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A New Species of *Macroderoides* (Trematoda: Macroderoididae) from Spotted Gar, *Lepisosteus oculatus* (Lepisosteidae), in the Big Thicket National Preserve and Surrounding Areas, Texas, U.S.A.

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ABSTRACT: *Macroderoides luki* n. sp. (Trematoda: Macroderoididae) is described from the intestine of spotted gar (*Lepisosteus oculatus*) collected from multiple locales on the Trinity River in eastern Texas, U.S.A. Members of the new species are most similar to *Macroderoides trilobatus* in that they possess an ovary composed of 3 lobes rather than an undivided ovary as in the other 6 species in North America. In the new species, the distance between the cirrus sac and ovary is far greater, the eggs are almost twice the size, and the body is longer than in *M. trilobatus*. This is the eighth species of *Macroderoides* named in North America and the largest specimens known from this group.

KEY WORDS: Macroderoides luki, spotted gar, Lepisosteus oculatus, Big Thicket National Preserve, Texas.

During an ongoing biodiversity inventory of the parasites of fishes from waters of the Big Thicket National Preserve and surrounding areas, Texas, U.S.A. (Barger, 2010a, b, 2014; Dutton and Barger, 2014, 2015; Barger and Wellenstein, 2015), specimens belonging to Macroderoides (Trematoda: Macroderoididae) were collected from several spotted gar (Lepisosteus oculatus) collected from sites on the Trinity River. Seven species of Macroderoides are known from North America: Macroderoides flavus (Van Cleave and Mueller, 1932); Macroderoides minutus Tkach and Kinsella, 2011; Macroderoides parvus (Hunter, 1932); Macroderoides spiniferus Pearse, 1924; Macroderoides texanus Tkach, Strand, and Froese, 2008; Macroderoides trilobatus Taylor, 1978; and Macroderoides typicus (Winfield, 1929). Tkach and Kinsella (2011) recently reviewed the species in North America and provided the first molecular phylogeny for 6 of the 7 species known at the time. The specimens of Macroderoides in the present investigation do not correspond to any described species in North America, and thus a new species is described herein.

MATERIALS AND METHODS

Ten spotted gar were collected by gill net from 3 sites on the Trinity River at, and downstream of, Lake Livingston, Polk and Liberty Counties, Texas, U.S.A., in 2014 and 2015. Worms were killed in hot 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 brightfield 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 highresolution 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 existing specimens from both the National Parasite Collection at the National Museum of Natural History, Washington, DC, U.S.A. (NMNH), and the Harold W. Manter Laboratory of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska, U.S.A. (HWML), as follows: *M. trilobatus* (NMNH 74618, 74619, holotype and paratypes; HWML 31616, voucher); *M. spiniferus* (HWML 20369, 20370, 20371, 30128, vouchers); *M. typicus* (HWML 22498, 23339, 31617, vouchers). The holotype and 2 paratype specimens of *M. trilobatus* were measured as per the new specimens for direct comparisons. Shape terms follow the recommendations of Clopton (2004) where possible. Measurements are presented in micrometers as the range followed by the mean and holotype parenthetically, except where otherwise noted.

RESULTS *Macroderoides luki* n. sp. (Figs. 1–7)

Description

Based on observation and measurements of 48 specimens. Body very elongate, 2.80–4.73 mm (3.93 mm; 4.24 mm) long, 228–415 (344; 375) wide at widest point. Widest point 1.05–3.48 mm (2.63 mm; 2.69 mm) from anterior end (29–86% of body length) at level of either the ventral sucker or in the region of the testes, tapering only slightly to a blunt

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Figure 1. Holotype of *Macroderoides luki* n. sp., ventral view. Most eggs omitted for clarity; vitelline follicles shown for only 1 plane of view, approximately midbody. Third lobe of ovary dorsal to remainder of ovary and obscured in drawing.

end. Tegument spinous, decreasingly so posteriorly. Forebody 0.93–1.45 mm (1.17 mm; 1.31 mm) long; hindbody 1.73–3.11 mm (2.58 mm; 2.78 mm) long; forebody:hindbody ratio 0.38–0.58 (0.46; 0.47). Oral sucker subterminal, 79–150 (120; 135) long, 95–162 (136; 157) wide. Pharynx muscular, 67–108 (86; 105) long, 71–104 (85; 86) wide. Cecal bifurcation 478– 752 (620; 752) from anterior end; ceca extending to near posterior end. Ventral sucker nearly circular, 128–

203 (162; 185) long, 125–198 (156; 196) wide. Testes intercecal, approximately ovoid, irregular, separated. Anterior testis 1.71-3.14 mm (2.35 mm; 2.49 mm) from anterior end, 207-391 (287; 329) long, 170-318 (239; 262) wide. Posterior testis 448–1,002 (762; 837) from posterior end, 225-396 (307; 330) long, 180-334 (249; 248) wide. Cirrus sac 502-766 (614; 558) long, 54-117 (95; 95) wide, extending dorsal to ventral sucker and posteriorly into anterior region of vitellaria, separated from ovary by 77-687 (308; 280). Vas efferens join cirrus sac separately; seminal vesicle bipartite. Ovary approximately midbody, trilobed, median to submedian, 118-232 (175; 216) long, 112-211 (155; 161) wide. Seminal receptacle observed in only a few specimens, usually appearing as broad convoluted tube, near Mehlis gland and small vitelline reservoir, immediately posterior to ovary. Vitelline fields lateral, beginning posterior of ventral sucker, 1.24-2.20 mm (1.55 mm; 1.63 mm) from anterior end to just posterior of posterior testis (in most cases), terminating 503-884 (689; 764) from posterior end. Vitelline follicles irregular and interconnected. Uterus extensive, passing into region between testes and descending on one side to posttesticular space. Common genital pore anterior to ventral sucker, median. Eggs 40-50 (45) long, 22-35 (27) wide.

Taxonomic Summary

Type host: Spotted gar, *Lepisosteus oculatus* Winchell, 1864.

Type locality: Trinity River upstream of confluence with Menard Creek, Liberty County, Texas, $30^{\circ}29'16.65''$ N, $94^{\circ}50'29.22''$ W.

Other localities: Menard Creek upstream of confluence with Trinity River, Menard Creek Corridor Unit, Big Thicket National Preserve, Liberty County, Texas, 30°29'17.09"N, 94°50'29.02"W; Lake Livingston at Brushy Creek near FM 356, Polk County, Texas, 30°50'03.84"N, 95°08'54.60"W.

Dates of collection: Two infected gar from type locality on 22 February 2014; 3 infected gar from Menard Creek on 9 July 2015; 1 infected gar from Lake Livingston on 12 March 2015.

Site of infection: Intestine.

Specimens deposited: Holotype (HWML 102966; BITH 17297) and 38 paratypes (HWML 102967; BITH 17298) deposited in the HWML. BITH numbers are National Park Service reference numbers.



Figures 2–7. Anatomy of *Macroderoides luki* n. sp. **2.** Tegumental spines at oral sucker of holotype. **3.** Tegumental spines at ventral sucker of holotype. **4.** Cirrus sac and uterus near gonopore of paratype. **5.** Terminus of male and female reproductive systems anterior of ventral sucker in paratype. **6.** Part of the seminal receptacle in relation to ovary in paratype, sinistral view. **7.** Mehlis gland in relation to ovary in paratype, same specimen as in panel 6. All images differential interference contrast: cs, cirrus sac; mg, Mehlis gland; ov, ovary; sr, seminal receptacle; ut, uterus; vit, vitelline follicle; vs, ventral sucker.

Etymology: The specific epithet is an honorific for Mr. Luke Dierks, nephew of M.A.B., whose interest in animals is admirable and likely extends even to worms in spotted gar, or at least to spotted gar themselves.

Remarks

Macroderoides luki n. sp. is most similar to *M. trilobatus*. Specimens of both species possess an ovary consisting of 3 distinct lobes, a feature that distinguishes each from all other members of the genus. Specimens of the new species can be distinguished from *M. trilobatus* based on the positional relationship of the cirrus sac to the ovary and on egg size. In specimens of *M. trilobatus*, the distal portion of the cirrus sac nearly abuts, abuts, or overlaps the ovary. The figure in the original species description (Taylor, 1978) (verified as the holotype by our examination) shows the cirrus sac and ovary as nearly touching. In our examination of the type series, the distance between the cirrus sac and ovary was 10 μ m for the holotype and 22 μ m for one paratype; in the remaining paratype, the ovary and cirrus sac overlapped. In contrast, the distance between these structures in specimens of *M. luki* n. sp. ranged from 77 μ m in the smallest gravid specimen to 687 μ m in the largest gravid specimen. In 42 of the 48 gravid specimens of *M. luki*, the distance between the cirrus sac and ovary was greater than 200 μ m.

The eggs of the new species are substantially larger than those of M. trilobatus as well. Taylor (1978) reported eggs as being 20-27 (22) µm by 10-17 (14) µm. Our measurements from the type series were close to Taylor's: 22-28 (25) µm by 12-16 (14) µm. Eggs of the new species are almost twice the size, 45 by 27 µm on average, with the width of eggs of the new species exceeding the length of most eggs of M. trilobatus. Additionally, the smallest gravid specimen of M. luki (2.8 mm long) is almost 800 µm longer than the largest specimen in the type series of M. trilobatus (1.8–2.1 mm), and the largest M. luki (almost 5 mm long) are longer than any other Macroderoides description from North America: M. flavus, 0.7 mm (Van Cleave and Mueller, 1932); M. minutus, 0.6 mm (Tkach and Kinsella, 2011); M. parvus, 1.8 mm (Hunter, 1932); M. spiniferus, 2.4 mm (Pearse, 1924); M. texanus, 2.7 mm (Tkach et al., 2008); M. typicus, 1.4 mm (Winfield, 1929).

Macroderoides texanus was described recently from gar in Texas (Tkach et al., 2008) and M. minutus from gar in Florida (Tkach and Kinsella, 2011). Combined with the new species described herein, these findings suggest that the genus is substantially more speciose than previously thought. New collections of the primary definitive hosts of Macroderoides in North America, including the gars (Lepisosteidae), bowfin (Amia calva), and pickerel (Esox spp.) to a lesser extent, will probably reveal additional species-level diversity. Tkach and Kinsella (2011) found some interesting patterns suggesting the evolution of smaller body sizes in 2 subclades of Macroderoides. The inclusion of DNA from specimens from additional species, including the very large M. luki n. sp., would allow this hypothesis to be tested.

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