2018

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Recommended Citation

McAllister, Chris T.; Cloutman, Donald G.; Choudhury, Anindo; Scholz, Tomas; Trauth, Stanley E.; Fayton, Thomas J.; and Robison, Henry W. (2018) "Parasites of the Spotted Sucker, *Minytrema melanops* (Cypriniformes: Catostomidae) from Arkansas and Oklahoma," *Journal of the Arkansas Academy of Science*: Vol. 72 , Article 16.

DOI: <https://doi.org/10.54119/jaas.2018.7224>

Available at: <https://scholarworks.uark.edu/jaas/vol72/iss1/16>

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Cover Page Footnote

We thank Drs. Scott L. Gardner and Gabor Racz (HWML) and Renn Tumblison (HSU) for expert curatorial assistance. Appreciation to members of the Oklahoma Department of Wildlife Conservation (ODWC), especially Matt Skoog and Trevor Starks, and Dr. David Neely (Tennessee Aquarium, Chattanooga, TN) and Uland Thomas (Chicago, IL) for assistance with collecting in Oklahoma and Arkansas, respectively. Thanks also to Dr. Michael A. Barger (Peru St. College NE) for identification of the acanthocephalan. The Arkansas Game & Fish Commission and ODWC provided Scientific Collecting Permits to CTM.

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Parasites of the Spotted Sucker, *Minytrema melanops* (Cypriniformes: Catostomidae) from Arkansas and Oklahoma

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Running Title: Spotted Sucker Parasites

Abstract

During October 2015, March and April 2016 and again between March and April 2017, 15 Spotted Sucker (*Minytrema melanops*) were collected from sites in the Ouachita ($n = 5$), Red ($n = 1$), and St. Francis ($n = 5$) river drainages, Arkansas, and the Arkansas River drainage, Oklahoma ($n = 4$), and examined for protozoan and metazoan parasites. Found were *Calyptospora* sp., *Myxobolus* sp., *Pseudomurraytrema alabarrum*, *Biacetabulum banghami*, *Penarchigetes oklensis*, and *Acanthocephalus* sp. New host and distributional records are documented for these parasites.

Introduction

The Spotted Sucker, *Minytrema melanops* (Rafinesque) is a moderately slim-bodied nearly terete sucker that ranges from the Lower Great Lakes basin (lakes Erie, Huron, and Michigan) and Ontario, Canada, the upper Mississippi Valley south to the Gulf slope drainages from Texas to Florida and north on the Atlantic coast to Cape Fear drainage of North Carolina (Gilbert and Burgess 1980). In Arkansas, *M. melanops* is widespread and common but nearly absent from the upper White River drainage (Robison and Buchanan 1988). In Oklahoma, the Spotted Sucker is found in about the eastern half of the state (Miller and Robison 2004). It is adapted to slow-moving slightly-turbid waterways of hard-bottomed creeks and small rivers. They feed on detritus, zooplankton, and various organisms such as mollusks and other invertebrates (White and Haag 1977).

There is a moderate amount of information on parasites of *M. melanops* from various North American localities (Mackiewicz 1968, 1969, Chien and Rogers 1970, Bangham 1972, Dechtiar 1972, Combs *et al.* 1976, 1977, Williams 1976, Christensen *et al.* 1982, Williams and Rogers 1984, Hoffman 1999, Dutton and Barger 2010, Fayton and Kritsky 2013, Gale *et al.* 2014, McAllister *et al.* 2013, 2015, Oros *et al.* 2016, 2018). However, most reports involve fish collected east of the Mississippi River and nothing is known of specimens from Arkansas. Here we report some new host and distributional records for parasites of *M. melanops* from Arkansas and Oklahoma.

Materials and Methods

During October 2015, March and April 2016 and again between March and April 2017, 15 *M. melanops* (mean $\pm$ 1SD total length [TL] = 179.0 $\pm$ 58.6, range 92–243 mm) were collected with a boat electrofisher from **OKLAHOMA**: Arkansas River drainage of the Illinois River, **Cherokee Co.** (35° 57' 30.042"N, 94° 52' 10.0272"W) ($n = 4$); and with seines from **ARKANSAS**: St. Francis River drainage of Crow Creek at Madison, **St. Francis Co.** (35° 00' 45.4752"N, 90° 43' 08.3346"W) ($n = 5$), Red River drainage of the Saline River, **Sevier Co.** (34° 05' 46.3128"N, 94° 05' 5.0496"W) ($n = 1$), Ouachita River drainage at Calion Lake Spillway, **Union Co.** (33° 19' 32.2608"N, 92° 31' 35.1912"W) ($n = 3$), West Tulip Creek, **Dallas Co.** (34° 01' 13.026"N, 92° 44' 4.1136"W) ($n = 1$), and Caddo River, **Montgomery Co.** (34° 23' 52.7742"N, 93° 37' 17.6226"W) ($n = 1$). Specimens were placed in aerated habitat water and necropsied within 24 hr. We

followed accepted guidelines for the use of fish in research (Use of Fishes in Research Committee 2014); specimens were overdosed with a concentrated chlorotone solution and a mid-ventral incision was made to expose the gastrointestinal (GI) tract and viscera. The GI tract was split longitudinally from esophagus to anus and all internal organs were placed in Petri dishes containing 0.9% saline and examined under a stereomicroscope. A species of monogenean was picked with minuten nadeln directly from the gills of fish ($n = 9$) previously preserved in 10% formalin then mounted in Gray and Wess medium stained with Gomori's trichrome. Cestodes from the intestine were fixed in hot tap water without coverslip pressure, transferred to 70–95% (v/v) DNA grade ethanol, stained in Mayer's carmine, dehydrated in an ethanol series, cleared with eugenol, and mounted in Canada balsam (Scholz *et al.* 2015). Tissues with encapsulated parasites were fixed in 10% neutral buffered formalin and processed using standard histological methods (Presnell and Schreibman 1997), with sectioning at 8–10 μm followed by staining with hematoxylin and eosin. Voucher specimens of parasites were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska, or the helminthological collection of the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic (IPCAS). Host voucher specimens were deposited in the Henderson State University (HSU) fish collection, Arkadelphia, Arkansas. Prevalence, mean intensity $\pm$ 1SD, and range of infection are provided in accordance with terminology given in Bush *et al.* (1997).

Results and Discussion

The following is an annotated list of collection and parasite data as follows:

PROTISTA: APICOMPLEXA: CALYPTOSPORIDAE

Calyptospora sp. (Figs. 1A–B)

Host and locality: 1 *M. melanops* (213 mm TL) collected on 21 Apr. 2017 from Crow Creek at Madison, St. Francis Co., Arkansas (35° 00' 45.4752"N, 90° 43' 08.3346"W).

Prevalence: 1/15 (7%) overall; 1/5 (20%) St. Francis Co.

Intensity: Sporulated and unsporulated oocysts too numerous to count.

Site of infection: Oocysts in hepatocytes of liver encapsulated in connective tissue (Fig. 1).

Figures 1A–B. *Calyptospora* sp. from *Minytrema melanops*. (A) Low power magnification showing oocysts in encapsulation of liver tissue; scale bar = 250 μm . (B) High power magnification of encapsulation showing hundreds of oocysts; scale bar = 125 μm .

Type-host and type locality: Gulf Killifish (*Fundulus grandis*), Ocean Springs, Jackson Co., Mississippi (Duszynski *et al.* 1979).

Other reported hosts: Several species of fundulid fishes are known as natural hosts, as well as the Inland Silverside (*Menidia beryllina*) and Gulf Toadfish (*Opsanus beta*) (Solangi and Overstreet 1980, Upton and Duszynski 1982, Fournie and Overstreet 1983, 1993, Hawkins *et al.* 1983, 1984, Oliveira *et al.* 1993, Whipps *et al.* 2012).

Geographic range: Arkansas (this report); Florida (Whipps *et al.* 2012); Louisiana (Duszynski *et al.* 1979); Mississippi (Duszynski *et al.* 1979, Fournie and Overstreet, 1983, 1993, Oliveira *et al.* 1993, Fournie *et al.* 2000).

Additional Arkansas records: None.

Specimens deposited: HWML 139429 (slide).

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Remarks: Infected liver tissue from our host appeared identical to those reported by Duszynski *et al.* (1979) for *Eimeria* (= *Calyptospora*) *funduli* (see their figs. 12–15). This coccidian requires palaemonid shrimp intermediate hosts (Fournie and Overstreet 1983, Overstreet *et al.* 1984, Fournie *et al.* 2000), and one, the Mississippi grass shrimp (*Palaemonetes kadiakensis*), occurs at our study site. Fournie *et al.* (1985) observed numerous motile sporozoites free in the intestinal contents of experimentally infected *P. kadiakensis* and suggested that benthic crustaceans are likely candidates for intermediate hosts in *Calyptospora empristica* Fournie, Hawkins and Overstreet, 1985 because the Starhead Topminnow (*Fundulus notti*) fish-host must apparently die before oocysts can be dispersed.

There are 2 eimerians previously reported from catostomid fishes, *E. catostomi* Molnár and Hanek, 1974 from the intestine of White Sucker (*Catostomus commersoni*) and Northern Hogsucker (*Hypentelium nigricans*) from Canada, and *E. fernandoae* Molnár and Hanek, 1974 from the same hosts and locality (see Hoffman 1999). To date, neither species have been determined to be *Calyptospora* sp. and more importantly, the site of infection was intestinal tissue, not liver. Therefore, the current report is the first time *Calyptospora* sp. has been reported from a catostomid fish and the initial report of this protist from Arkansas and west of the Mississippi River.

CNIDARIA: MYXOBOLIDAE

Myxobolus sp. (Figs. 2A–B)

Hosts and localities: 4 *M. melanops* (222.8 ± 17.1, 205–243 mm TL) collected on 21 Apr. 2017 from Crow Creek at Madison, St. Francis Co., Arkansas (35° 00' 45.4752"N, 90° 43' 08.3346"W); 2 *M. melanops* (116, 120 mm) collected on 13 Mar. 2017 from Illinois River, Cherokee Co., Oklahoma (35° 57' 30.042"N, 94° 52' 10.0272"W).

Prevalence: 6/9 (67%) overall; 4/5 (80%) St. Francis Co., Arkansas; 2/4 (50%) Cherokee Co., Oklahoma.

Intensity: Too numerous to count.

Site of infection: Gills.

Geographic range: The genus is cosmopolitan.

Additional Arkansas records: None.

Specimens deposited: HWML photovoucher 139414.

Remarks: Bangham (1972) reported an unidentified myxozoan from *M. melanops* from Lake Erie. However, a single described species of myxozoan was described 30 yr earlier from Spotted Sucker. Meglitsch (1942) described *M.* (syn. *Myxosoma*) *microthecum*

Figures 2A–B. *Myxobolus* sp. from gills of *Minytrema melanops*. (A) Cyst (arrow); scale bar = 100 μ m. (B) Spores from cyst; scale bar = 20 μ m.

from the mesenteries of *M. melanops* from Illinois. The spores of specimens found on the gills of *M. melanops* in this study are smaller on average (mean length 8.7, range 7.4–11.5 μ m, $n = 30$) than those from the mesenteries in Illinois (11.7, 10.0–12.5 μ m) (Meglitsch 1942). Although the ranges partially overlap, the sites of infection are clearly different. Of the other 10 species of *Myxobolus* reported from the gills of North American suckers (Eiras *et al.* 2005), *Myxobolus globosus* Gurley, 1893, from the Eastern Creek Chubsucker (*Erimyzon oblongus*) is most similar to *Myxobolus* sp. on the gills of *M. melanops* in size and shape of the spores. The spores of *M. globosus* are globose or subcircular, 7–8 μ m long and 6–7 μ m wide (Kudo 1920, Eiras *et al.* 2005). The cysts from these 2 species of *Myxobolus* differ in shape from elongate-ellipsoidal to rod-shaped in *M. globosus* vs. spheroidal in our *Myxobolus* sp. There appears to be considerable site specificity as well as host specificity among fish myxozoans. Therefore,

our form is likely a new species that will require additional study.

MONOGENOIDEA: DACTYLOGYRIDA:

PSEUDOMURRAYTREMATIDAE

Pseudomurraytrema alabarrum Rogers, 1966 (Figs. 3A–C)

Syn. *Murraytrema muelleri* Price, 1967

Hosts and localities: 2 *M. melanops* (205, 216 mm TL) collected on 21 Apr. 2017 from Crow Creek at Madison, St. Francis Co., Arkansas (35° 00' 45.4752"N, 90° 43' 08.3346"W).

Prevalence: 2/9 (22%) overall; 2/5 (40%) St. Francis Co., Arkansas.

Intensity: 2 and 3 worms.

Site of infection: Gills.

Type-host and type locality: *M. melanops*, Lee County, Alabama (Rogers 1966).

Other reported hosts: None.

Geographic range: Alabama (Rogers 1966); Arkansas (this report); Ohio (Mergo and White 1984).

Additional Arkansas records: None.

Specimens deposited: HWML 139418 (4 slides).

Figures 3A–C. *Pseudomurraytrema alabarrum* from the gills of *Minytrema melanops*. (A) Whole mount; scale bar = 150 μ m. (B) Haptor showing marginal hook (MH), dorsal anchors (DA), dorsal bars (DB), ventral anchors (VA), and ventral bar (VB); scale bar = 25 μ m. (C) Reproductive organs showing male copulatory organ (MCO), accessory piece (AP), prostatic glands (PG), and vagina (V); scale bar = 25 μ m.

Remarks: In Alabama, *P. alabarrum* was described from *M. melanops* (Rogers 1966). For the first time we document *P. alabarrum* from Arkansas and west of the Mississippi River.

CESTODA: CARYOPHYLLIDEA: CARYOPHYLLAEIDAE

Biacetabulum banghami Mackiewicz, 1968

Hosts and localities: 1 *M. melanops* (230 mm TL) collected on 21 Apr. 2017 from Crow Creek at Madison, St. Francis Co., Arkansas (sample no. US 672) (35° 00' 45.4752"N, 90° 43' 08.3346"W); 1 *M. melanops* (190 mm) collected on 10 Apr. 2016 from Caddo River, Montgomery Co., Arkansas (sample no. US 593) (34° 23' 52.7742"N, 93° 37' 17.6226"W).

Prevalence: 2/15 (13%) overall; 1/5 (20%) St. Francis Co.; 1/1 (100%) Montgomery Co.

Intensity: 1 and 2 worms.

Site of infection: Intestinal tract.

Type-host and type locality: *M. melanops*; Opintalocca Creek, 3.2 km NE Tuskegee, Macon Co., Alabama (Mackiewicz 1968).

Other reported host: Golden Redhorse, *Moxostoma erythrurum*.

Geographic range: USA (Alabama (Mackiewicz 1968), Arkansas (this report), Oklahoma (Mackiewicz 1968).

Additional Arkansas records: None.

Specimens deposited: IPCAS C-000/1 (slide).

Remarks: *Biacetabulum banghami* was described by Mackiewicz (1968) from *M. melanops* (type host) and *M. erythrurum* from Alabama and Oklahoma. The species is characterized by the presence of 2 papilliform processes on each side of the scolex and vitelline follicles arranged in 2 lateral rows (Mackiewicz 1968). We document *B. banghami* from Arkansas for the first time.

Penarchigetes oklensis Mackiewicz, 1969

Host and locality: 1 *M. melanops* (213 mm TL) collected on 21 Apr. 2017 from Crow Creek at Madison, St. Francis Co., Arkansas (sample no. US 671) (35° 00' 45.4752"N, 90° 43' 08.3346"W).

Prevalence: 1/15 (7%) overall; 1/5 (20%) St. Francis Co.

Intensity: 1 worm.

Site of infection: Intestinal tract.

Type-host and type locality: *M. melanops*; Northeastern Outing Club Lake, 22 km NE of Tahlequah on OK St. Hwy. 10, Cherokee Co., Oklahoma (Mackiewicz 1969).

Other reported hosts: None.

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Geographic range: Arkansas (this report); Oklahoma (Mackiewicz 1969).

Additional Arkansas records: None.

Specimens deposited: IPCAS C-000/1 (slide).

Remarks: *Penarchigetes* was erected by Mackiewicz (1969) to accommodate the new species, *P. oklensis* from *M. melanops* (type and only host) in Oklahoma. Since then, another 2 species were described from Lake Chubsucker, *Erimyzon sucetta*, namely *P. fessus* Williams, 1979 in southeastern USA and *P. macrorchis* Christensen and Calentine, 1983 in New York and Oklahoma (Hoffman 1999).

In addition to the 2 above-listed species, *M. melanops* has been reported to host another 4 species of caryophyllideans. *Promonobothrium ulmeri* (Calentine and Mackiewicz 1966) (syn. *Monobothrium ulmeri*) was described from Northern Hogsucker, *Hypentelium nigricans* in Iowa. The species has recently been reported from *M. melanops* in Mississippi by Oros *et al.* (2016). *Promonobothrium minytremi* Mackiewicz, 1968, was described from *M. melanops* in Oklahoma and then found in the same fish species from Mississippi, South Carolina and Wisconsin (Oros *et al.* 2016) and *Isoglaridacris agminis* sensu Williams and Rogers, 1972 was reported from *M. melanops* from Alabama (Williams 1976). A larval *Glaridacris* sp. was reported from *M. melanops* from Lake Erie by Bangham (1972).

ACANTHOCEPHALA: EOACANTHOCEPHALA: NEOECHINORHYNCHIDAE

Neoechinorhynchus sp.

Host and locality: 1 *M. melanops* (92 mm TL) collected on 22 Apr. 2016 from Calion Lake Spillway, Union Co., Arkansas (33° 19' 32.2608"N, 92° 31' 35.1912"W).

Prevalence: 1/15 (7%) overall; 1/3 (33%) Union Co.

Intensity: 2 male worms.

Site of infection: Intestinal tract.

Other reported hosts: Numerous fishes of various taxa (see Hoffman 1999).

Geographic range: Cosmopolitan.

Additional Arkansas records: Acanthocephalans of the genus *Neoechinorhynchus* have been reported from various Arkansas fishes (see McAllister *et al.* 2016, 2018).

Specimens deposited: None (specimens retained in senior author's collection).

Remarks: Three acanthocephalans have been previously reported from *M. melanops*, *Pomphorhynchus bulbocolli* from Lake Erie (Dechtiar

1972), *Leptorhynchoides thecatus* from Alabama (Williams and Rogers 1982) and *Pomphorhynchus lucyae* from Alabama and Florida (Williams and Rogers 1984). Since females were not collected in our study, it is not possible to provide a species designation. However, we document a new host record for *Neoechinorhynchus* sp.

In summary, we provide the first parasitological survey of *M. melanops* from Arkansas by documenting 2 new host and 4 new distributional records for some of its parasites. In an attempt to collect additional specimens, the senior author (CTM) revisited the St. Francis County site noted herein on 22 July 2018 and did not locate any *M. melanops*. Indeed, larger samples sizes are needed as well as collections of Spotted Suckers from other drainages in Arkansas and Oklahoma. Future studies will undoubtedly report additional records, including the possibility of description of new taxa.

Acknowledgments

We thank Drs. Scott L. Gardner and Gabor Racz (HWML) and Renn Tumilson (HSU) for expert curatorial assistance. Appreciation to members of the Oklahoma Department of Wildlife Conservation (ODWC), especially Matt Skoog and Trevor Starks, and Dr. David Neely (Tennessee Aquarium, Chattanooga, TN) and Uland Thomas (Chicago, IL) for assistance with collecting in Oklahoma and Arkansas, respectively. Thanks also to Dr. Michael A. Barger (Peru St. College NE) for identification of the acanthocephalan. The Arkansas Game & Fish Commission and ODWC provided Scientific Collecting Permits to CTM.

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