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## Morphological Confirmation of *Homalometron* (Trematoda: Apocreadiidae) Species in Freshwater Fishes in Southeastern Texas, U.S.A., with Description of Two Species

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**ABSTRACT:** Recent work using DNA sequence data considered *Homalometron armatum* (McCallum, 1895) Manter, 1947 (Trematoda: Apocreadiidae) to consist of 3 species. The goal of the present investigation was to test, using new collections, whether these 3 putative species could be recognized and differentiated morphometrically. Newly collected worms from freshwater drum (*Aplodinotus grunniens*) and redear sunfish (*Lepomis microlophus*) in Texas, U.S.A., were combined with existing museum collections in multivariate analyses of morphometric characteristics. Principal components analysis on new collections suggested that worms did group by morphometric characteristics relativized by worm length. Discriminant function analyses demonstrated a high degree of fidelity between a priori classification of worms and the ability to discriminate among populations using morphometric data. Based on these results, the 2 as-yet undescribed species of *Homalometron* are named herein.

**KEY WORDS:** *Homalometron armatum*, *Homalometron currani*, *Homalometron microlophi*, *Aplodinotus grunniens*, *Lepomis microlophus*, freshwater drum, redear sunfish, Texas, Big Thicket National Preserve.

Curran et al. (2013) studied new collections of *Homalometron* collected from freshwater fishes in Mississippi and Tennessee and discovered 2 previously undescribed species via DNA sequence comparisons. *Homalometron armatum* (McCallum, 1895) Manter, 1947, was found in freshwater drum (*Aplodinotus grunniens*), as was a second species (referred to as “species A”); a third species occurred in redear sunfish (*Lepomis microlophus*) (referred to as “species B”). These latter 2 species were not formally named because of a lack of morphological differentiation. Significantly, the ranges of most of the characteristics that were measured overlapped among the 3 species.

Specimens of *Homalometron* were collected from drum and redear sunfish as part of an ongoing biodiversity survey in and around the Big Thicket National Preserve, Texas, U.S.A. The new collections conform to the predicted species diversity of Curran et al. (2013) in that there appear to be 3 distinct forms: 2 in drum and 1 in redear sunfish. Multivariate analyses of morphometric data confirmed that specimens fall into 3 natural groupings, and the 2 unnamed species are named herein.

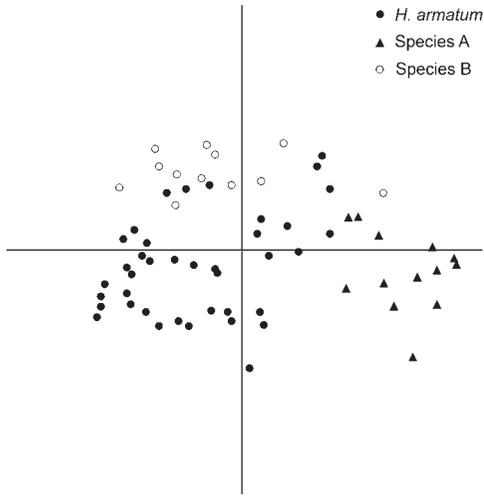
### MATERIALS AND METHODS

Specimens of *Homalometron* were collected from freshwater drum (*Aplodinotus grunniens*) and redear sunfish (*Lepomis microlophus*) from 4 locales in southeastern Texas,

U.S.A.: Village Creek at Highway 96 north of Lumberton, Village Creek Unit, Big Thicket National Preserve (30°17'8.63"N; 94°11'32.99"W); Pine Island Bayou at Highway 96 north of Beaumont, Pine Island Bayou Unit, Big Thicket National Preserve (30°10'45.63"N; 94°11'11.54"W); Neches River upstream of the confluence with Pine Island Bayou north of Beaumont, Lower Neches River Unit, Big Thicket National Preserve (30°10'16.32"N; 94°6'44.99"W); and Long King Creek at Highway 190 in Livingston (30°42'59.24"N; 94°57'32.75"W). Worms from 7 drum and 9 redear sunfish were utilized in the present investigation. Specimens were borrowed from the Harold W. Manter Laboratory of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska, U.S.A. (HWML 775, 776, 1259, and 38240) and the National Parasite Collection, U.S. Department of Agriculture, Beltsville, Maryland, U.S.A. (NPC 77899, 101911, 101913, 102981, 106461, 106462).

Live worms were killed in hot water, fixed and stored in 70% ethanol, stained in carmalum, and mounted on glass slides in damar balsam. Measurements were made on each adult worm (with at least 1 fully formed egg en utero); worms that were contorted or immature were not utilized in subsequent analyses or in the calculation of summary statistics. The following measurements were taken on each worm: total length, maximum width, location of maximum width (distance from anterior), prepharynx length, esophagus length, pretesticular space, post-testicular space, forebody (anterior end to anterior of ventral sucker), postcecal space, space between ovary and anterior testis, and the length and width of the oral sucker, ventral sucker, anterior testis, posterior testis, pharynx, seminal vesicle, seminal receptacle, and ovary. Museum specimens were measured, as well. Each worm was tentatively classified as *H. armatum* species A or species B by comparison with measurements and figures provided in Curran et al. (2013). Sixty-five of 82 newly collected worms and 22 worms from museum collections were utilized in subsequent analyses.

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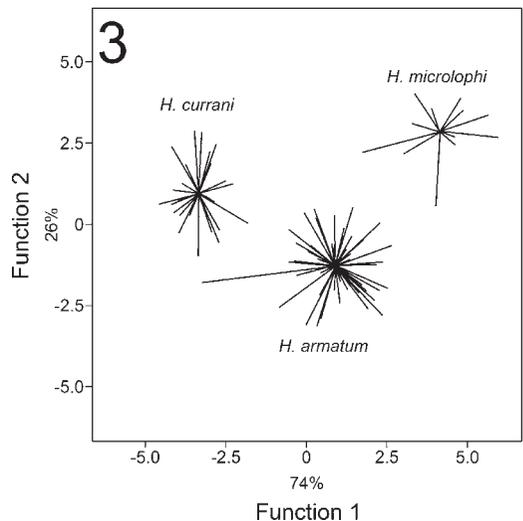
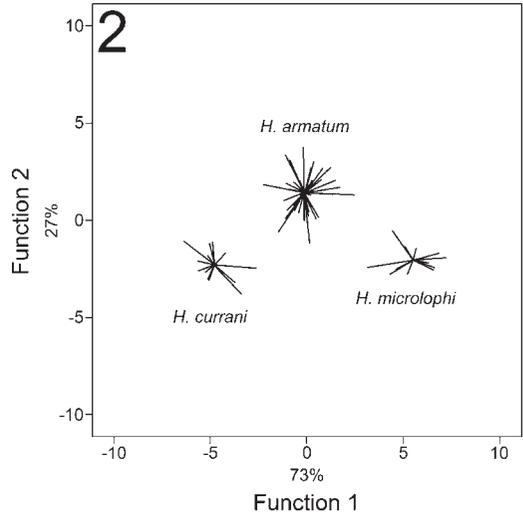


**Figure 1.** Principal components analysis of relativized morphometric data on newly collected *Homalometron* specimens. Species designations are based on comparison of specimens to descriptions and figures in Curran et al. (2013).

Principal components analysis (PCA) and discriminant function analysis (DFA) were used to determine whether specimens that conformed to the species of Curran et al. (2013) could be reliably differentiated using morphological features. PCA was run using raw measurement data and using a data set in which each measurement was relativized by worm length on newly collected worms only. Tentative species identifications (from above) were utilized to code each species as 1 of the 3 species of Curran et al. (2013) in a data set of relativized data. DFA was then run on the data set including only new worms and on the data set including new worms and museum worms using relativized data. Shape terms follow the recommendations of Clopton (2004).

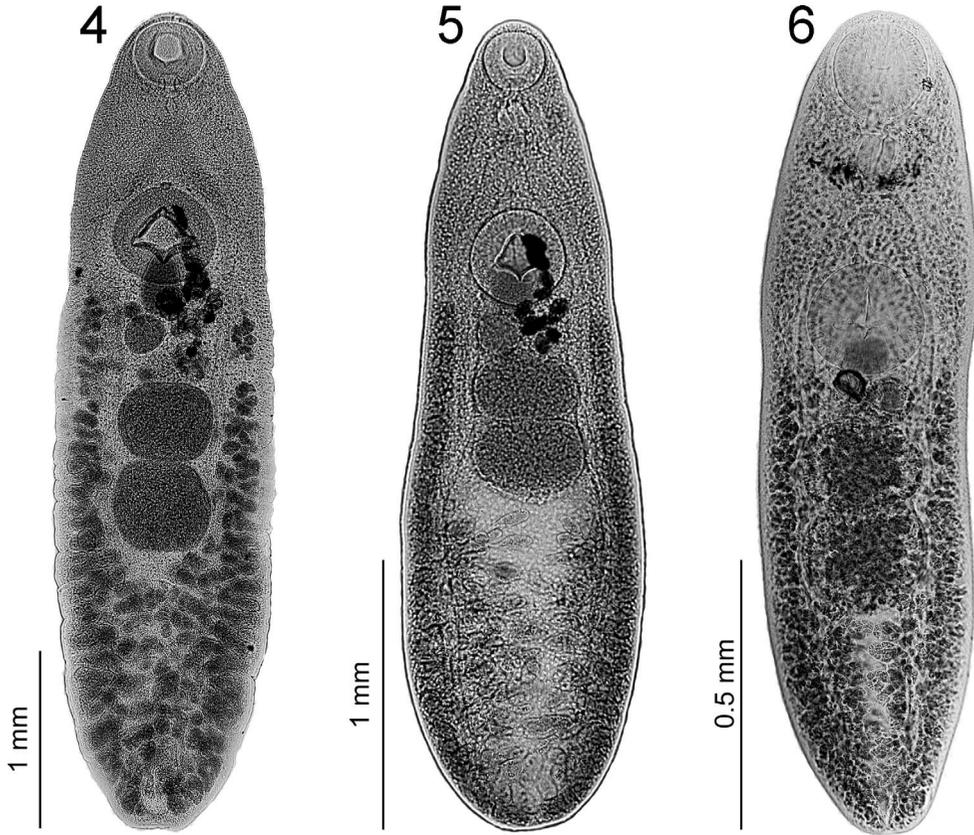
## RESULTS

The first axis of the PCA on raw data accounted for 71% of the variance in the data set and was correlated primarily with worm length and all other measures that were themselves either positively or negatively correlated with worm length. Relativizing the data set by worm length resulted in a PCA in which the first 2 axes accounted for 48% of the variance, and there appeared to be some grouping of worms in the plot by their putative identities (Fig. 1). The first principal component was negatively correlated with relativized sizes of the oral sucker, ventral sucker, ovary, and pharynx, as well as the pretesticular space; the post-testicular space was positively correlated with the first axis. The second principal component was primarily composed of negative correlations with relativized maximum width and the location of the maximum width.



**Figures 2, 3.** Discriminant function analysis of relativized morphometric data of *Homalometron armatum*, *Homalometron currani* n. sp., and *Homalometron microlophi* n. sp. **2.** Newly collected specimens only. **3.** Newly collected and existing museum specimens. Percentages represent fraction of total variation accounted for by function.

DFA on newly collected specimens resulted in clear separation among all 3 putative species (Fig. 2); 100% of original cases were correctly classified. Most relativized measures were significantly different among groups, with the exceptions being: widths of the anterior and posterior testes, length of the esophagus, lengths and widths of the seminal vesicle and seminal receptacle, and distance between the



**Figures 4–6.** Representatives of *Homalometron* species collected in the present investigation. **4.** *Homalometron armatum*. **5.** *Homalometron currani* n. sp. **6.** *Homalometron microlophi* n. sp.

ovary and anterior testis. The relativized sizes of the oral sucker, ventral sucker, and the pre- and post-testicular spaces were most different among groups.

DFA on newly collected and museum specimens returned similar results (Fig. 3); 98.9% of original cases were correctly classified. However, 1 specimen originally identified as *H. armatum* (far left in that group in Fig. 3) was misclassified. The same variables as above were strongly correlated with group differences: Pre- and post-testicular spaces were most strongly correlated with the first function, whereas position of the maximum width and pharynx width were most strongly correlated with the second function.

Collection data and deposited specimens for the newly named species are included next; *H. armatum* specimens were collected from *A. grunniens* from: Long King Creek, Polk County, Texas, U.S.A. (30°42'59.24"N; 94°57'32.75"W), Village Creek, Hardin County, Texas, U.S.A. (30°17'8.63"N; 94°11'32.99"W), and the Neches River, Orange County,

Texas, U.S.A. (30°10'16.32"N; 94°6'44.99"W) and deposited in the Harold W. Manter Laboratory of Parasitology (HWML 75066–75069) (Fig. 4).

***Homalometron currani* n. sp.**  
**(= Species A of Curran et al., 2013)**  
**(Fig. 5; figs. 2 and 4B of Curran et al., 2013)**

**Description**

Based on observations and measurements of 13 newly collected specimens and 15 museum specimens. See Curran et al. (2013) and Table 1 for summary statistics.

**Taxonomic summary**

*Type host:* *Aplodinotus grunniens* Rafinesque, 1819, freshwater drum.

*Type locality:* Pearl River, Pearl River County, Mississippi, U.S.A. (30°29'9.50"N; 89°44'22"W); see Curran et al. (2013).

**Table 1.** Measurements of *Homalometron armatum*, *Homalometron currani*, and *Homalometron microlophi*, including newly collected and museum specimens. Values are means  $\pm$  95% confidence intervals.

	<i>H. armatum</i> <i>n</i> = 47	<i>H. currani</i> <i>n</i> = 28	<i>H. microlophi</i> <i>n</i> = 12
Body length	2,505 $\pm$ 224	3,853 $\pm$ 429	1,683 $\pm$ 309
Maximum width	710 $\pm$ 58	1,228 $\pm$ 122	435 $\pm$ 66
Position of max width	1,711 $\pm$ 144	2,567 $\pm$ 313	806 $\pm$ 281
Oral sucker length	240 $\pm$ 16	287 $\pm$ 17	172 $\pm$ 24
Oral sucker width	253 $\pm$ 18	294 $\pm$ 18	174 $\pm$ 26
Prepharynx length	67 $\pm$ 14	94 $\pm$ 18	51 $\pm$ 11
Pharynx length	122 $\pm$ 8	165 $\pm$ 12	76 $\pm$ 10
Pharynx width	125 $\pm$ 8	149 $\pm$ 11	68 $\pm$ 9
Esophagus length	47 $\pm$ 8	107 $\pm$ 28	32 $\pm$ 6
Ventral sucker length	354 $\pm$ 24	432 $\pm$ 25	215 $\pm$ 27
Ventral sucker width	371 $\pm$ 26	449 $\pm$ 25	219 $\pm$ 25
Forebody (%)	0.25 $\pm$ 0.12	0.21 $\pm$ 0.15	0.29 $\pm$ 0.25
Post-testicular space (%)	0.31 $\pm$ 0.13	0.42 $\pm$ 0.18	0.29 $\pm$ 0.25
Anterior testis length	247 $\pm$ 26	334 $\pm$ 42	183 $\pm$ 35
Anterior testis width	344 $\pm$ 88	546 $\pm$ 59	201 $\pm$ 43
Posterior testis length	289 $\pm$ 32	377 $\pm$ 50	197 $\pm$ 40
Posterior testis width	319 $\pm$ 26	599 $\pm$ 64	212 $\pm$ 44
Seminal vesicle length	184 $\pm$ 28	356 $\pm$ 38	115 $\pm$ 31
Seminal vesicle width	122 $\pm$ 32	192 $\pm$ 20	82 $\pm$ 17
Ovary length	157 $\pm$ 12	240 $\pm$ 19	96 $\pm$ 20
Ovary width	154 $\pm$ 10	201 $\pm$ 17	103 $\pm$ 21
Seminal receptacle length	133 $\pm$ 18	210 $\pm$ 35	97 $\pm$ 28
Seminal receptacle width	67 $\pm$ 8	113 $\pm$ 14	48 $\pm$ 12

*Date of collection:* June 2013; February 2014.

*Localities:* Long King Creek, Polk County, Texas, U.S.A. (30°42'59.24"N; 94°57'32.75"W), Village Creek, Hardin County, Texas, U.S.A. (30°17'8.63"N; 94°11'32.99"W), and the Neches River, Orange County, Texas, U.S.A. (30°10'16.32"N; 94°6'44.99"W).

*Site of infection:* Intestine.

*Specimens deposited:* Holotype is designated from specimens deposited by Curran et al. (2013) (NPC 106461; storage no. SH231:13-77), now housed in the National Museum of Natural History, Smithsonian Institution, Washington, D.C. (USNM 1251721). The remainder of the specimens in this series may be treated as the paratype series (USNM 1251722–1251724). Additional specimens from the present investigation were deposited in the Harold W. Manter Laboratory of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska, U.S.A. (HWML 75070).

*Etymology:* The specific epithet recognizes Dr. Stephen Curran's contribution to trematode taxonomy in general, and his recognition of this taxon specifically.

## Remarks

Measurements of the newly collected worms conform closely to those in Curran et al. (2013).

This worm is usually the largest of the 3 species considered herein: Curran et al. (2013) reported a maximum length of nearly 6 mm, and this was confirmed in the present investigation. This worm can usually be recognized by a combination of the position of the maximum width (posterior of the midline; see Fig. 5), the relatively smaller suckers, and the anterior displacement of the testes (or, conversely, the relatively large post-testicular space). In addition to these quantitative measures, the vitellaria in the hindbody of specimens of *H. currani* n. sp. tend to wrap around the large excretory bladder with finger-like projections, whereas in the other 2 species, the post-testicular space is simply filled with globular vitelline follicles (Figs. 4–6; see also fig. 2 of Curran et al., 2013).

***Homalometron microlophi* n. sp.  
(= Species B of Curran et al., 2013)  
(Fig. 6; figs. 3 and 4C of Curran et al., 2013)**

## Description

Based on observations and measurements of 12 newly collected specimens and 1 museum specimen. See Curran et al. (2013) and Table 1 for summary statistics.

## Taxonomic summary

*Type host:* *Lepomis microlophus* (Gunther, 1859), redear sunfish.

*Type locality:* Pascagoula River at Wilkerson's Ferry, George County, Mississippi, U.S.A. (30°48'53"N; 88°44'43"W); see Curran et al. (2013).

*Date of collection:* June 2013; May 2014.

*Localities:* Pine Island Bayou, Hardin County, Texas, U.S.A. (30°10'45.63"N; 94°11'11.54"W), and the Neches River, Orange County, Texas, U.S.A. (30°10'16.32"N; 94°6'44.99"W).

*Site of infection:* Intestine.

*Specimens deposited:* Holotype is designated from specimens deposited by Curran et al. (2013) (NPC 106462), now housed in the National Museum of Natural History, Smithsonian Institution, Washington, D.C. (USNM 1251725). Additional specimens from the present investigation were deposited in the Harold W. Manter Laboratory of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska, U.S.A. (HWML 75071; 75072).

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

## Remarks

Measurements of the newly collected worms conform closely to those in Curran et al. (2013). This worm is usually the smallest of the 3 species considered herein: Curran et al. (2013) reported a maximum length of 2.33 mm, and the largest worm in the present investigation was merely 2.64 mm. In comparison, none of the newly collected specimens and only 2 museum specimens of gravid *H. currani* n. sp. were less than 2.64 mm. Morphometrically, specimens of *H. microlophi* n. sp. have a relatively larger forebody (Figs. 4–6; Table 1), as was noted by Curran et al. (2013), and a smaller post-testicular space. Curran et al. (2013) also noted that the body spines on specimens of *H. microlophi* n. sp. are minute compared to those on *H. armatum* or *H. currani* n. sp. (fig. 4 of Curran et al, 2013), and examination of our specimens confirms this observation. So far, this worm has only been found in *L. microlophus*.

## DISCUSSION

Overlap of metric ranges among putative species precluded Curran et al. (2013) from naming the 2 species of *Homalometron* that they discovered using molecular sequence data. Additional specimens collected herein, along with multivariate analyses of

anatomical metrics, produced a more robust morphological complement to the conclusions of Curran et al. (2013), i.e., that there exist 3 species of *Homalometron* in the freshwater drum and redear sunfish of the region. Naming these species and analyzing their morphological characters in a multivariate context supplement the work of Curran et al. (2013) and should be helpful to future taxonomic work within the genus.

Upon first inspection, overall worm size is the most notable distinguishing characteristic among these species, with *H. currani* n. sp. the largest, *H. armatum* intermediate, and *H. microlophi* n. sp. the smallest. However, *H. armatum* overlaps both other species in size range and can be as large, or larger, than *H. currani* n. sp. (Curran et al., 2013; Figs. 4, 5). When all other measurements are relativized by worm length, however, almost all of them are relatively smaller in *H. currani* n. sp., intermediate in *H. armatum*, and largest in *H. microlophi* n. sp. (Figs. 4–6). Distinguishing *H. currani* n. sp. from the other 2 species is relatively easy, assuming that gravid worms are available: The shape of the worm combined with a very large post-testicular space and finger-like vitelline follicles around the excretory bladder are diagnostic.

The morphological distinctions between *H. armatum* and *H. microlophi* n. sp. are more subtle, even though they are the most different in ITS (internal transcribed spacer) sequence data (Curran et al., 2013). The most stable anatomical diagnostic characters in the newly collected specimens are the relative size of the forebody (larger in *H. microlophi* n. sp., but overlapping) and the location of the maximum width (more anterior in *H. microlophi* n. sp., but highly variable in *H. armatum*). Both are subtle and probably subject to artifact due to fixation technique, etc., but specimens of *H. microlophi* n. sp. tend to be lomentiform (peanut-shaped), whereas those of *H. armatum* are more uniform midbody and taper gradually at both the anterior and posterior ends. For routine identification, worms collected from *L. microlophus* should be suspected as *H. microlophi* n. sp. and then confirmed through examination of body size, the extent of the forebody, the size of cuticular spines, and/or through comparison to existing DNA sequence data as prescribed by Curran et al. (2013).

As improved techniques and additional sampling result in discovery of species-level taxa previously presumed to be unified, tension will increase between the routine need and desire to identify individuals (i.e., place a species name on each collected

specimen, often using ever-more-subtle morphological characters) and the broader goal of recognizing the taxon groups themselves (i.e., asserting and verifying the existence of a population that can be recognized as a species). The approach taken herein is a population-level one, in which the understandable desire to place a name on each specimen is subordinated to the goal of recognizing and differentiating species as populations. This approach will fail to correctly place names on individuals at some unknown rate, but neither taxonomic theory nor practice requires that every individual be identified or that every diagnostic characteristic be foolproof.

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