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# First Report of the Asian Fish Tapeworm, *Bothriocephalus acheilognathi* (Cestoda: Bothriocephalidea:Bothriocephalidae) from Oklahoma with New Host Records in Non- Hatchery Fishes in Arkansas

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**Abstract:** The Asian fish tapeworm, *Bothriocephalus acheilognathi* is an invasive pathogen in North America and beyond. It first appeared in North America in the mid-1970s likely arriving in shipments of introduced Grass Carp, *Ctenopharyngodon idella* from China to control aquatic vegetation. This tapeworm now can be found in many parts of the world (except Antarctica) where it infects over 200 species of fishes, including several raised in commercial hatcheries. In examining 67 fishes, including 11 Bluntnose Minnow (*Pimephales notatus*), one Spotted Sucker (*Minytrema melanops*), 17 Western Mosquitofish (*Gambusia affinis*) and 22 Creek Chub (*Semotilus atromaculatus*) from four watersheds in Arkansas, and 16 *G. affinis* from a watershed in Oklahoma, we found *B. acheilognathi* in 15 (22%) of them. In addition, examination of 256 fishes from the same watersheds where other fishes were infected with this tapeworm yielded no *B. acheilognathi*. Here, we report, for the first time, *B. acheilognathi* in an Oklahoma fish, document two new host records, and confirm the parasite in Arkansas in non-hatchery fishes. ©2015 Oklahoma Academy of Science

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## Introduction

*Bothriocephalus acheilognathi* Yamaguti, 1934, the Asian fish tapeworm, is a potentially pathogenic cestode species native to eastern Asia in Japan, China, and Russia (Scholz 1997); the first report of *B. acheilognathi* in North America was in 1975, likely in shipments from China of introduced Grass Carp (*Ctenopharyngodon idella*) to control aquatic vegetation (Hoffman 1999). Since then, this parasite has spread in the Western Hemisphere and been reliably reported in numerous fishes from at least 16 U.S. states (including Hawaii, and four states in hatcheries only), Manitoba, Canada, Honduras, Panama, Puerto Rico, Mexico, Argentina and Brazil (Bunkley-Williams and Williams 1994; Font and Tate 1994; Salgado-Maldonado and Pineda-López 2003; Choudhury et al. 2006; Salgado-Maldonado 2006; Salgado-Maldonado et al. 2015). This tapeworm is established on all continents except Antarctica and currently infects about 235 species (19 families) of freshwater fish, primarily cyprinids and poeciliids (Salgado-Maldonado and Pineda-López 2003; Scholz et al. 2012; Choudhury and Cole 2015).

The life cycle of *B. acheilognathi* includes four stages: (1) eggs which are shed in feces from a definitive host fish, (2) a free-swimming coracidium that is swallowed by an intermediate host, usually a cyclopoid copepod (of at least five genera), (3) a proceroid that develops in the intermediate host, and (4) an adult worm that develops from a proceroid in the definitive host (either smaller cyprinid fishes that complete the life cycle or piscivorous fishes that eat smaller fish and complete the life cycle) (Marcogliese and Esch 1989; Kline et al. 2009).

In Arkansas, *B. acheilognathi* has been documented in fish hatcheries/commercial ponds (Scott and Grizzle 1979; Choudhury et al. 2006) but not yet in native fish populations. Rogers (1976) reported transfer of this tapeworm from Arkansas to Florida in *C. idella* and Golden Shiners (*Notemigonus crysoleucus*). In addition, bait minnows (*N. crysoleucus*) said to have originated from Arkansas were reported to be infected with *B. acheilognathi*

obtained from bait shops in Nevada (Heckmann et al. 1993). However, we are not aware of any previous reports of *B. acheilognathi* reported from non-hatchery fishes from Arkansas, nor are there published reports of this tapeworm in any fish from Oklahoma. Here, we report, for the first time, *B. acheilognathi* in an Oklahoma fish and confirm the parasite in Arkansas but in non-hatchery raised (native) fishes.

## Methods

Between July 2014 and August 2015, 67 fishes were collected with seine (3.7 m, 1.6 mm mesh), dipnet or electroshocker as follows: 11 Bluntnose Minnow (*Pimephales notatus*) from East Branch of Gulpha Creek, Garland County, Arkansas (Ouachita River drainage, 34.470655°N, 92.986162°W), 22 Creek Chub (*Semotilus atromaculatus*) from East Flint Creek at Springtown, Benton County, Arkansas (Arkansas River drainage, 36.261867°N, 94.421601°W), one Spotted Sucker (*Minytrema melanops*) from the Arkansas River at Pendleton, Desha County, Arkansas (Arkansas River drainage, 33.987451°N, 91.362222°W), 17 Western Mosquitofish (*Gambusia affinis*) from the Rolling Fork River, Sevier County, Arkansas (Red River drainage, 34.064701°N, 94.38003°W) and 16 *G. affinis* from Yashau Creek at Memorial Drive, McCurtain County, Oklahoma (Red River drainage, 34.011507°N, 94.749615°W). An additional 256 fishes from the same watersheds (Appendix I) were also examined. Fish were placed in containers with cool aerated habitat water; all were necropsied within 24 hr following accepted guidelines for the use of fish in research (AFS 2004). Specimens were overdosed with a concentrated Chloretone solution and a mid-ventral incision was made to expose the gastrointestinal tract, which was split lengthwise, and internal viscera. Tapeworms were rinsed in saline, fixed in nearly boiling distilled water, preserved in DNA grade ethanol (70-95%), stained with acetocarmine, and mounted in Canada balsam. Voucher specimens of parasites were deposited in the Harold W. Manter Laboratory

Table 1. Fishes infected with *Bothriocephalus acheilognathi* from four watersheds of Arkansas and one watershed of Oklahoma.

Species	County Location	Watershed	Intensity <sup>1</sup>	Prevalence <sup>2</sup>
<i>Semotilus atromaculatus</i> <sup>3,4</sup>	Benton Co., AR	E. Flint Creek	1.5 ± 0.6 (1-2)	4/22 (18%)
<i>Minytrema melanops</i> <sup>3,4</sup>	Desha Co., AR	Arkansas River	2.0 ± 0.0 (2)	1/1 (100%)
<i>Pimephales notatus</i> <sup>4</sup>	Garland Co., AR	Gulpha Creek	1.3 ± 0.5 (1-2)	6/17 (35%)
<i>Gambusia affinis</i> <sup>4</sup>	Sevier Co., AR	Rolling Fork River	1.0 ± 0.0 (1)	3/11 (27%)
<i>G. affinis</i> <sup>4</sup>	McCurtain Co., OK	Yashau Creek	6.0 ± 0.0 (6)	1/16 (6%)
			<b>overall = 15/67 (22%)</b>	

<sup>1</sup>mean ± 1SD (range).<sup>2</sup>infected/examined × 100 = percent.<sup>3</sup>New host record.<sup>4</sup>New distributional record.

of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska. Host voucher specimens were deposited in the Henderson State University Museum (HSU), Arkadelphia, Arkansas. Prevalence, mean intensity, and range of infection are provided in accordance with terminology given in Bush et al. (1997).

## Results and Discussion

Of the 67 individual fishes originally examined (Table 1), 15 (22%) were found to harbor tapeworms fitting the description of *B. acheilognathi*. This species is easily distinguished from other *Bothriocephalus* spp. reported from fishes based on the scolex and strobilar morphology; it possesses an arrowhead-shaped or heart-shaped, fleshy scolex with anterolaterally directed narrow slit like openings and a medial position of the genital opening, which is unique among *Bothriocephalus* spp. (Scholz 1997). A total of 12 *B. acheilognathi* (overall mean  $\pm$  1SD intensity =  $1.3 \pm 0.5$ , range 1–2) were removed from the intestinal tract of these four hosts.

Of the four fish species found to be infected, *G. affinis* and *P. notatus* have previously been reported as hosts of *B. acheilognathi*. The Asian fish tapeworm is known from *G. affinis* in California (Choudhury et al. 2006), Louisiana (W. Font pers. comm.), North Carolina (Granath and Esch 1983; Scholz 1997), Texas (Bean and Bonner 2010) and China (syn. *Coelobothrium gambusiense* Yang, Wang, Peng, Zhou and Liu, 2005 per Kuchta et al. 2008). Marcogliese (2008) reported *B. acheilognathi* from *P. notatus* from the Great Lakes, Michigan. However, we document *B. acheilognathi* in *M. melanops* and *S. atromaculatus* for the first time.

We examined other fishes ( $n = 256$ ) from the watersheds from which a species was reported positive for *B. acheilognathi*, but none of them were found to be harboring this tapeworm (Appendix I). Those examined included 72 cyprinids which are usually not suitable hosts for other North American *Bothriocephalus* spp. (Choudhury et al. 2006) and some have been previously reported as hosts of this helminth

(Hoffman 1999). Therefore, although prevalence and intensity of infection is relatively low for the fishes we examined from four sites (three river drainages) in two states, this tapeworm should still be considered a potential pathogenic species that could spread into new drainages (most recently the Rio Grande of North America, see Bean and Bonner 2010) and contribute to the global spread of this invasive parasite. Indeed, *B. acheilognathi* was found infecting two IUCN-listed endangered fishes, including the Humpback Chub (*Gila cypha*) in Arizona (Stone et al. 2007) and the Woundfin (*Plagopterus argentissimus*) in Utah (Heckmann et al., 1986; Heckman 2009); further research indicated that *G. cypha* could maintain this parasite in the Little Colorado River without the presence of carp or other non-native fish species (Hoffnagle et al. 2006; Stone et al. 2007). Ultimately, certain populations of fish could be negatively affected and this tapeworm could inhibit their recovery.

In summary, we provide the first record of *B. acheilognathi* from Oklahoma and the first report of this tapeworm in non-hatchery fishes in Arkansas, including two new host records (*M. melanops*, *S. atromaculatus*). It is now documented herein that the Asian tapeworm occurs in fish hosts in three major river systems in Arkansas, including the lower Arkansas River that drains directly into the Mississippi River. Additional research in both states, particularly Oklahoma, will surely increase the host list and watersheds affected by this invasive parasite.

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**Appendix I. Other fishes ( $n = 256$ ) from same watersheds examined for *Bothriocephalus acheilognathi* and found to be negative.**

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**E. Flint Creek, Benton Co., AR ( $n = 76$ )**

- 1 *Ambloplites rupestris*
- 12 *Campostoma anomalum*
- 6 *Chrosomus erythrogaster*
- 4 *Cottus carolinae*
- 17 *Etheostoma spectabile*
- 17 *Luxilus cardinalis*
- 7 *Notropis boops*
- 4 *N. nubilis*
- 8 *Noturus exilis*

**Arkansas River, Desha Co., AR ( $n = 11$ )**

- 1 *Carpionodes velifer*
- 8 *Cyprinella venusta*
- 2 *Moxostoma erythrurum*

**Gulpha Creek, Garland Co., AR ( $n = 5$ )**

- 2 *Campostoma spadiceum*
- 3 *Fundulus catenatus*

**Rolling Fork River, Sevier Co., AR ( $n = 9$ )**

- 1 *Lepomis megalotis*
- 1 *Lythrurus umbratilis*
- 7 *Noturus gyrinus*

**Yashau Creek, McCurtain Co., OK ( $n = 155$ )**

- 17 *Ameiurus melas*
- 5 *A. natalis*
- 4 *Aphredoderus sayanus*
- 1 *C. spadiceum*
- 6 *Esox americanus*
- 2 *Etheostoma radiosum*
- 101 *Fundulus notatus*
- 2 *Lepomis cyanellus*
- 2 *L. gulosus*
- 1 *L. megalotis*
- 1 *L. umbratilis*
- 5 *Luxilus chrysocephalus*
- 8 *Notemigonus crysoleucus*