

## ALLOGLOSSIDIUM FLORIDENSE N. SP. (DIGENEA: MACRODEROIDIDAE) FROM A SPRING RUN IN NORTH CENTRAL FLORIDA

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**ABSTRACT:** A new species of *Alloglossidium* is described from the intestines of 2 madtom species (*Noturus leptacanthus* and *Noturus gyrinus*) that were collected from the run of a small, unnamed spring system that drains into the Santa Fe River, Florida. *Alloglossidium floridense* n. sp. is morphologically very similar to other nonprecocious *Alloglossidium* spp. that use ictalurids as definitive hosts, but can be distinguished by a combination of its smaller overall size (length and width), large eggs in relation to its small body size, position of the vitellaria, ovary shape, and position of the ovary in relation to the cirrus sac. A comparison of nuclear rDNA sequences (spanning partial 18s, complete ITS1, 5.8s, ITS2, and partial 28s regions) showed that *A. floridense* n. sp. diverged by 0.70–3.17% from the other 4, nonprecocious species that infect ictalurids (*Alloglossidium corti*, *Alloglossidium fontii*, *Alloglossidium geminum*, and *Alloglossidium kenti*). The new species of *Alloglossidium*, described herein, is the first of the genus to be reported from Florida and the first to be recorded from *N. leptacanthus*. In light of the subtle morphological differences among the nonprecocious species that infect ictalurids, we discuss how previous descriptions of species traits that are not supported with genetic data are difficult to interpret because of the possible past nonrecognition of distinct species.

Within the digenean genus *Alloglossidium* Simer, 1929 (Macroderoididae McMullen, 1937) there is among-species variation in terms of the number of hosts needed to complete the life cycle (2 or 3 hosts) and the types of definitive host species utilized (catfishes, leeches, or crustaceans). The genus is unique among trematodes in that a large proportion of its species exhibit a 2-host life cycle that involves either a crustacean or hirudinid definitive host. Of the 16 nominal species of *Alloglossidium*, 12 exhibit a 2-host pattern, undergoing precocious development to reach sexual maturity in what would typically be considered the second intermediate host. Precocious species can be found in both crustacean (Sullivan and Heard, 1969; Font and Corkum, 1975; Corkum and Turner, 1977; Turner and McKeever, 1993; Font, 1994; Poinar et al., 1995) and leech definitive hosts (Schmidt and Chaloupka, 1969; Beckerdite and Corkum, 1974; Fish and Vande Vusse, 1976; Neumann and Vande Vusse, 1976; Timmers, 1979). Of the precocious species, gravid *Alloglossidium progeneticum* Sullivan and Heard, 1969 has also been reported free in the intestines of a single brown bullhead, *Ameiurus nebulosus* (LeSueur, 1819) (Font and Corkum, 1975). Adult worms of the remaining 4 species (*Alloglossidium kenti* Simer, 1929, *Alloglossidium corti* (Lamont, 1921), *Alloglossidium geminum* (Mueller, 1930), and *Alloglossidium fontii* Tkach and Mills, 2011) have been reported from the intestines of various ictalurid (catfish, madtoms, or bullheads) hosts. Of these 4 species, only the life cycle of *A. corti* has been determined (Crawford, 1937). Because no additional data are available, we work under the assumption that like *A. corti*, the other 3 (*A. kenti*, *A. geminum*, and *A. fontii*) have a typical digenean 3-host life cycle. Thus, for simplicity, we refer to the latter 4 species as nonprecocious species of ictalurids. We note this designation is subject to change pending future studies.

Prior to 2011, no DNA data had been published from the genus *Alloglossidium*, and the only recognized species in the genus that were known to reach maturity in fish were *A. corti*,

*A. geminum*, and *A. progeneticum*. However, both *A. corti* and *A. geminum* have been reported from a broad host and/or geographic range (see Carney and Brooks, 1991; Hoffman, 1999). Pérez-Ponce de León and Choudhury (2010) highlight that molecular prospecting (Vilas et al., 2005) for cryptic species (morphologically similar, but genetically distinct) is particularly relevant in parasitic species that display a wide geographic distribution or broad host associations. Indeed, the rDNA sequence obtained by Tkach and Mills (2011) provided unambiguous evidence that supported the resurrection of *A. kenti*, which was previously synonymized with *A. corti* by Van Cleave and Mueller (1934), and the description of a new species, *A. fontii*. Here, we provide both morphological and DNA sequence evidence for a new species of *Alloglossidium* that infects madtoms from a freshwater artesian spring run in Florida. Including the present new species along with that of Tkach and Mills (2011), there are now 6 species of *Alloglossidium* that can be found as gravid specimens in an ictalurid host. Morphologically the new species is very similar to the 4 nonprecocious *Alloglossidium* spp. that use ictalurids as definitive hosts (thus excluding the precocious *A. progeneticum*). In our discussion, we reiterate the sentiment by Tkach and Mills (2011) that sequence data will be needed to facilitate the validity of subtle morphological differences among the species of *Alloglossidium* that infect catfishes.

### MATERIALS AND METHODS

In June 2012, 21 speckled madtoms, *Noturus leptacanthus* Jordan, 1877, and 8 tadpole madtoms, *Noturus gyrinus* (Mitchell, 1817) were sampled from a small spring feeding into the Santa Fe River in Gilchrist County, Florida. A new digenean species belonging to the genus *Alloglossidium* was recovered from intestines of both host species. Specimens were killed with hot water or heat-fixed underneath a coverslip without pressure to orient the worms ventrodorsally, and stored in either 70 or 95% ethanol. Subsequently, specimens were stained with Semichon's acetic carmine, dehydrated in a graded ethanol series, cleared with the use of xylene, and mounted on slides with the use of Damar gum. Figures were made with the aid of a drawing tube. All measurements were taken from mounted specimens and are given in micrometers; the mean is given for each measurement followed by the range in parentheses. Museum specimens were deposited with the United States National Parasite Collection, Beltsville, Maryland (USNPC) and the Harold W. Manter Laboratory, Lincoln, Nebraska (HWML).

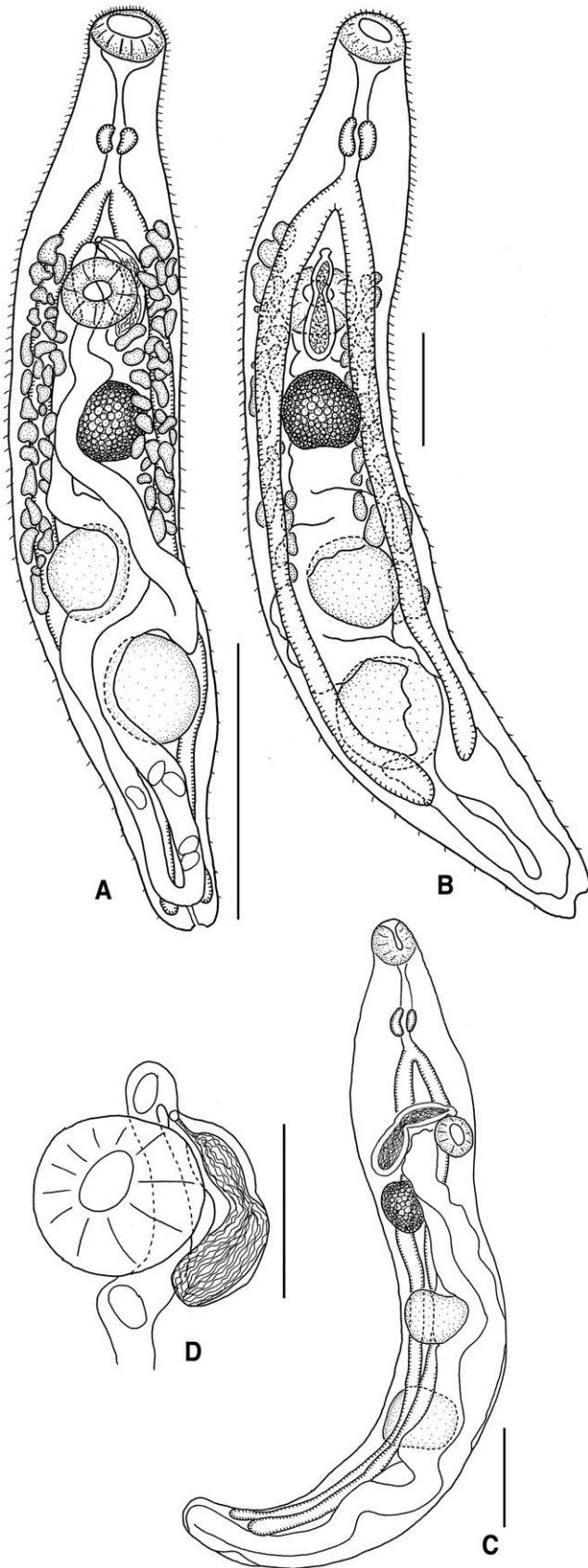
Four specimens of the new species, which were sampled from 2 individuals each of *N. leptacanthus* and *N. gyrinus*, were used to obtain

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rDNA sequence data. DNA was extracted by placing individual worms in 200  $\mu$ l of 5% chelex containing 0.2 mg/ml of proteinase K, incubated for 2 hr at 56 C and boiled at 100 C for 8 min. Polymerase chain reaction (PCR) amplifications were performed, resulting in DNA fragments of approximately 2,450 base pairs (bp) spanning from the 3' end of the 18s nuclear rDNA gene through the internal transcribed spacer region 1 (ITS1), 5.8s, and ITS2 genes to the 5' end of the 28s gene (including domains D1–D3). Twenty-five-microliter reactions containing 3  $\mu$ l of template extract, 15.25  $\mu$ l water, 2.5  $\mu$ l 10x buffer, 2.5  $\mu$ l  $MgCl_2$  [25 mM], 0.5  $\mu$ l dNTP [10 mM/each], 0.5  $\mu$ l of each primer [20  $\mu$ M], and 0.25  $\mu$ l of OmegaTaq polymerase [5 units/ $\mu$ l] were used following the thermocycler profile described in Tkach et al. (2003). An *Alloglossidium*-based primer that we designed and that sits near the 3' end of the 18s, CC41 (5'-GATTGAATGGTTTAGCAAGG-3'), and the general trematode 28s reverse primer 1,500R (5'-GCTATCCTGAGGAACTTCG-3') of Olson et al. (2003) were used for the PCR. In addition to the PCR primers, 3 internal forward primers (CC48 [5'-TGTCGATGAAGAGTGCAGC-3'] developed herein, and 900F [5'-CCGCTTTGAAACACGGACCAAG-3'] and 300F [5'-CAAGTACCGTGAGGAAAGTT G-3'] from Olson et al., 2003) were used for sequencing. PCR products were purified with the use of the Ultra Clean PCR clean-up Kit (MO BIO Laboratories, Inc., Solana Beach, California) and sent to the DNA Analysis Facility on Science Hill at Yale University (New Haven, Connecticut) for sequencing. Contiguous sequences from individuals were assembled using Sequencher™ (GeneCodes Corp., ver. 4.1.4) and submitted to GenBank under accession number KC812276.

We compared our sequences to those of Tkach and Mills (2011) (GenBank JF440783.1, *A. corti*; JF440765.1, *A. fonti*; JF440771.1, *A. geminum*; and JF440808.1, *A. kenti*). Sequences of *A. floridense* n. sp. were aligned against those of *A. corti*, *A. kenti*, *A. geminum*, and *A. fonti* with the use of Clustal W within the BioEdit program, version 7.1.8 (Hall, 1999). Tkach and Mills (2011) noted that *A. fonti* had 1 C/T intraspecific polymorphism in the 28s. The other 4 species in our analyses had a T at this position. Our analyses included the *A. fonti* sequence that had a C at that position. The aligned sequences were examined for pairwise differences with individual gaps treated as differences. Pairwise comparisons were performed using DnaSP version 5.10 (Librado and Rozas, 2009). The alignment is available from the corresponding author upon request.

## DESCRIPTION

### *Alloglossidium floridense* n. sp.

(Fig. 1; Tables I–III)

*Diagnosis* (based on 20 total mounts and 4 specimens used for sequencing): Body elongate, 977 (682–1,263) long  $\times$  197 (126–288) wide at level of ventral sucker; body length to width ratio 5.1:1 (3.6–6.8:1). Tegument spines cover body and decrease in number toward posterior end. Oral sucker 67 (35–91)  $\times$  85 (56–121), subterminal. Ventral sucker 78 (61–101)  $\times$  80 (56–101) located approximately 1/3 of body length from anterior end; forebody length (anterior end of the body to the anterior margin of the ventral sucker) 269 (142–349). Prepharynx 54.6 (20–91) long, roughly twice as long as esophagus. Muscular pharynx 38 (30–56)  $\times$  43 (25–56). Esophagus 22 (5–35) long. Cecal bifurcation 199 (121–258) from anterior end; ceca extend posttesticularly terminating 90 (40–131) from posterior end. Two testes spherical to oval, intercecal, tandem to slightly oblique and larger than ovary; anterior testis 92 (71–116)  $\times$  96 (56–141), posterior testis usually slightly larger, 101 (66–152)  $\times$  99 (66–147). Cirrus sac 105 (76–121)  $\times$  24 (15–35), mostly overlapped by ventral sucker,

FIGURE 1. *Alloglossidium floridense* n. sp. (A) Ventral view of the holotype with 5 representative eggs depicted. (B) Dorsal view of a paratype. (C) Lateral view of a paratype, vitellaria, and spination not shown. Prior to mounting, specimens were either heat-fixed underneath a cover slip without pressure (A, B) or placed in hot water directly (C). (D) Male terminal reproductive structures. Drawing of ovarian complex not shown because details were not discernable in stained specimens. Scale bars = 400  $\mu$ m (A), 150  $\mu$ m (B, C), 100  $\mu$ m (D).

TABLE I. Morphological traits that may be useful for identifying the nonprecocious *Alloglossidium* species described from ictalurid hosts. All trait descriptions for *Alloglossidium floridense* were derived from this study and those of *Alloglossidium fonti* from Tkach and Mills (2011). Note, symbols on the traits of the remaining 3 species indicate source of data (see text for justification). Ranges and means (in parentheses) are provided when available.

Characters	<i>A. floridense</i> n. sp.	<i>Alloglossidium corti</i>	<i>Alloglossidium kenti</i>	<i>Alloglossidium geminum</i>	<i>A. fonti</i>
Body length	687–1,263 (977)	1,000–2,090*	3,159*	1,527*, 1,600†	1,005–1,820 (1,356.2)
Body width	126–288 (197)	213–400*	518*	213*, 455†	167–311 (243.6)
Anterior extent of the vitellaria	Just anterior to the genital pore to the anterior end of the ventral sucker	Pharynx to cecal bifurcation*	Esophagus to cecal bifurcation*,‡	Posterior margin of ventral sucker or further posteriorly*	Anterior margin of ventral sucker or slightly lower
Posterior extent of the vitellaria	Posterior margin of anterior testis	Anterior margin of posterior testis*	Middle to posterior margin of anterior testis*,‡	Between the two testes to the anterior margin of the posterior testis*,§	Posterior margin of anterior testis or midway between testes
Ovary shape	Spherical/oval	Spherical/oval*	Oval /lobed *	Oval*	Oval
Ovary placement	Postcirrus by <half the ovary diameter	Immediately postcirrus*	Immediately postcirrus *	Immediately postcirrus *	Postcirrus by ~half the ovary diameter
Egg length	25–30 (27)	28 §, (34)	(24)‡	24–32§	22–27 (25.1)
Egg width	13–17 (14)	16 §, (20)	(12)‡	12–16§	9–14 (11.8)

\* Tkach and Mills (2011) was the first paper to support morphological trait differences with DNA sequences for species of *Alloglossidium*; thus more weight is given to traits reported in Tkach and Mills (2011) (see text for justification). We used the line drawings and scales provided in Tkach and Mills (2011) to determine positions of organs or to calculate body length and width measurements. The ranges given for *A. corti* are based on 3 drawings and encompass the measurements given in the original species description of Lamont (1921) and in the discussion of Mueller (1930). The measurements for both *A. kenti* and *A. geminum* were based on a single drawing.

† Mueller (1930) did not provide body length/width or egg length/width measurements in his description of *A. geminum*; however, we were able to measure the line drawing in the original description to determine size.

‡ Based on the description of *A. kenti* (Simer, 1929). Measured traits were based on 5 specimens (Simer, 1929). However, no body length or width measurements were reported and we were unable to determine size based off of the line drawing because of the missing figure 8 legend and its scale legend. We also note that although the species description described the ovary as ovoid, the generic description based on *A. kenti* within the same article described the ovary as being “slightly lobed.”

§ Based on the description or measurements given in Van Cleave and Mueller (1934).

|| Means based on 5 specimens used in the description of *Plagiorchis ameirensis* (McCoy, 1928), which was synonymized with *A. corti* (Mueller, 1930).

containing a bipartite seminal vesicle. Genital pore ventral, median, immediately anterior to ventral sucker. Ovary pretesticular, spherical to slightly elongate, 76 (46–101) × 68 (46–101); located posterior to cirrus sac by less than half the diameter of the ovary. Seminal receptacle absent. Laurer’s canal not observed. Vitellaria in 2 lateral follicular bands ventral and lateral to ceca (Fig. 1A, B). Vitellaria in 2 lateral follicular bands ventral and lateral to ceca (Fig. 1A, B) with anterior extent ranging from just anterior to genital pore to anterior end of ventral sucker. Vitellaria extend posteriorly to the anterior margin of anterior testis with 1 band extending to the posterior margin of the anterior testis. The posterior end of the vitellaria extends to the anterior margin of anterior testis with 1 band extending to the posterior margin of the anterior testis. Uterus extending postovarian to posterior of body, overlapping ovary, testes, and ceca when gravid but otherwise lacking coiling between ascending and descending arms. Metraterm poorly differentiated. Eggs oval, numerous, 27 (25–30) long × 14 (13–17) wide. Excretory bladder I-shaped; excretory pore terminal.

#### Taxonomic summary

*Type host:* Speckled madtom, *Noturus leptacanthus* Jordan, 1877.

*Other host:* Tadpole madtom, *Noturus gyrinus* (Mitchill, 1817).

*Type locality:* Run of unnamed spring (29°51′32.03″N, 82°44′5.64″W) feeding into the Santa Fe River, Gilchrist County, Florida.

*Site of infection:* Intestine.

*Prevalence and intensity:* Of the hosts examined, 20 of 21 *N. leptacanthus* were infected (intensity range = 1–25, mean = 10.5) and 7 of 8 *N. gyrinus* were infected (intensity range = 1–5, mean = 2.7).

*Type specimens:* Holotype USNPC 106781; paratypes, USNPC 106782, 106783, Manter Museum HWML-49774, HWML-49775.

*Etymology:* The species epithet is derived from the geographic region of the type locality.

#### Remarks

Morphologically, the features of *Alloglossidium floridense* n. sp. are consistent with the diagnosis of *Alloglossidium* (see Vande Vusse, 1980). Particularly, the presence of tegument spination, a subterminal oral sucker, cirrus sac containing a bipartite seminal vesicle, genital pore immediately anterior of ventral sucker, the absence of a seminal receptacle, lateral vitellaria, and I-shaped excretory bladder support placement within this genus. It should also be noted that after reexamining our specimens with DIC, bright field, and phase contrast microscopes, we were unable to distinguish the ductwork associated with the ovarian complex and for many specimens were not able to clearly see the Mehlis gland. Additionally, for those specimens in which the Mehlis gland was visible, it appeared more ovoid than glandular in nature, which we believe to be an artifact of the fixing or staining process. Because we were unable to obtain a clear, detailed view of the ovarian complex we did not include an illustration.

Within the genus, the new species is most similar to the nonprecocious species of *Alloglossidium* known to infect catfishes: *A. corti*, *A. kenti*, *A. geminum*, and *A. fonti*. Table I lists a summary of morphological traits that may be most useful in distinguishing *A. floridense* n. sp. from the latter species and among the 5 nonprecocious species in general. However, Table I should be regarded as a tentative guide. We caution that many of these differences are subtle such that trait characteristics may overlap among species and no 1 trait separates all the species simultaneously. Moreover, only *A. floridense* n. sp. and *A. fonti* are based on type locality and type host data where both morphological and genetic data are available (Tkach and Mills, 2011). Tkach and Mills (2011) also support morphological trait differences with genetic data for *A. corti*, *A. kenti*, and *A. geminum*. Though they did not use specimens of the latter 3 species from the type localities, we put more emphasis on the data from Tkach and Mills (2011) because they provide a set of morphological traits that



TABLE II. Percent pairwise sequence comparison of *Alloglossidium floridense* n. sp. and 4 other *Alloglossidium* species. Number of variable sites within the ITS1 (519–base pairs [bp] alignment; above diagonal) and 18S (150-bp alignment; below diagonal) gene regions of nuclear ribosomal DNA.

	<i>A. floridense</i> n. sp.	<i>Alloglossidium corti</i>	<i>Alloglossidium kenti</i>	<i>Alloglossidium geminum</i>	<i>Alloglossidium fonti</i>
<i>A. floridense</i> n. sp.		1.7	2.1	5.4	3.5
<i>A. corti</i>	0.7		2.7	8.5	3.7
<i>A. kenti</i>	0	0.7		5.0	3.7
<i>A. geminum</i>	0	0	0.7		7.1
<i>A. fonti</i>	0	0	0	0.7	

are clearly tied to distinct genetic entities. Reasons for less emphasis on past trait descriptions of *A. corti*, *A. kenti*, and *A. geminum* in Table I are given in the Discussion.

Most noticeably, *A. floridense* n. sp. is the smallest of the 5 species. Despite its small body size, its eggs are similar in size to the other species listed in Table I. Thus, egg size relative to body size appears to distinguish *A. floridense* n. sp. from the other nonprecocious species that infect ictalurids. Tkach and Mills (2011) noted that *A. kenti* has a lobed ovary (see their fig. 3). We too have observed this trait in molecularly verified *A. kenti* from Texas (E. L. Kasl, unpubl. data). In contrast, Simer (1929) described and illustrated the ovary as ovoid in the original description of *A. kenti*; however, Simer's (1929) description was based on only 5 specimens and Simer's (1929) generic description states the ovary as being "slightly lobed" (discussed below). The spherical/oval ovary of *A. floridense* n. sp. distinguishes it from the lobed condition of *A. kenti*, as reported by Tkach and Mills (2011), but not the remaining species. Tkach and Mills (2011) also report the ovary of *A. fonti* is a substantial distance posterior from the cirrus sac, whereas in *A. kenti*, *A. corti*, and *A. geminum*, the ovary nearly abuts the posterior end of the cirrus sac. The location of the ovary (posterior to the cirrus sac by less than half the diameter of the ovary) appears to be more variable in *A. floridense* n. sp., with some specimens showing greater distance between the posterior margin of the cirrus sac and the ovary (compare Fig. 1A, B). The extent of the vitellaria was shown to be useful for distinguishing species of *Alloglossidium* that infect catfishes (Tkach and Mills, 2011). This pattern holds true for *A. floridense* n. sp., with the anterior and posterior extents of the vitellaria serving as separate characters with which to differentiate species. When considering the anterior extent of the vitelline fields, *A. floridense* n. sp. begins just anterior to the genital pore to the anterior end of the ventral sucker, which is further anterior than that of *A. fonti* and *A. geminum* (which originate at the anterior and posterior margins of the ventral sucker, respectively), but posterior to that of *A. corti* and *A. kenti* (which can extend from the region of the pharynx to the cecal bifurcation). The posterior extent of the vitellaria of *A. floridense* n. sp. is most similar to that of *A. kenti* and *A. fonti*, with all 3 ending around the posterior margin of the anterior testis. In contrast, in both *A. corti* and *A. geminum* the vitellaria extend further posteriorly; in *A. geminum* the vitelline fields extend to a level between the 2 testes to the anterior margin of the posterior testis while in *A. corti* they extend fully to anterior margin of the posterior testis.

In correlation with the morphological differences, we found strong molecular support for the establishment of *A. floridense* n. sp. (Tables II, III). Across the studied rDNA fragment (2,300 bp from the 3'–18s to the 5'–28s) no intraspecific variability was observed among the 4 specimens of *A. floridense* n. sp., and only the 5.8s was invariable among the compared

species. A comparison of the pairwise sequence variability among the nonprecocious species of *Alloglossidium* is presented in Tables II and III. *Alloglossidium kenti* was the least overall divergent (0.70%, *p*-distance including gaps) from *A. floridense* n. sp., whereas *A. geminum* was the most overall divergent (3.17%). Vilas et al. (2005) note that many morphologically recognized pairs of congener digeneans exhibit about 1% divergence in the ITS regions, which tend to be less conserved than the 18s or 28s. When combining ITS1 and ITS2, *A. floridense* n. sp. shows this approximate level of divergence from *A. kenti* and *A. corti* (1.41 and 1.88%, respectively; Tables II, III). Thus, there is concordance between the molecular and morphological data, which present a strong argument for the establishment of *A. floridense* n. sp.

## DISCUSSION

The subtle morphological variation among the nonprecocious species of *Alloglossidium* that infect ictalurids can make conclusive identification of species difficult with the use of morphology alone. Table I is our attempt to highlight potential characters that may be useful in identifying these species. However, the data in Table I are tentative and several issues need to be addressed before they can be deemed more reliable. First, how is one to know if the observed morphological variation is environmentally induced (e.g., host-induced plastic response), represents standing intraspecific genetic variation, or characterizes distinct species? Investigator-induced variation caused by differences in the fixation and mounting of flatworm specimens can also distort key morphological traits (Bakke, 1988; Criscione and Font, 2001). The latter was an issue in the description of *A. corti* (Lamont, 1921). In particular, when synonymizing *Plagiorchis ameiuensis* McCoy, 1928 with *A. corti*, Mueller (1930) made note that Lamont's "inadequate study of unsuitable preparations" caused her to misrepresent morphological traits. This led Mueller (1930) to redescribe a few key characteristics (i.e., overall size and extent of the vitellaria). A second issue we encountered in trying to identify diagnostic interspecific morphological traits is the lack of reported details in some of the original species descriptions. The description of *A. kenti* provides no mention of the overall body size of the worm and is missing a scale legend on the

TABLE III. Percent pairwise sequence comparison of *Alloglossidium floridense* n. sp. and 4 other *Alloglossidium* species. Number of variable sites within the ITS2 (334–base pair [bp] alignment; above diagonal) and 28S (1,297-bp alignment of the 5' end; below diagonal) gene regions of nuclear ribosomal DNA.

	<i>A. floridense</i> n. sp.	<i>Alloglossidium corti</i>	<i>Alloglossidium kenti</i>	<i>Alloglossidium geminum</i>	<i>Alloglossidium fonti</i>
<i>A. floridense</i> n. sp.		2.1	0.3	3.9	0.6
<i>A. corti</i>	0.3		1.8	4.5	1.8
<i>A. kenti</i>	0.3	0.2		3.6	0.3
<i>A. geminum</i>	2.5	2.5	2.5		3.9
<i>A. fonti</i>	0.6	0.6	0.5	2.8	

corresponding figure (i.e., the legend of fig. 8 in Simer, 1929 is missing), and the description of *A. geminum* by Mueller (1930) gives only a vague discussion (and somewhat different description from the line drawing itself) of the extent of the vitellaria and provides no information as to the overall size of the worm or the size of its eggs. The egg data for *A. geminum* in Table I came from a subsequent report (Van Cleave and Mueller, 1934). Third, further confounding matters, the name *A. kenti* spent decades lumped together with *A. corti*, starting with the synonymy by Van Cleave and Mueller (1934) until its resurrection by Tkach and Mills (2011). Thus, morphological measurements and descriptors (e.g., Carney and Brooks, 1991; Smythe and Font, 2001) as well as host and geographic distribution records over this period are enmeshed under a single species name. Given that *A. fonti* was reported to be most similar to *A. corti* and *A. geminum* (Tkach and Mills, 2011), its variation may also be subsumed under previous reports of *A. corti* or *A. geminum*. Because of this synonymy and the possible past nonrecognition of cryptic species, the data in Table I are more heavily drawn upon from Tkach and Mills (2011) than from past studies based on morphology alone.

To resolve the issue of intraspecific versus interspecific variation in the genus *Alloglossidium*, we advocate the approach of Tkach and Mills (2011), whereby genetic data are used to corroborate observed morphological differences. Although *A. floridense* n. sp. has morphological differences that distinguish it from previously described species in the genus, these differences are subtle. To reiterate Tkach and Mills (2011), without the sequence data, we might have viewed such differences as representing intraspecific variability. To address the lack of details or improper fixation methods in the original descriptions of *A. corti*, *A. geminum*, and *A. kenti*, we suggest that new collections from the type host and the type locality (if possible) are warranted in order to redescribe or amend the previous descriptions and to provide sequence data to support the morphology. Although Tkach and Mills (2011) clearly supported morphological differences with genetic data, their samples of *A. corti*, *A. geminum*, and *A. kenti* did not come from the type localities. Although we think their results will hold, it cannot be certain that their specimens represent the “true” type of each species. As a case in point, the original species description and drawing of *A. kenti* by Simer (1929) describes the ovary as being ovoid. This contrasts with the generic description of the genus *Alloglossidium*, which is based on *A. kenti*, in stating the ovary is “slightly lobed” (Simer, 1929). Tkach and Mills (2011) report a lobed ovary for *A. kenti*. Thus, is this intraspecific variation, developmental stage variation, or did Tkach and Mills (2011) uncover a cryptic species different from that originally described as *A. kenti*? Additional data from the type locality will help resolve these issues. We are in the process of field collections that will help correlate patterns of genetic variation with patterns of morphological variation from type locality specimens of *A. corti*, *A. geminum*, and *A. kenti*. Lastly, once there is a clear picture of trait differences that are supported by DNA-based data, museum specimens can be reexamined in order to ensure their correct identification. The latter will enable more accurate depictions of host and geographic distributions.

In summary, we have identified a new species of *Alloglossidium* that uses ictalurids as a definitive host. The description is based on both morphology and DNA sequence data. Importantly, it appears no 1 morphological trait is simultaneously diagnostic among all the nonprecocious species. Rather a combination of

morphological traits is needed (as given by the tentative list in Table I). Prior to the use of DNA-based data, only 2 species of nonprecocious *Alloglossidium* were recognized from ictalurid hosts. Furthermore, the broad host range and geographic ranges of *A. corti* and *A. geminum* raised the possibility of cryptic species. Indeed, the work of Tkach and Mills (2011) and our current study have added 3 species from ictalurid hosts and thus supported the hypothesis of cryptic species. It remains to be seen if the currently recognized species, or those potentially to be discovered, have any host or geographic affiliation. It will also be interesting to see if the addition of molecular data and/or new species will alter our current understanding of the patterns of life-cycle evolution in this intriguing genus (Smythe and Font, 2001).

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## LITERATURE CITED

- BAKKE, T. A. 1988. Morphology of adult *Phyllodistomum umblae* (Fabricius) (Platyhelminthes, Gorgoderidae): The effect of preparation, killing and fixation procedures. *Zoologica Scripta* **17**: 1–13.
- BECKERDITE, F. W., AND K. C. CORKUM. 1974. *Alloglossidium macrobdellensis* sp. n. (Trematoda: Macroderoididae) from the leech, *Macrobdella ditetra* Moore, 1953. *Journal of Parasitology* **60**: 434–436.
- CARNEY, J. R., AND D. R. BROOKS. 1991. Phylogenetic analysis of *Alloglossidium* Simer, 1929 (Digenea: Plagiorchiiformes: Macroderoididae) with discussion of the origin of truncated life cycle patterns in the genus. *Journal of Parasitology* **77**: 890–900.
- CORKUM, K. C., AND H. M. TURNER. 1977. *Alloglossoides cardiacola* gen. et sp. n. (Trematoda: Macroderoididae) from Louisiana crayfish. *Proceedings from the Helminthological Society of Washington* **44**: 176–178.
- CRAWFORD, W. W. 1937. A further contribution to the life history of *Alloglossidium corti* (Lamont), with especial reference to dragonfly naiads as second intermediate hosts. *Journal of Parasitology* **23**: 389–399.
- CRISCIONE, C. D., AND W. F. FONT. 2001. Artifactual and natural variation of *Oochoristica javaensis*: Statistical evaluation of in situ fixation. *Comparative Parasitology* **68**: 156–163.
- FISH, T. D., AND F. J. VANDE VUSSE. 1976. *Hirudicolotrema richardsoni* gen. et sp. n. (Trematoda: Macroderoididae) from Minnesota hirudinid leeches. *Journal of Parasitology* **62**: 899–900.
- FONT, W. F. 1994. *Alloglossidium greeri* n. sp. (Digenea: Macroderoididae) from the Cajun dwarf crayfish, *Cambarellus schufeldti*, in Louisiana, U.S.A. *Transactions of American Microscopical Society* **113**: 86–89.
- , AND K. C. CORKUM. 1975. *Alloglossidium renale* sp. nov. (Digenea: Macroderoididae) from a fresh-water shrimp and *A. progeneticum* n. comb. *Transactions of the American Microscopical Society* **94**: 421–424.
- HALL, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95–98.
- HOFFMAN, G. L. 1999. *Parasites of North American freshwater fishes*, 2nd ed. Cornell University, Press, Ithaca, New York, 173 p.
- LAMONT, M. E. 1921. Two new parasitic flatworms. *Occasional Papers of the Museum of Zoology, University of Michigan* **93**: 1–4.

- LIBRADO, P., AND J. ROZAS. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**: 1451–1452.
- McCoy, O. R. 1928. Life history studies on trematodes from Missouri. *Journal of Parasitology* **14**: 207–228.
- MUELLER, J. F. 1930. The trematode genus *Plagiorchis* in fishes. *Transactions of the American Microscopical Society* **49**: 174–177.
- NEUMANN, M. P., AND F. J. VANDE VUSSE. 1976. Two new species of *Alloglossidium* Simer 1929 (Trematoda: Macroderoididae) from Minnesota leeches. *Journal of Parasitology* **62**: 556–559.
- OLSON, P. D., T. H. CRIBB, V. V. TKACH, R. A. BRAY, AND D. T. J. LITTLEWOOD. 2003. Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). *International Journal for Parasitology* **33**: 733–755.
- PÉREZ-PONCE DE LEÓN, G., AND A. CHOUDHURY. 2010. Parasite inventories and DNA-based taxonomy: Lessons from helminths of freshwater fishes in a megadiverse country. *Journal of Parasitology* **96**: 236–244.
- POINAR JR., G. O., S. S. SCHWARTS, AND G. CAMERON. 1995. *Alloglossidium anomophagis* sp. n. (Trematoda: Plagiorchidae) exhibiting progenesis in water fleas (Anomopoda: Daphniidae). *Experientia* **51**: 388–390.
- SCHMIDT, G. D., AND K. CHALOUKKA. 1969. *Alloglossidium hirudicola* sp. n., a neotentic trematode (Plagiorchidae) from leeches, *Haemopsis* sp. *Journal of Parasitology* **55**: 1185–1186.
- SIMER, P. H. 1929. Fish trematodes from the lower Tallahatchie River. *American Midland Naturalist* **11**: 563–588.
- SMYTHE, A. B., AND W. F. FONT. 2001. Phylogenetic analysis of *Alloglossidium* (Digenea: Macroderoididae) and related genera: Life-cycle evolution and taxonomic revision. *Journal of Parasitology* **87**: 386–391.
- SULLIVAN, J. J., AND R. W. HEARD III. 1969. *Macroderoides progeneticus* n. sp., a progenetic trematode (Digenea: Macroderoididae) from the antennary gland of the crayfish, *Procambarus spiculifer* (LeConte). *Transactions of the American Microscopical Society* **88**: 304–308.
- TIMMERS, S. F. 1979. *Alloglossidium schmidtii* sp. n. (Trematoda: Macroderoididae) from hirudinid leeches in Manitoba. *Proceedings from the Helminthological Society of Washington* **46**: 459–461.
- TKACH V. V., D. T. J. LITTLEWOOD, P. D. OLSON, J. M. KINSELLA, AND Z. SWIDERSKI. 2003. Molecular phylogenetic analysis of the Microphalloidea Ward, 1901 (Trematoda: Digenea). *Systematic Parasitology* **56**: 1–15.
- , AND A. M. MILLS. 2011. *Alloglossidium fonti* sp. nov. (Digenea, Macroderoididae) from black bullheads in Minnesota with molecular differentiation from congeners and resurrection of *Alloglossidium kenti*. *Acta Parasitologica* **56**: 154–162.
- TURNER, H. M., AND S. MCKEEVER. 1993. *Alloglossoides dolandi* n. sp. (Trematoda: Macroderoididae) from crayfish *Procambarus epicyrtus* in Georgia. *Journal of Parasitology* **79**: 353–355.
- VAN CLEAVE, H. J., AND J. F. MUELLER. 1934. Parasites of Oneida Lake fishes. Part III. A biological and ecological survey of the worm parasites. *Roosevelt Wildlife Annals* **3**: 163–334.
- VANDE VUSSE, F. J. 1980. Revision of *Alloglossidium* Simer 1929 (Trematoda: Macroderoididae) and description of *A. microspinatum* sp. n. from a leech. *Journal of Parasitology* **66**: 667–670.
- VILAS, R., C. D. CRISCIONE, AND M. S. BLOUIN. 2005. A comparison between mitochondrial DNA and the ribosomal internal transcribed regions in prospecting for cryptic species of platyhelminth parasites. *Parasitology* **131**: 839–846.