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## A New Species of *Azygia* (Trematoda: Azygiidae) from Pirate Perch, *Aphredoderus sayanus* (Aphredoderidae), in the Big Thicket National Preserve, Texas, U.S.A.

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**ABSTRACT:** *Azygia aphredoderi* n. sp. is described from the stomach of the pirate perch *Aphredoderus sayanus* (Aphredoderidae) collected from the Menard Creek Corridor Unit of the Big Thicket National Preserve in Texas, U.S.A. The new species is similar to *Azygia angusticauda* in that the ventral sucker and gonads are placed posteriorly, characteristics that allow both species to be distinguished from all others in North America. Members of the new species are characterized by a ventral sucker that is larger than the oral sucker, small body size (<2 mm), small length-to-width ratio, and a forebody-to-hindbody ratio of nearly 1.0, all of which allow them to be distinguished from *A. angusticauda*.

**KEY WORDS:** *Azygia aphredoderi*, Trematoda, *Aphredoderus sayanus*, pirate perch, Big Thicket National Preserve, Texas.

During a biodiversity inventory of the parasites of fishes from waters of the Big Thicket National Preserve, Texas, U.S.A., specimens of *Azygia* that do not correspond to any described species in North America were recovered from pirate perch (*Aphredoderus sayanus*). The new species is described herein.

### MATERIALS AND METHODS

Five pirate perch were collected by seine and minnow trap from Menard Creek at FM 943 in the Menard Creek Corridor Unit of the Big Thicket National Preserve, Polk County, Texas, U.S.A. from 2–8 August 2013. Worms were killed in near-boiling water, fixed, and stored in 70% ethanol, stained in carmalum, dehydrated in an ethanol series, cleared in xylene, and mounted on glass slides in damar balsam. Mounted worms were examined with bright-field and differential interference contrast microscopy, and photographs were taken with an Olympus DP-73 camera mounted on an Olympus BX53 compound microscope. Line drawings of individual structures were made from high-resolution photographs taken in a series of adjacent focal planes, and drawings were then assembled in Adobe Photoshop CS software (Adobe Systems Inc., San Jose, California, U.S.A.) to produce composite line drawings.

Comparisons were made to various lots of *Azygia angusticauda* from both the United States National Parasite Collection (USNPC) and the Harold W. Manter Laboratory of Parasitology (HWML) including: NPC 10525, 10679, 51402, 51412, 76648, 78925, 81477, 89380, 95776, 95778, 96780, 100247, 101451, 101908, and 101931; HWML 1528, 20372, and 31615. In addition, specimens in vials of NPC 38405, 38409, 38410, 38411, and 38413 (all from the Oneida Lake collections of Van Cleave and Mueller, 1934) were mounted for the first time and examined. Measurements were taken for all specimens verified as *A. angusticauda* from these collections.

Shape terms follow the recommendations of Clopton (2004). Measurements are presented in micrometers ( $\mu\text{m}$ ; except where noted) as the range followed by the mean parenthetically.

### RESULTS

#### *Azygia aphredoderi* n. sp. (Fig. 1)

#### Description

Based on observation and measurements of 20 specimens. With characters of the genus. Body short, robust, tapering towards posterior end, 1,380 (1,145–1,855) long, 356 (298–435) wide at widest point, near ventral sucker. Oral sucker subterminal, 233 (191–284) long, 250 (213–298) wide; prepharynx absent; pharynx 88 (75–106) long, 93 (78–110) wide; esophagus very short; ceca convoluted anteriorly, medial and dorsal to vitellaria in posterior portion of body, terminating 118 (76–176) from posterior end. Ventral sucker at midbody, 547 (450–710) from anterior end, 259 (220–303) long, 275 (223–332) wide. Excretory bladder terminal 132 (100–186) long. Ratio of forebody to hindbody (excluding ventral sucker) 0.97 (0.84–1.14); ratio of length of ventral sucker to oral sucker 1.11 (1.07–1.21); ratio of width of ventral sucker to oral sucker 1.11 (1.05–1.17). Gonads contiguous, located in posterior quarter of body, often “squeezed” together, varying in shape from circular to subtriangular. Testes tandem, median in posterior half of hind body; anterior testis 67 (53–76) long, 95 (69–123) wide; posterior testis 83 (68–110) long, 91 (62–120) wide. Seminal vesicle and pars prostatica tubular, contained within prostatic sac immediately anterior to ventral sucker or slightly overlapping, 87 (67–108) long, 82 (69–94) wide; common gonopore empties into sinus with broad, flat opening. Ovary immediately pretesticular, 78 (65–86) long, 93 (76–107) wide. Vitellaria follicular, lateral and extra-cecal, extending from posterior 1/3 of

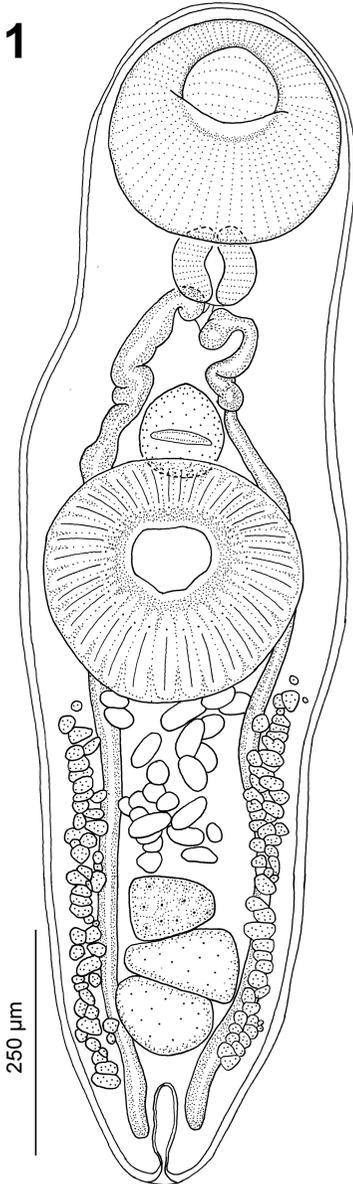


Figure 1. Holotype of *Azygia aphredoderi* n. sp.

ventral sucker to 118 (76–176) from posterior end; vitelline reservoir dorsal to ovary; Mehlis' gland in region of ovary, usually slightly anterior, but obscured by eggs and difficult to observe. Laurer's canal and seminal receptacle not observed, but presumed present (Gibson, 2002). Uterus loops anterior of ovary, filling space between ceca; eggs 53 (46–60) long, 29 (24–34) wide.

### Taxonomic summary

*Type host*: *Aphredoderus sayanus* (Gilliams, 1824), pirate perch.

*Type locality*: Menard Creek, Menard Creek Corridor Unit, Big Thicket National Preserve, Polk County, Texas, 30°34'9.35"N; 94°42'16.95"W.

*Date of collection*: 2–8 August 2013.

*Site of infection*: Stomach, 20 worms in 5 fish (range: 1–7).

*Specimens deposited*: Holotype and paratypes deposited in the Harold W. Manter Laboratory of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska (HWML 49933, 49934).

*Etymology*: The specific epithet references the type host.

### Remarks

The new species most closely resembles *A. angusticauda* (Stafford, 1904) and, with it, can be distinguished from all other species in the genus by the midbody position of the ventral sucker and the posterior position of the gonads. All other species of *Azygia* possess a ventral sucker that is substantially displaced anteriorly and gonads that are placed more anteriorly. The new species can be distinguished from *A. angusticauda* by the fact that the ventral sucker is larger in both dimensions than the oral sucker: the mean ratio for the specimens of *A. angusticauda* in this study was 0.90 (length) and 0.93 (width) compared to 1.11 for both dimensions in the new species. The overall size of the new worm is substantially smaller than what is reported for *A. angusticauda* in the literature and from the specimen base. The smallest specimen of *A. angusticauda* measured in the present study was not gravid and was 1.97 mm long, whereas all gravid specimens were longer than 3 mm. In contrast, the largest gravid specimen of the new species was 1.85 mm long. The ratio of body length to width ranged from 3.58–4.23 (mean = 3.87) whereas in *A. angusticauda* it was 2.90–8.06 (mean = 5.95). Although the ranges overlap, it is clear from examining the specimen base of *A. angusticauda* that these worms are comparatively thin for their length, whereas worms of the new species are stouter. The smaller values of this ratio observed for *A. angusticauda* are for worms that were fixed poorly and had contracted.

### DISCUSSION

The genus *Azygia* has a long and complicated taxonomic history (Manter, 1926; Stunkard, 1956;

Wootton, 1957; Gibson, 2002), with differing judgments regarding what constitutes species-level differences. Manter (1926) synonymized *Azygia loossii* Marshall and Gilbert, 1905 with *A. angusticauda* and 7 other names with *Azygia longa* (Leidy, 1851), and along with *Azygia acuminata* Goldberger, 1911 considered the genus to have 3 representative species in North America. Van Cleave and Mueller (1934) suggested that *A. acuminata* was a synonym of *A. longa*. However, Stunkard (1956) rejected this decision, re-erected *Azygia sebago* Ward, 1910 from Manter's synonymy with *A. longa*, and suggested that *A. acuminata* was a synonym of *A. sebago*. Wootton (1957) resurrected *A. acuminata*, studied the life cycle of this worm, and provided a dichotomous key to the species he considered valid. Much of the confusion in this genus surrounds the assumption that members of these species can infect multiple host species and that they display sometimes massive differences in some morphological traits because of this.

Despite this overall confusion, and the synonymy with *A. loossii* described above, the identity of *A. angusticauda* as a distinct form has been recognized by all previous authors (Wootton, 1957). In all other species of *Azygia* in North America, the gonads are as close or closer to midbody as is the ventral sucker, i.e., both the gonads and the ventral sucker are situated very far to the anterior. In contrast, specimens of *A. angusticauda* possess gonads and a ventral sucker positioned posteriorly, i.e., the gonads are close to the posterior end and the ventral sucker is close to midbody. This difference is apparently not the result of differential growth in different regions of the body (at least, not entirely) because both Sillman (1962) for *A. longa* and Stunkard (1956) for *A. sebago* diagrammed juvenile worms with the ventral sucker and gonads to the anterior, as in the adult forms. Similarly, there was no correlation between the forebody-to-hindbody ratio and worm length either in the specimens of the new species ( $R = -0.38$ ;  $P = 0.18$ ) or in the specimens of *A. angusticauda* studied herein ( $R = -0.15$ ;  $P = 0.50$ ).

*Azygia aphredoderi* joins *A. angusticauda* as the second species in the genus with a ventral sucker and

gonads situated more posteriorly. In fact, the ventral suckers in specimens of *A. aphredoderi* are almost exactly centered at midbody (forebody:hindbody ratio = 0.97), whereas the ventral sucker in *A. angusticauda* is slightly more anterior (forebody:hindbody ratio = 0.76). The new species is also the only known member of the genus in which the ventral sucker is larger than the oral sucker, the reverse of which is cited by Gibson (2002) as a character of the genus *Azygia*.

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