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Research Note

New Host Records for *Alloglossidium progeneticum* (Digenea: Alloglossiidae) in Crayfishes (Decapoda: Cambaridae) from Arkansas and Oklahoma, U.S.A.

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ABSTRACT: We examined 125 individual crayfish representing 9 species from Arkansas, Oklahoma, and Texas, U.S.A., for the presence of the antennal gland digenetic trematode *Alloglossidium*. Fifteen (12%) individuals were found to harbor *Alloglossidium progeneticum*, which is reported for the first time from the following second intermediate hosts: 2 of 7 (29%) *Orconectes longidigitus* (longpincered crayfish), 3 of 53 (6%) *Orconectes ozarkae* (Ozark crayfish), 3 of 15 (20%) *Orconectes punctimanus* (spothanded crayfish), 4 of 7 (57%) *Procambarus ouachitae* (Ouachita River crayfish) from Arkansas, and 3 of 10 (30%) *Orconectes menae* (Mena crayfish) from Oklahoma. Four other crayfish species were negative for infection.

KEY WORDS: *Alloglossidium progeneticum*, antennal glands, crayfish, Trematoda, Digenea, Alloglossiidae, *Orconectes*, *Procambarus*, Arkansas, Oklahoma, Texas.

The digenetic trematode *Alloglossidium progeneticum* Sullivan and Heard was originally described from gravid specimens encysted within the antennal glands of the white-tubercled crayfish (*Procambarus spiculifer*) from Calls Creek, Georgia, U.S.A. (Sullivan and Heard 1969). A subsequent study in Calls Creek by Font and Corkum (1975) found adults free in the intestinal tract of brown bullheads (*Ameiurus nebulosus*), thus demonstrating a facultative, 2-host precocious or 3-host life cycle. More recently, Kasl et al. (2015), reported *A. progeneticum* from snail bullheads (*Ameiurus brunneus*) as well as other ictalurid fishes (*Noturus* and *Ameiurus* spp.) from Alabama, Arkansas, Georgia, Louisiana, Oklahoma, or Texas, U.S.A. In addition, Kasl et al. (2015) found that the facultative precocious life cycle was restricted to a few locations in Georgia whereas the other locations appeared to have an obligate 3-host life cycle (i.e., metacercariae were not gravid within surveyed crayfish). Here, we report new host records for this digenean in several species of cambarid crayfishes from Arkansas and Oklahoma. None of these encysted metacerariae was gravid, thus reaffirming that in this part of the geographic range, *A. progeneticum* likely has an obligate 3-host life history.

Between June 2010 and April 2015, 125 individual crayfish from Arkansas, Oklahoma, and Texas were examined for antennal worms as follows: 12 Hubbs crayfish (*Cambarus hubbsi*), 7 longpincered crayfish (*Orconectes longidigitus*), 6 Mammoth Spring crayfish (*Orconectes marchandi*), 7 Neosho midget crayfish (*Orconectes macrus*), 10 Mena crayfish (*Orconectes menae*), 53 Ozark crayfish (*Orconectes ozarkae*), 15 spothanded crayfish (*Orconectes punctimanus*), 8 red swamp crayfish (*Procambarus clarkii*), and 7 Ouachita River crayfish (*Procambarus ouachitae*). When metacercarial cysts were observed in antennal glands, metacercariae (all were nongravid) were teased from capsules using 25-ga insulin needles, placed in Petri dishes containing tap water, and heat-fixed in near boiling tap water without coverslip pressure. Fixed metacercaria were stored in 70% ethanol for morphological studies or in DNA

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grade 95% ethanol for molecular analyses per detailed methods of Kasl et al. (2015). For photomicroscopy, we used a Swift model M10 series light microscope with a digital camera (Swift Optical Instruments, San Antonio, Texas, U.S.A.).

Due to the subtle morphological differences among species of *Alloglossidium* known to infect catfishes (Kasl et al., 2014; Tkach and Mills, 2011), we sequenced at least 1 worm/crayfish species to confirm species identification. The mitochondrial NADH-dehydrogenase subunit 1 gene (ND1) and a fragment of ribosomal DNA spanning the 18s and first internal transcribed spacer region (ITS1) were sequenced, assembled using Sequencher™ (GeneCodes Corp., ver. 4.1.4), and compared to those taken from the type locality (Calls Creek, Georgia, U.S.A.) for positive identification of *A. progeneticum*. Molecular methods and primers are given in Kasl et al. (2015).

All specimens used for morphological analyses were stained with Semichon's acetocarmine, dehydrated in a graded ethanol series, cleared using xylene, and mounted on slides using either Damar gum or Canada balsam. Voucher specimens of crayfishes are deposited in the Henderson State University (HSU) collection, Arkadelphia, Arkansas, U.S.A., and voucher specimens of *A. progeneticum* were deposited in the Harold W. Manter Laboratory of Parasitology, Lincoln (HWML), Nebraska, U.S.A. The ND1 sequences were submitted to GenBank under accession numbers KU728083–KU728091.

Fifteen (12%) individual crayfish were found to harbor *A. progeneticum*; 4 crayfish species ($n = 33$ individuals) were not found to be infected with antennal worms (Table 1).

Trematoda: Digenea: Alloglossiidae *Alloglossidium progeneticum* (Sullivan and Heard, 1969) Font and Corkum, 1975 (Fig. 1–2)

(Syn. *Macroderoides progeneticus* Sullivan and Heard, 1969.)

Hosts and localities: See Table 1.

Site of infection: Antennary glands.

Additional Arkansas crayfish records: White River crayfish (*Procambarus acutus*), Red River crayfish (*Procambarus natchitochae*), red-spotted crayfish (*Orconectes acares*), gapped ringed crayfish (*Orconectes neglectus*), western painted crayfish (*Orconectes palmeri longimanus*), gray-speckled crayfish (*Orconectes palmeri palmeri*) (McAllister et al., 2011; Kasl et al., 2015).

Additional Oklahoma crayfish records: P. acutus, Ouachita Mountain crayfish (*Procambarus tenuis*), *O. p. longimanus* (McAllister et al., 2011; Kasl et al., 2015).

Type host and locality: White-tubercled crayfish, *Procambarus spiculifer* (LeConte, 1856); Calls Creek, Oconee County, Georgia (Sullivan and Heard, 1969).

Other reported hosts: Several species of ictalurid fishes (see Kasl et al., 2015).

Geographic range: Alabama, Arkansas, Georgia, Louisiana, Oklahoma, Texas (Kasl et al., 2015)

Specimens deposited: HWML 101964–101967 (slides).

Remarks

All ITS1 sequences obtained in these surveys ($n = 9$) were identical and the same to that reported in Kasl et al. (2015) as GenBank KT455826. From the 9 sequenced worms, 8 unique ND1 haplotypes were found (Table 1). Although 5 of these haplotypes were new compared to the study of Kasl et al. (2015), all new ND1 haplotypes fell well within the range (no more than 1 or 2 mutations off previously reported ND1 haplotypes; Fig. 3) typically observed for trematode intraspecific mtDNA variation (i.e., $<2\%$ *p*-distance; Vilas et al., 2005). Thus, these molecular data confirm the species identification as *A. progeneticum*.

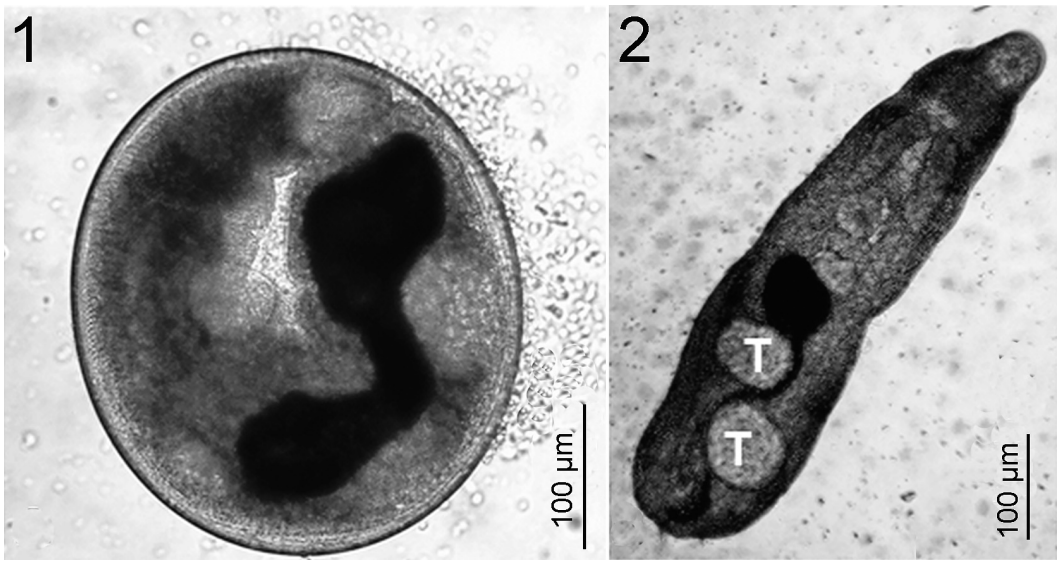
McAllister et al. (2011) reported *Alloglossidium corti* (Lamont, 1921) Van Cleave and Mueller, 1932 from *O. acares* and *O. p. longimanus* from Arkansas and *P. tenuis* from Oklahoma. These metacercariae were identified based strictly on morphological characters in the absence of complimentary molecular techniques. Given the detailed study of Kasl et al. (2015) on similar crayfishes from Arkansas and Oklahoma (including sequenced metacercariae from *O. p. longimanus* in Oklahoma), we are confident that the antennal worms reported by McAllister et al. (2011) represent *A. progeneticum*, thus extending the crayfish hosts known to harbor this worm.

McAllister et al. (2011) noted that some of the stream crayfishes they surveyed for antennal worms were Ozark Mountain endemics from the Interior Highlands of Arkansas and that these crayfishes were found to be without antennal worms. However, with the current study, we can now add several species from the Ozark Mountains of Arkansas to the list of crayfishes known to harbor *A. progeneticum*. Additional crayfish species remain to be surveyed and we expect to add further to our knowledge of intermediate hosts infected with antennal worms from this region of North America.

Table 1. Crayfish examined for *Alloglossidium progeneticum* with their collection data, prevalence, and intensities. All individuals were sequenced at ITS1 and ND1. Given are the sequence accession numbers and haplotype IDs for ND1 (see Fig. 2). AR, Arkansas; OK, Oklahoma; TX, Texas; (–), none.

Crayfish	Dates collected	Locality	Prevalence* (%)	Intensity†	No. sequenced	ND1 accession no.	ND1 haplotypes
Cambarus hubbsi	Nov 2011	AR: Sharp Co., Rock Creek	0/8 (0)	–	–	–	–
	Jul 2012	AR: Searcy Co., Water Creek	0/4 (0)	–	–	–	–
Orconectes longidigitus	Jul 2011	AR: Marion Co., Crooked Creek	0/1 (0)	–	–	–	–
	May 2012, Jun 2013	AR: Searcy Co., Water Creek	2/6 (33)	2, 3	2	KU728090, KU728089	Hap11, Hap12
Orconectes macrus	Nov 2011	AR: Benton Co., Spavinaw Creek	0/7 (0)	–	–	–	–
Orconectes menae	Sep 2011	OK: McCurtain Co., Mt. Fork River	3/10 (30)	4.7 ± 4.7 (1–10)‡	1	KU728091	Hap26
Orconectes marchandi	Nov 2011	AR: Sharp Co., Rock Creek	0/6 (0)	–	–	–	–
Orconectes ozarkae	Jun 2010, Jul 2011	AR: Marion Co., Crooked Creek	0/45 (0)	–	–	–	–
	Jul 2011	AR: Independence Co., Departee Creek	1/5 (20)	1	–	–	–
	Apr 2012	AR: Izard Co., Strawberry River	2/3 (67)	1, 3	1	KU728088	Hap42
Orconectes punctimanus	Jul 2011	AR: Baxter Co., Mill Creek	0/10 (0)	–	–	–	–
	Apr 2012	AR: Baxter Co., Moccasin Creek	1/2 (50)	15	1	KU728085	Hap39
	Apr 2012	AR: Fulton Co., Spring River	2/3 (67)	1 ,5	2	KU728086, KU728087	Hap40, Hap41
Procambarus clarkii	Feb 2012	TX: Tom Green Co., Concho River	0/8 (0)	–	–	–	–
Procambarus ouachitae	Jul 2012	AR: Polk Co., Abernathy Springs	2/2 (100)	3, 4	2	KU728083, KU728084	Hap38
	Apr 2015	AR: Clark Co., Saline Bayou	2/5 (40)	2, 5	–	–	–

* Number infected/number examined.
† Number found in individual hosts.
‡ Mean ± 1 SD (range).



Figures 1, 2. *Alloglossidium progeneticum* from crayfishes. **1.** Light microscopic view of single metacercarial cyst. **2.** Ventral view of metacercaria removed from cyst showing testes (T) but without ova.

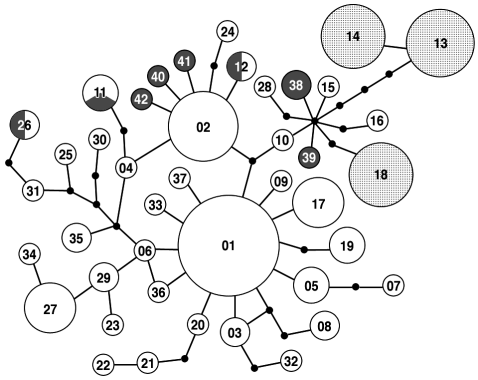


Figure 3. Statistical parsimony network of ND1 mtDNA haplotypes. Numbers correspond to haplotype IDs. The grey haplotypes are those found in the current study (Table 1) and all are from nongravid metacercariae. The white and dotted haplotypes (13, 14, and 18) are data from Kasl et al. (2015). The dotted haplotypes are from facultative precocious populations whereas most of the white haplotypes are from locations where nongravid metacercariae were found (i.e., they have an obligate 3-host life cycle); location and host details are given in Kasl et al. (2015). Each connection is a single mutational step with small black circles representing inferred haplotypes. Circles are scaled according to the number of individuals with that haplotype. The network is not intended to be a final representation of phylogeographic patterns due to opportunistic surveys resulting in uneven sampling. We used TCS version 1.21 to construct the network (Clement et al., 2000).

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