electron

transfer in molecular devices (Kadish et al., 2000).

Methods

General

Melting points (mp) were determined using a Stuart SMP10 instrument. ^{1}H NMR spectra were acquired in $DCCl_3$ or DMSO-d_6 using a Varian 400 MHz or a Gemini 300 MHz spectrometer. Chemical shifts (δ) are expressed in ppm relative to residual chloroform (^{1}H: 7.26 ppm) or to DMSO (^{1}H: 2.49 ppm). Column chromatography occurred on silica gel (Sorbent Technologies, 230-400 mesh), and TLC was performed with polyester sheets precoated with silica gel (Sorbent Technologies). MALDI-TOF experiments were performed on a Voyager Spec instrument. Methyl 3-formylbenzoate, nicotinaldehyde, benzaldehyde, *p*-nitrobenzaldehyde, *p*-aminobenzyl alcohol, 3-chloro-3-methyl-1-butyne (Sigma-Aldrich), trifluoroacetic acid (Alfa Aesar), DDQ, oxalyl chloride, Boc anhydride, and *p*-hydroxybenzaldehyde (Alfa Aesar) were used as received unless otherwise indicated. All final products showed one spot on TLC analysis.

General Procedure for the Synthesis of Porphyrins using Acetic acid

In a single-necked, round-bottomed flask fitted with a condenser and magnetic stirrer were placed dipyrromethane **1** (1.0 equiv) and the corresponding aldehyde (1.0 equiv). Acetic acid (100 volumes w.r.t dipyrromethane) was added to the solution which was refluxed with stirring at 120 ˚C for 4-5 h. The reaction contents became a thick, black solution which was allowed to cool to room temperature. The solvent was evaporated, and the residue was dissolved in ethyl acetate (20 mL). The ethyl acetate solution was treated with 1 *M* NaOH, followed by brine. The organic layer was separated, dried (Na_2SO_4) and evaporated to dryness to yield a black solid. The product was redissolved in CH_2Cl_2 (20 mL), and the solution was passed through a pad of silica to remove most of the impurities. Evaporation of the solvent under vacuum afforded the base porphyrin.

5,15-Bis(3-Methoxycarbonylphenyl)-10,20-bis(4-phenyl)porphinato-Zinc(II) (3).

Compound **1** (0.1 g, 0.35 mol) and benzaldehyde (0.037 g, 0.35 mmol) were treated with acetic acid (20 mL) according to the general procedure. Metallation of the porphyrin occurred when a solution of the product in CH_2Cl_2:MeOH mixture (1:1, 4 mL) was stirred with $Zn(OAc)_2{\cdot}2H_2O$ (0.1 g, 0.45 mol) for 12 h at room temperature. The solvent was evaporated, and the residue was extracted (ethyl acetate, 2 x 20 mL). The combined extracts were washed with brine, dried (Na_2SO_4), and concentrated to a dark purple solid which was then flash chromatographed using ethyl acetate:hexane (1:3) to afford the zinc porphyrin **3**, (79 mg , 28%); mp >300 ˚C. ^{1}H NMR (300 MHz $DCCl_3$): δ 8.96 (d, 4H, J = 4.8 Hz, pyrrole), 8.89 (s, 2H, Ar-CO_2Me), 8.86 (d, 4H, J = 4.8 Hz, pyrrole), 8.49 (d, 2H, J = 7.5 Hz, Ar-CO_2Me), 8.42 (d, 2H, J = 7.5 Hz, Ar-CO_2Me), 8.21 (d, 4H, J = 5.7 Hz, *o*-phenyl), 7.84 (t, 2H, J = 7.5 Hz, Ar-CO_2Me), 7.75 (m, 6H, *m,p*-phenyl), 3.96 (s, 12H, -OCH_3-ester). MALDI-TOF ($C_{48}H_{32}N_4O_4Zn$): Calculated: 792.17. Found: 792.16.

5, 10-Bis(3-Pyridyl)-15, 20-bis(3-methylcarboxyphenyl)porphyrin (4).

Compound **1** (0.8 g, 2.8 mmol) and nicotinaldehyde (0.31 g, 2.8 mmol) were refluxed in acetic acid (350 mL) for 5 h via the general procedure to give **4** as a purple solid (0.51 g, 24%). Reaction of a 1:1 ratio of dipyromethane **2** and methyl 3-formylbenzoate using the same procedure (Scheme 1) gave a low yield of **4** (6%); mp >300 ˚C. ^{1}H NMR (300 MHz, $DCCl_3$): δ 9.46 (d, J = 1.5 Hz, 2H, 2-py), 9.06 (dd, 2H, *J* = 5.2 Hz, J = 1.5 Hz, 6-py), 8.89-8.81 (m, 10H, 8H-pyrrole, 2H-Ar-CO_2Me), 8.53-8.49 (m, 2H, 4 py), 8.41 (d, 2H, *J* = 7.5 Hz, Ar-CO_2Me), 7.86 (t, 2H, *J* = 7.8 Hz, Ar-CO_2Me), 7.76 (td, 2H, *J* = 5.1 Hz, J = 2.4 Hz, 5-py), 3.96 (s, 6H, OCH_3-Ar-CO_2Me), -2.82 (s, 2H, -NH-Porph). MALDI-TOF ($C_{46}H_{32}N_6O_4$): Calcd. 732.24. Found: 732.18.

5, 10-Bis(3-Pyridyl)-15, 20-bis(3-carboxyphenyl)porphyrin (5).

To a stirred solution of porphyrin **4** (0.35 g, 0.47 mmol) in THF (10 mL) was added KOH (4 mL, 1 *M*/methanol). The resulting mixture was stirred at 35 ºC for 6 h. After evaporation of the solvent, water (75 mL) was added, and the reaction mixture was acidified with 1 *M* HCl to pH 4-6. The solution was extracted (ethyl acetate, 3 x 100 mL) and the combined extracts were evaporated to dryness to afford porphyrin **5** (0.32 g, 95%) as a purple solid; mp: >300 ˚C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.28 (s, 2H), 9.36 (d, 2H, J = 1.5 Hz, 2-py), 9.03 (dd, 2H, *J* = 5.2 Hz, 1.5 Hz, 6-py), 8.83 (m, 10H, 8H-pyrrole, 2H-Ar-CO_2Me), 8.67 (m, 2H, 4 py), 8.39 (d, 4H, *J* = 7.5 Hz, Ar-CO_2Me), 7.95 (t, 2H, *J* = 7.8 Hz, Ar-CO_2Me), 7.88 (td, 2H, *J* = 5.1 Hz, J = 2.4 Hz, 5-py), -2.82 (s, 2H, -NH-Porph). MALDI-TOF ($C_{44}H_{28}N_6O_4$): Calculated: 704.21. Found: 704.26.

Synthesis of 4-[N-(tert-Butyloxycarbonyl)amino]benzyl Alcohol (6).

Although **6** was reported (Rai et al., 1992), the following procedure is much easier to perform, A solution of *p*-aminobenzyl alcohol (3.0 g, 0.024 mol) in THF (50 mL) was taken in a two-necked, 100-mL, round-bottomed flask fitted with a magnetic stirrer and was stirred for 5 min. To the solution was added triethylamine (6.7 mL, 0.0487 mol) and Boc anhydride (7.97 g, 0.0365 mol). The resulting solution was stirred at room temperature for 24 h while reaction progress was monitored by TLC. After disappearance of the starting materials via TLC analysis, water (35 mL) was added to the mixture, and the product was extracted (ethyl acetate, 2 x 30 mL). The extracts were washed with brine, were separated, dried (Na_2SO_4), filtered, and evaporated to give **6** as a pale yellow liquid (4.5 g, 83%). The ^{1}H NMR spectrum was identical to the reported (Rai et al., 1992).

Synthesis of 4-[N-(tert-Butyloxycarbonyl)amino]benzaldehyde (7)

Although reported (Rai et al., 1992), **7** was obtained by a more facile procedure. In a 100-mL, two-necked, round-bottomed flask fitted with a rubber septum and an addition funnel was placed a mixture of CH_2Cl_2 (1.15 mL) and DMSO (0.54 mL, 7.73 mmol). The solution was cooled to –78 ˚C (dry ice-acetone bath). Oxalyl chloride (0.71 mL, 8.2 mmol) was added dropwise, and the solution was stirred (30 min), maintaining the temperature at -78 ˚C. Compound **6** (1.15 g, 5.1 mmol) dissolved in CH_2Cl_2 (3 mL) was added dropwise to the reaction mixture which was stirred at -78 ˚C (30 min). TEA (3.59 mL, 25.7 mmol) was then added, and the pale yellow solution generated was stirred for 15 min. The product was extracted (ethyl acetate, 2 x 10 mL), and the combined extracts were washed with brine, dried (Na_2SO_4), filtered and evaporated under reduced pressure to give **7** as a white solid (0.98 g, 87%); mp 136-138 ˚C. (lit[6] mp 138 ˚C). The ^{1}H NMR spectrum was identical to that reported (Rai et al., 1992).

cis- and trans-5,15-Bis[(4-tert-Butoxycarbonylamino)phenyl]-10,20-bis(3-methoxycarbonyl-phenyl)porphinato-Zinc(II) (8a, 8b).

A sample of **1** (0.5 g, 1.7 mmol) and aldehyde **7** (0.39 g, 1.78 mmol) were dissolved in CH_2Cl_2 (190 mL) in a 100-mL, single-necked, round-bottomed flask fitted with a magnetic stirrer. Then TFA (0.243 mL, 3.18 mmol) was added, and the reaction mixture was stirred at room temperature (30 min). Then dichlorodicyanobenzoquinone (DDQ, 0.606 g, 0.00267 mol) was added, and the reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, as judged by TLC analysis, the mixture was neutralized with TEA (3.0 mL). Filtration of the crude mixture through a pad of silica was followed by washing of the pad with CH_2Cl_2. The liquid filtrate was evaporated to dryness to give a dark purple solid. A mixture of methanol and CH_2Cl_2 (10 mL, 1:1) and $Zn(OAc)_2.2H_2O$ (0.5 g, 0.0022 mol) was added to the purple solid, and the resulting solution was stirred overnight at room temperature. Evaporation of the solvent

gave the crude solid which was extracted (CH_2Cl_2 (30 mL). The extract was washed with brine, dried (Na_2SO_4), filtered and evaporated under reduced pressure. Column chromatography of the metallated porphyrin mixture using ethyl acetate:hexane (1:8) afforded a purple solid mixture of *cis*- and *trans*-substituted porphyrins. The isomers were further separated by column chromatography using toluene:methanol (20:1). The *cis*-isomer eluted from the column first. *cis*-**8a:** yield: 0.12 g (7%); mp >300˚C. ^{1}H NMR (400 MHz, $DCCl_3$): δ 8.95 (m, 6H, 4H-pyrrole), 8.85 (s, 2H, Ar-CO_2Me), 8.85 (m, 4H, pyrrole), 8.48 (d, 2H, J = 8 Hz, Ar-CO_2Me), 8.42 (d, 2H, J = 8 Hz, Ar-CO_2Me), 8.13 (d, 4H, J = 8.4 Hz, Ar-NHBoc), 7.84 (t,2H, J = 7.6 Hz, Ar-CO_2Me), 7.72 (d, 4H, J = 8.4 Hz, Ar-NHBoc), 3.96 (s, 6H, -OCH_3-ester), 1.63 (s, 18H, -CH_3-Boc). MALDI-TOF ($C_{58}H_{50}N_6O_8Zn$): Calculated: 1022.29. Found: 1022.24. *trans*-**8b:** yield: 0.5 g (28%); mp: >300 ˚C. ^{1}H NMR (400 MHz, $DCCl_3$): δ 8.96 (d, 4H, J = 4.8, pyrrole), 8.86 (s, 2H, Ar-CO_2Me), 8.85 (d, 4H, J = 4.8, pyrrole), 8.45 (d, 2H, J = 8 Hz, Ar-CO_2Me), 8.42 (d, 2H, J = 8 Hz, Ar-CO_2Me), 8.12 (d, 4H, J = 8.4 Hz, Ar-NHBoc), 7.82 (t, 2H, J = 8 Hz, Ar-CO_2Me), 7.72 (d,4H, J = 8.4 Hz, Ar-NHBoc), 1.65 (s, 18H, -CH_3-Boc), 3.92 (s, 6H, -OCH_3-ester). MALDI-TOF ($C_{58}H_{50}N_6O_8Zn$): Calculated: 1022.29. Found: 1022.25.

5,15-Bis[(4-Amino)phenyl]-10,20-bis(3-methoxycarbonylphenyl)porphine (9).

For deprotection of **8b**, a solution of **8b** (0.1 g, 0.097 mmol) in CH_2Cl_2 (10 mL) was cooled to 0 ˚C in a 100-mL, single-necked, round-bottomed flask fitted with a magnetic stirrer. Then TFA (0.021 mL) was added dropwise. The solution changed from purple to a green color. While stirring the mixure for 2 h at room temperature, the process was monitored by TLC analysis via following a polar spot corresponding to the amine. The reaction mixture was neutralized with 0.1 *M* NaOH (3 mL) and was then extracted (ethyl acetate, 2 x 10 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated to dryness to afford the di-amino,di-ester porphyrin **9** (0.05 g, 72%) as a purple solid; mp >300 ˚C. ^{1}H NMR (400 MHz, $DCCl_3$): δ 8.94 (d, 4H, J = 4.8 Hz, pyrrole), 8.89 (s, 2H, Ar-CO_2Me), 8.74 (d, 4H, J = 4.8 Hz, pyrrole), 8.46 (d, 2H, J = 8 Hz, Ar-CO_2Me), 8.38 (d, 2H, J = 8 Hz, Ar-CO_2Me), 7.97 (d, 4H, J = 8 Hz, Ar-NH_2), 7.82 (t, 2H, J = 7.6 Hz, Ar-CO_2Me), 7.06 (d, 4H, J = 8 Hz, Ar-NH_2), 3.92 (s, 6H, -OCH_3-ester), -2.78 (s, 2H, NH- Porph). MALDI-TOF ($C_{48}H_{36}N_6O_4$): Calculated: 760.28. Found: 760.24.

5, 10-Bis(3-Pyridyl)-15, 20-bis(4-nitrophenyl)porphyrin (10).

The starting materials, dipyrromethane **2** (0.2 g, 0.89 mmol) and *p*-nitrobenzaldehyde (0.13 g, 0.89 mmol), were placed in a 100-mL, single necked, round bottomed flask fitted with a condenser. A mixture of acetic acid and propionic acid (50 mL, 1:0.5) was added, and the resulting pale yellow solution was refluxed for 4 h. After the reaction mixture was cooled to room temperature, evaporation of the solvent under reduced pressure gave a crude solid. Column chromatography of this crude solid using ethyl acetate:hexane (3:1) afforded the target porphyrin **10** as a purple solid (0.39 g, 61%); mp >300 ˚C. ^{1}H-NMR (300 MHz, $DCCl_3$): δ 9.43 (d, 2H, J = 1.5 Hz, 2-py), 9.07 (dd, 2H, *J* = 5.1, 1.5 Hz, 6-py), 8.85 (d, 4H, J = 4.8 Hz, pyrrole), 8.80 (d, 4H, J = 4.8 Hz, pyrrole), 8.64 (d, 4H, *J* = 7.8 Hz, Ar-NO_2), 8.53 (d, 2H, *J* = 7.5 Hz, 4-py), 8.38 (d, 4H, *J* = 7.8 Hz, Ar-NO_2), 7.78 (td, 2H, *J* = 5.1 Hz, 2.4 Hz, 5-py), -2.84 (s, 2H, -NH). MALDI-TOF ($C_{42}H_{26}N_8O_4Zn$): Calculated: 706.21. Found: 706.23.

5, 10-Bis(3-Pyridyl)-15, 20-bis(4-aminophenyl)porphyrin (11).

To the stirred mixture of HCl/ether (20 mL, 1 *M*) in a 50-mL, round-bottomed flask was added $SnCl_2.H_2O$ (1.5 g). Compound **10** (0.1 g, 0.14 mmol) dissolved in $CHCl_3$ (10 mL) was added to the above mixture which was then stirred at room temperature for 8 h. After completion of the reaction, the crude, thick mass obtained was poured onto crushed ice. When the ice melted, $HCCl_3$ (50 mL) was added. The resulting organic layer was separated, washed with water (20 mL),

washed with 1 *M* NaOH (20 mL), washed with brine, dried (Na_2SO_4), filtered, and concentrated to afford porphyrin **11** (0.083 g, 90%) as a purple solid. mp >300 ˚C. ^{1}H-NMR (300 MHz, $DCCl_3$): δ 9.44 (d, 2H, J = 1.5 Hz 2-py), 9.03 (dd, 2H, *J* = 5.1 Hz, 1.5 Hz 6-py), 8.97 (d, 4H, *J* = 4.8 Hz, pyrrole), 8.77 (d, 4H, *J* = 4.8 Hz, pyrrole), 8.50 (d, 2H, *J* = 7.5 Hz, 4-py), 7.97 (d, 4H, *J* = 7.8 Hz, Ar-NO_2), 7.74 (td, 2H, *J* = 5.1 Hz, 2.4 Hz, 5-py), 7.08 (d, 4H, *J* = 7.8 Hz, Ar-NO_2), -2.78 (s, 2H, NH). MALDI-TOF ($C_{42}H_{30}N_8$): Calculated: 646.25. Found: 646.18.

4-[(2-Methylbut-3-yn-2-yl)oxy]benzaldehyde (12).

A mixture of *p*-hydroxybenzaldehyde (1.0 g, 0.0081 mol) and 3-chloro-3-methyl-1-butyne (12.34 g, 0.1210 mol) was placed in a 100-mL, two-necked, round-bottomed flask fitted with a condenser and a magnetic stirrer. Dry DMF (40 mL) was added, and the system was purged with Ar for 15 min. Anhydrous K_2CO_3 (2.0 g, 0.014 mol) and KI (2.28 g, 0.0137 mol) were added, and the reaction mixture was stirred at 65 ˚C for 24 h under Ar. The heterogenous mixture and the combined extracts were allowed to cool to RT and were then filtered. The filtrate was extracted (ethyl acetate, 2 x 20 mL), and the combined extracts were washed with brine, dried (Na_2SO_4), filtered, and evaporated under vacuum to give the crude material. Further purification was achieved by flash chromatography using ethyl acetate:hexane (1:4) to give **12** as an yellow oil (1.31 g 86%). The material was used immediatelly in the next step.

5-(4-[(2-Methylbut-3-yn-2-yl)oxy])phenyl-10,15,20-tris(3-methoxycarbonylphenyl)porp-hyrin (13a), 5,15-(4-[(2-Methylbut-3-yn-2-yl)oxy])phenyl-10,20-tris(3-methoxycarbonyl-phenyl)porphyrin (13b).

To a 100-mL, single-necked, round-bottomed flask fitted with a gas bubbler and a magnetic stirrer was added a solution of **12** (0.067 g, 0.35 mmol) and dipyrromethane **1** (0.1 g, 0.35 mmol) dissolved in dry CH_2Cl_2 (50 mL). The system was degassed for about 15 min with Ar. Then BF_3 ethereate (0.09 mL, 0.7 mmol) was added slowly to the reaction mixture which was stirred at RT for 12 h. A change in color was observed from pale yellow to a dark brown solution. The flask was covered with aluminium foil, and the system was stirred at RT for 1 h during which time a color change occurred from a dark brown to a purple solution. Then DDQ (0.12 g, 0.53 mmol) was added to the reaction, and the solution was purged with Ar with continuous stirring for 1.3 h. The solvent was evaporated, and the crude solid was placed on a pad of silica–celite and was washed with CH_2Cl_2:ethyl acetate (3:1, 450 mL). The liquid filtrate was evaporated to dryness, and the product was further purified by column chromatography using ethyl acetate:hexane (1:5) to give two fractions from which porphyrins **13a** and **13b** were isolated as purple solids. Compound **13a** eluted first from the column. **13a**: yield: (0.024 g, 8%); mp >300 ˚C. ^{1}H NMR (300 MHz, $DCCl_3$): δ 8.92 (d, 2H, J = 4.8 Hz, pyrrole), 8.90 (s, 2H, Ar-CO_2Me), 8.79 (s, 6H, pyrrole), 8.48 (d, 3H, J = 7.8, Ar-CO_2Me), 8.39 (d, 3H, J = 7.8 Hz, Ar-CO_2Me), 8.10 (d, 2H, J = 8.4 Hz, Ar-Alkyne), 7.83 (t, 3H, J = 7.8 Hz, Ar-CO_2Me), 7.60 (d, 2H, J = 8.4 Hz, Ar-Alkyne), 3.99 (s, 9H, -OCH_3-ester), 2.76 (s, 1H, -CH-Alkyne), 1.88 (s, 6H, -CH_3-Alkyne), -2.78 (s, 2H, NH-Porph). MALDI-TOF ($C_{55}H_{42}N_4O_7$): Calculated: 870.30. Found: 870.31. **13b**: yield: 0.038 g (12%); mp: >300 ˚C. ^{1}H NMR (400 MHz, $DCCl_3$): δ 8.90 (bs, 6H, 4H-pyrrole, 2H- Ar-CO_2Me), 8.77 (d, 4H, J = 4.8 Hz, pyrrole), 8.47 (d, 2H, J = 8.1 Hz, Ar-CO_2Me), 8.39 (d, 2H, J = 8.1 Hz, Ar-CO_2Me), 8.10 (d, 4H, J = 8.4 Hz, Ar-Alkyne), 7.83 (t, 2H, J = 8.1 Hz, Ar-CO_2Me), 7.60 (d, 4H, J = 8.4 Hz, Ar-Alkyne), 3.99 (s, 6H, -OCH_3-ester), 2.76 (s, 2H, -CH-Alkyne), 1.88 (s, 12H, -CH_3-Alkyne), -2.78 (s, 2H, NH-Porph). MALDI-TOF ($C_{55}H_{42}N_4O_7$): Calculated: 894.3417. Found: 894.3512.

Conclusions

We have developed procedures to obtain 10 different *meso*-substituted porphyrins containing peripheral functional groups. The structures of all synthesized compounds were characterized by ^{1}H NMR and MALDI–TOF

spectrosocopy. The new porphyrins may find use as building blocks for the construction of novel supramolecular assemblies which can potentially be applied in various bioorganic model systems and molecular devices.

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Abstracts of the
94th Oklahoma Academy of Science Technical Meeting
November 7th, 2014
Northeastern Oklahoma State University – Broken Arrow

GAME THEORY TO IMPROVE SECURITY

Patrick Harrington, Northeastern State University, Tahlequah, OK

This presentation addresses the issue of computer security and explores the use of game theory as one solution to improve it. Game theory has advantages over other solutions in that the game designer models the problem and evaluation criteria, and players' moves optimize the environment once the game is set into motion, reaching an equilibrium solution. Furthermore, game theory allows for modeling complex problems that would be more difficult using other approaches.

SYNTACTIC AND GRAMMATICAL STRUCTURE IN THE CHATTER OF THE EUROPEAN STARLING, STURNUS VULGARIS

Richard Smedley, Northeastern State University, Broken Arrow, OK

Introduction: The European Starling, *Sturnus vulgaris*, is a very social and vocal avian species common in the United States. Its vocalizations can be divided into two distinct categories – song and chatter. Song is non-directed, and although structured, is not language-like. Chatter, on the other hand, is structured and largely directed to other conspecifics. Numerous researchers have analyzed starling song structure. However, the chatter has received little attention. Starling chatter has a distinctive staccato-like chirp and cannot clearly be followed by humans until it is slowed by a factor of four. In its slowed form, structure in the chatter becomes evident. In this study over one thousand chatter motifs were analyzed for syntactic and grammatical structure. Comparisons to both human language and dolphin whistles were made using the methods of McCowan et al. (1999).

Methods: Chatter samples were collected over a four-year period from 2011 to 2014 from an urban nesting site in Broken Arrow, Oklahoma. Discrete chatter samples were extracted from the larger audio files. Custom software, "YakTalk", was developed to extract, enhance, organize and analyze the samples. Over 1,000 chatter samples were extracted and analyzed.

Results: Starling chatter shows a high-level of structure as demonstrated by syllable frequency counts, the Zipf statistic and a recursive grammatical structure. Over 50 syllables were identified. Syllables used for the statistical analysis were "simple" syllables with relatively few frequency shifts. Other syllables dubbed "hyper-syllables" consisted of vocalizations with 90+ bps frequency changes. These were largely excluded from this study, as the software was not designed to match on such complex utterances. Generation of a grammar for the chatter motifs revealed recursion, a feature thought only present in human languages (Hauser, Chomsky and Fitch 2002).

Conclusion: Starling chatter is very language-like statistically and in audible perception. With its similar Zipf statistic, starling chatter entropy is largely indistinguishable from human language and dolphin whistles. Considering the discovery of recursion in the chatter grammar, it becomes tempting to call it a language. The meanings of the utterances are unknown, so more

work needs to be done. The hyper syllables potentially contain a large amount of tightly packed encoded information, so the software needs modifications to parse them. Recursion in the grammar needs a more in-depth look with analysis of longer motifs and at least double the number of chatter samples to resolve higher entropy orders.

SNOOPY: PORTABLE SOFTWARE FOR CAPTURE-RECAPTURE SURVEYS

Richard Smedley; Erik Terdal, Ph.D., Department of Natural Sciences, Northeastern State University, Broken Arrow, OK

Introduction: Camera trapping capture-recapture surveys have become a very important data gathering technique in recent years. Data collected from these surveys is important in animal species abundance, distribution, richness and behavioral studies. As these surveys often produce large amounts of media captures, software to catalog and organize the media has become increasingly important. The available software is limited. The goal of this project was to design and build a comprehensive and portable software suite for capture-recapture surveys.

Methods: The initial stage of the project was requirements gathering and identification of features in currently available software. Software architecture was modeled in Paradigm, a UML modeling package. The software was coded in the Java language on top of an ODBC (Open Database Connectivity) backend using MySQL for the database. This architecture ensured portability to Windows, Mac X and Unix platforms. Comparisons were made continuously to the existing software project, CameraBase, to include desired features and modify others for improved flexibility.

Results: The completed software includes desired features identified from existing software packages as well as advanced features such as environmental and weather-related data. The software was architected to include media recorders other than photographic including audio and video, as well as the ability to add environmental recorders.

Conclusion: Snoopy is a capture-recapture study platform that can be built upon for future enhancements as well as a comprehensive tool for existing surveys. The software is portable to the common operating system platforms and includes a highly configurable database backend. Data can be stored in any ODBC-compliant database of the user's choice including MySQL, MS Access and SQL Server. The user-interface in Snoopy is object-oriented, context-sensitive and intuitive. Future enhancements planned include image recognition and GIS data.

NAÏVE NARCISSISTS OR AFFABLE ALTRUISTS? PERSONALITY CHARACTERISTICS OF THE MILLENNIAL GENERATION

Leah Tecle[1] and **Jennifer Kisamore**[2]

[1] Department of Psychology, Rogers State University, Claremore, OK 74017
[2] Department of Psychology, University of Oklahoma-Tulsa, Tulsa, OK

Millennials, those born between 1982 and 2002, are typically portrayed as tech-savvy narcissists who crave constant feedback and attention (Chambers, 2010; Deal, Altmann & Rogelberg, 2010; Twenge 2011). The current study examined whether these characteristics are really more representative of Millenials than individuals from another generation. Thus far, research on Millenials has predominantly relied on data gathered from college student samples (Deal et al., 2010; Twenge, 2013). Thus, some researchers have suggested that differences in characteristics of Millenials and members of other generations may be a function of different levels of maturation or reliance on non-representative, student-based samples rather than real differences

in personality between members of different generations. This study used archival data gathered from young, working American adults between the ages of 23 and 29 to compare personality characteristics of members of Generation X (those born between 1962 and 1983) to characteristics of members of the Millenial generation. The archival data set included responses from approximately 26,000 Millennials and 9,000 members of Generation X on Hogan Assessment Systems Motives, Values, and Preferences Inventory (MVPI). Based on characteristic typically ascribed to Millenials, five of the 10 MVPI personality factors were compared: recognition, hedonism, altruism, security and affiliation. Significant differences between the generations were noted on hedonism, altruism and affiliation with Millenials scoring slightly but significantly higher than members of Generation X on those three personality characteristics. Thus, Millenials may have higher leisure preferences than members of Generation X, but they also are likely to be more giving and sociable the Gen Xers. Because the current study is based on archival data, causal conclusions are not warranted. Additionally, because the data was collected over several decades, the differences noted between respondents from different generations may be reflective of subtle response biases due to changes in societal values rather than real interpersonal differences.

CULTIVATION STUDIES ON THE GASTROINTESTINAL TRACT FROM AN INDIGENOUS PERUVIAN COMMUNITY YIELDS SEVERAL NOVEL BACTERIAL TAXA

N. B. Patel[1], R. Y. Tito[2], A. J. Obregón-Tito[2], O. Trujillo-Villaroel[3], L. Marin-Reyes[4], L. Troncoso-Corzo[2], E. Guija-Poma[2], C. M. Lewis Jr.[5], P. A. Lawson[1]; [1]University of Oklahoma-Department of Microbiology & Plant Biology, Norman, OK, [2]Universidad Científica del Sur, Lima, Peru, [3]Centro Nacional de Salud Intercultural, Instiuto Nacional de Salud, Lima, Peru, [4]Centro Nacional de Salud Publica, Instituto Nacional de Salud, Lima, PERU, [5]University of Oklahoma-Department of Anthropology, Norman, OK.

While the literature contains many examples of studies focused on the human gut microbiome of individuals from western populations, indigenous populations with a “non-western” diet and lifestyles are underrepresented. In order to truly determine if there is a core human microbiome, individuals with a variety of diets and geographic regions also need to be included in these investigations. The primary purpose of this study is to test the hypothesis that traditional communities from remote regions harbor novel microorganisms influenced by diet, health, and environmental conditions. We used rRNA-based road maps generated in our laboratories to target previously uncultivated bacterial groups to investigate their phylogenetic, physiological, biochemical, and chemotaxonomic properties. Freshly voided fecal samples were collected from members of the Afro-Peruvian community of Cruz Verde in Tambo de Mora, region Ica, in Peru. Multiple enrichments using an array of substrates were constructed and inoculated with 1 ml of fecal slurry. All isolates recovered from the enrichments were maintained on blood agar plates and were screened using 16s gene sequence analysis. A number of isolates yielded relatively low sequence similarity values to those in DNA databases; phylogenetic tree topologies demonstrated that a number of isolates belonged to a group of organisms known as the anaerobic Gram-positive cocci. The nearest relatives included members of the genera Peptoniphilus, Finegoldia, Gallicola and Parvimonas. To date, our studies have identified two novel genera and a new species belonging to the genus Peptoniphilus recovered from a single individual. Our investigations demonstrate that remote indigenous communities harbor novel microbial taxa and further studies employing culture-based approaches of human gut microbiomes of diverse communities are encouraged to augment the insights provided by molecular investigations. Cultivation and characterization of novel organisms from these unique communities will help to

gain a deeper understanding of ecological and functional diversity of the gastrointestinal tract of indigenous communities.

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STATEMENT OF REVENUES COLLECTED AND EXPENSES PAID FOR THE YEAR ENDED 31 DECEMBER 2013

REVENUES COLLECTED

Membership Dues		
Dues		$5,117.00
Investment Income		
Interest CD	1,364.99	$1,364.99
Other Income		
POAS Income	3,771.00	
Woody Plants		
Other	1,053,42	$4,824,42
Registration- Spring Meeting	4,679,00	
Registration -Fall Meeting	6,510.00	
Registration - Technical Meeting	5,804.96	$16,993.96
Total revenue collected		$28,300.37

EXPENSES PAID

Stipend and Other Compensation		
Stipend	6,141,24	
Social Security	824.60	
Medicare	192.84	$7,158.68
Professional Fees		
Audit	400.00	
Tax Preparation	360.00	$760.00

STATEMENT OF REVENUES COLLECTED AND EXPENSES PAID FOR THE YEAR ENDED 31 DECEMBER 2013 (Continued)

Other Expenses		
Spring Meeting	3,201.22	
Fall Meeting	4,927.74	
Technical Meeting	2,434.32	$10,563.28
Insurance	561.80	$561.80
Dues		
AAAS	198.00	
NAAS	450.00	
POAS	3,966.94	
Others	1,782.97	$ 6,397.41
Total Expenses		$25,441.17
Revenues Collected over Expenses Paid		**$2,859.20**

.

OKLAHOMA ACADEMY OF SCIENCE

STATEMENT OF ASSETS, LIABILITIES AND FUND BALANCE ARISING FROM CASH TRANSCATIONS 31 DECEMBER 2013

ASSETS

Cash:		
Checking account	$ 21,851.55	
Savings account	1.186.96	
Savings account	3,273.27	
		$26,311.78
Investments:		
Certificate of Deposit	60,000.00	$60,000.00
Total Assets		$86.311.78

LIABILITIES AND FUND BALANCE

Liabilities	$0.00
Fund balance:	
Beginning operation fund balance	$83,450.84
Excess revenues collected over expenses	2,860.94
	$86,311.78
	$86,311.78

INDEPENDENT AUDITOR'S REPORT

Executive Committee
Oklahoma Academy of Science

I have audited the accompanying statements of assets, liabilities and fund balance arising from cash transactions of the Oklahoma Academy of Science as of December 31, 2013, and the related statements of revenue collected and expenses paid for the year than ended. These financial statements are the responsibility of the Company's management. My responsibility is to express an opinion on these financial statements based on the audit.

I have conducted an audit in accordance with generally accepted auditing standards. An audit to obtain reasonable assurance about whether the financial statements are free of material misstatement and examining, on a test basis evidence supporting the amounts and disclosures in the financial statements. These financial statements were prepared on the basis of cash receipts and disbursements and this report prepared only for the internal use of the Executive Committee of the Oklahoma Academy of Science.

I find the financial statements referred to above present fairly, in all material respects, the assets, liabilities and fund balance arising from cash transactions of The Oklahoma Academy of Science as of December 31, 2013and its revenue collected and expenses paid during the year then ended.

E. Pace, Retired
Assistant County Auditor

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The *Proceedings of the Oklahoma Academy of Science* is published by the Oklahoma Academy of Science. Its editorial policies are established by the Editor and Associate Editors, under the general authority of the Publications Committee. The Editor is appointed by the Executive Committee of the Academy; Associate Editors are appointed by the Publications Committee in consultation with the Editor. The suitability for publication in the *Proceedings* of submitted manuscripts is judged by the Editor and the Associate Editors.

All manuscripts must be refereed critically. The *POAS* Editors have an obligation to the membership of the Academy and to the scientific community to insure, as far as possible, that the *Proceedings* is scientifically accurate. Expert refereeing is a tested, effective method by which the scientific community maintains a standard of excellence. In addition, expert refereeing frequently helps the author(s) to present the results in a clear, concise form that exceeds minimal standards.

The corresponding author is notified of the receipt of a manuscript, and the Editor sends the manuscript to at least two reviewers, anonymous to the author(s). After the initial review, the Editor either accepts the manuscript for publication, returns it to the author for clarification or revision, sends it to another referee for further review, or declines the manuscript.

A declined manuscript will have had at least two reviews, usually more. The Editors examine such manuscripts very carefully and take full responsibility. There are several grounds for declining a manuscript: the substance of the paper may not fall within the scope of the *Proceedings*; the work may not meet the standards that the *Proceedings* strives to maintain; the work may not be complete; the experimental evidence may not support the conclusion(s) that the author(s) would like to draw; the experimental approach maybe equivocal; faulty design or technique may vitiate the results; or the manuscript may not make a sufficient contribution to the overall understanding of the system being studied, even though the quality of the experimental work is not in question.

A combination of these reasons is also possible grounds for declining to publish the MS. In most cases, the Editors rely on the judgment of the reviewers.

Reviewer's Responsibilities

We thank the reviewers who contribute so much to the quality of these *Proceedings*. They must remain anonymous to assure their freedom in making recommendations. The responsibilities or obligations of these reviewers are

- Because science depends on peer-reviewed publications, every scientist has an obligation to do a fair share of reviewing.
- A reviewer who has a conflict of interest or a schedule that will not allow rapid completion of the review will quickly return the manuscript; otherwise, the review will be completed and returned promptly.
- A reviewer shall respect the intellectual independence of the author(s). The review shall be objective, based on scientific merit alone, without regard to race, religion, nationality, sex, seniority, or institutional affiliation of the author(s). However, the reviewer may take into account the relationship of a manuscript under consideration to others previously or concurrently offered by the same author(s).
- A reviewer should not evaluate a manuscript by a person with whom the reviewer has a personal or professional connection if the relationship could reasonably be perceived as influencing judgment of the manuscript.
- The manuscript is a confidential document. If the reviewer seeks an opinion or discusses the manuscript with another, those consultations shall be revealed to the Editor.
- Reviewers must not use or disclose unpublished information, arguments, or interpretations contained in a manuscript under consideration, or in press, without the written consent of the author.
- Reviewers should explain and support their judgments and statements, so both the Editor and the author(s) may understand the basis of their comments.

Brief Instructions to Authors

The instructions to authors wishing to publish their research in the Proceedings of the Oklahoma Academy of Science are listed below. We ask the authors to recognize that the intent is not to establish a set of restrictive, arbitrary rules, but to provide a useful set of guidelines for authors, guidelines that, in most cases, are also binding on the Editors in their task of producing a sound and respected scientific journal.

A. Submission Process.

The *POAS* has transitioned into an online submission platform. The online Journal system (OJS), operated by the Oklahoma State University library will now be the platform through which manuscripts are submitted, handled, and eventually published. Starting in 2015 all manuscripts should be submitted through the online Journal system (OJS) website: http://ojs.library.okstate.edu/osu/index.php/OAS.

B. Types of Manuscripts.

A manuscript may be a paper (report), review, note (communication), a technical comment, or a letter to the editor.

Paper (a report; traditional research paper). A Paper may be of any length that is required to describe and to explain adequately the experimental observations.

Review. The Editor will usually solicit review articles, but will consider unsolicited ones. The prospective writer(s) of reviews should consult the Editor; in general, the Editor needs a synopsis of the area proposed for review and an outline of the paper before deciding. Reviews are typically peer- reviewed.

Note (Communication). The objective of a *Note* is to provide an effective form for communicating new results and ideas and/or describing small but complete pieces of research. Thus, a *Note* is either a preliminary report or a complete account of a small investigation. *Notes* must not exceed four printed pages including text, figures, tables, and references. One journal page of standard text contains about 600 words; hence, there is space for presentation of considerable experimental detail. *Notes* are peer-reviewed.

Technical Comment. Technical comments (one journal page) may criticize material published in an earlier volume of *POAS* or may offer additional useful information. The author(s) of the original paper are asked for an opinion on the comment and, if the comment is published, are invited to reply in the same volume.

Letter to the Editor. Letters are selected for their pertinence to materials published in *POAS* or because they discuss problems of general interest to scientists and/or to Oklahomans. Letters pertaining to material published in *POAS* may correct errors, provide support or agreements, or offer different points of view, clarifications, or additional information.

Abstract. You may submit an abstract of your presentation at the OAS Technical Meeting. For specific instructions, contact the Editor. Even though abstracts are not peer-reviewed, they must align with the policies and scope of the *Proceedings*. The quality or relevance of work may not be in question, but the printed material is still subject to scientific accuracy.

The same guidelines that apply to manuscripts and notes submitted for peer-review, also apply to abstracts submitted for print. Just as manuscripts and notes are subject to thorough testing, so are comments written in abstracts (supported by data). The *Proceedings* understands that all disciplines are in a search for a deeper understanding of the world some of which are through creative expression and personal interpretation. Science is a system by which one discovers and records physical phenomena, dealing with hypotheses that are testable. The domain of "science" while working within nature is restricted to the observable world. There are many valid and important questions to be answered but lie outside the realm of science.

C. Manuscript Organization.

1. General organization.

For papers (reports), the subsections should typically include the following: Abstract, Introduction, Experimental Procedures (or Methods), Results, Discussion, Acknowledgments (if any), and References. In the case of notes or short papers, you may combine some headings, for example, "Results and Discussion":

I. The title should be short, clear, and informative; it should not exceed 150 characters and spaces (three lines in the journal), and include the name of the organism, compound, process, system, enzyme, etc., that is the major object of the study.

II. Provide a running title of fewer than 60 characters and spaces.

III. Spell out either the first or second given name of each author. For example, Otis C. Dermer, instead of O.C. Dermer, or H. Olin Spivey, instead of H.O. Spivey.

IV. Every Paper must begin with a brief Abstract (up to 200 words) that presents clearly the plan, procedure, and significant results of the investigation. The Abstract should be understandable alone and should provide a comprehensive overview of the entire research effort.

V. The Introduction should state the purpose of the investigation and the relationship with other work in the same field. It should not be an extensive review of literature, but provide appropriate literature to demonstrate the context of the research.

VI. The Experimental Procedures (or Methods) section should be brief, but adequate for repetition of the work by a qualified experimenter. References to previously published procedures can reduce the length of this section. Refer to the original description of a procedure and describe any modifications.

VII. You may present the Results in tables or figures or both, but note that it is sometimes simpler and clearer to state the observations and the appropriate experimental values directly in the text. Present a given set of results *in only one form:* in a table, or figure, or the text.

VIII. The Discussion section should interpret the Results and how these observations fit with the results of others. Sometimes the combination of Results and Discussion can give a clearer, more compact presentation.

IX. Acknowledgments of financial support and other aid are to be included.

X. References are discussed below.

1. References

POAS uses the name-year system for citing references. Citations in the text, tables and figure legends include the surname of the author or authors of the cited document and the year of publication. The references are listed alphabetically by authors' surnames in the reference list found at the end of the text of the article. Below are given several examples of correct formats for citing journal articles, books, theses and web resources. For Additional information regarding the name-year system, consult the CBE Manual [Scientific *Style and Format: The CBE Manual for Authors, Editors, and Publishers,* 6th edition]. Abbreviate journal names according to the *International List of Periodical Title Word Abbreviations.*

If it is necessary to refer to a manuscript that has been accepted for publication elsewhere but is not yet published, use the format shown below, with the volume and page numbers absent, the (estimated) publication year included and followed by the words *in press* for papers publications and *forthcoming* for all other forms (CBE 30.68). If the materials are published before the manuscript with that reference is published in *POAS,* notify the Editor of the appropriate volume and page numbers and make the changes as you revise.

Responsibility for the accuracy of bibliographic references rests entirely with the author(s); confirm all references through comparison of the final draft of the manuscript with the original publications. *We expect that the only changes in galley proof will be for typographical errors.* Any mention of *manuscript in preparation, unpublished experiments,* and *personal communication* should be in parenthesis. Use of *personal communication* should be with written permission of the communicator and should be entered only in the text, not in the Reference list.

Examples of References in CBE Style and Format

Journal Articles
Miller LF, Chance CJ. 1954. Fishing in the tail waters of TVS dams. Prog Fish-Cult 16:3-9.

Ortenburger AI, Hubbs CL. 1927. A report on the fishes of Oklahoma, with descriptions of new genera and species. Proc Okla Acad Sci 6:123-141.

Books

Book with Authors:
Miller RJ, Robison HW. 1980. The fishes of Oklahoma. Stillwater (OK): Oklahoma State University Press. 246 p.

Book with Editors:
Gilman AG, Rall TW, Nies AS, Taylor P, editors. 1990. The pharmacological basis of theraputics. 8th ed. New York: Pergamon. 1811 p.

Book with Organization as Author:
International Union of Pure and Applied Chemistry, Physical Chemistry Division. 1993. Quantities, units, and symbols in physical chemistry. 3rd. Oxford (UK): Blackwell Science. 166 p.

Chapter in Book with Editors:
Hamilton K, Combs DL, Randolph JC. 1985. Sportfishing changes related to hydro- power generation and non-generation in the tailwater of Keystone Reservoir, Oklahoma. In: Olsen FW, White RG, Hamre RH, editors. Proceedings of the symposium on small hydropower and fisheries. Bethesda (MD): American Fisheries Society. p 145-152.

Theses: Knapp MM. 1985. Effects of exploitation on crappie in a new reservoir [MSc thesis]. Stillwater (OK): Oklahoma State University. 84 p. Available from: OSU Library.

Internet: Oklahoma Climatological Survey. 2003. Climate of Oklahoma [online]. Available from: http://climate.ocs.ou.edu. (Accessed August 15, 2005).

D. Review Process.

The Editors review the MS and carefully select reviewers for all submitted manuscripts. All referee and editorial opinions are anonymous. A decision to accept, revise, or reject the manuscript is made by the editor after careful consideration of reviewers' comments and recommendations. If a "revise" decision is reached, the authors will be allowed to resubmit a revised version of the manuscript within a given time window. The authors are considered to address all reviewers' comments and concerns, or provide compelling reasons to explain why they chose not to do so. A point-by-point rebuttal letter is required with each revised manuscripts, which clearly indicates the nature and locations of corrections within the revised manuscript. All authors should approve all revisions, with the corresponding author being responsible for insuring that all authors agree to the changes.

E. Page Charges

The OAS will publish accepted MSs with the implicit understanding that the author(s) will pay a charge per published page. Page charges are billed at the cost per page for the given issue: current rates of $90 per page for nonmembers of the Academy and $35 for members. All authors are expected to honor these page charges. Billing for page charges and receipt of payment are handled by the

Business Manager, who is also the Executive Secretary and Treasurer for the Academy.

Under exceptional circumstances, when no source of grant funds or other support exists, the author(s) may apply, at the time of submission, for a waiver of page charges.

F. Copyright Transfer

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