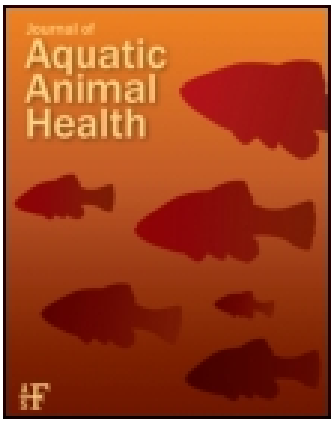

Journal of Aquatic Animal Health
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/uahh20

First Record of Bothriocephalus acheilognathi in the Rio Grande with Comparative Analysis of ITS2 and V4-18S rRNA Gene Sequences

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Published online: 09 Jan 2011.

To cite this article: Megan G. Bean, Andrea Škeříková, Timothy H. Bonner, Tomáš Scholz & David G. Huffman (2007) First Record of Bothriocephalus acheilognathi in the Rio Grande with Comparative Analysis of ITS2 and V4-18S rRNA Gene Sequences, Journal of Aquatic Animal Health, 19:2, 71-76

To link to this article: http://dx.doi.org/10.1577/H06-035.1

Journal of Aquatic Animal Health 19:71–76, 2007

DOI: 10.1577/H06-035.1

[Communication]

First Record of *Bothriocephalus acheilognathi* in the Rio Grande with Comparative Analysis of ITS2 and V4-18S rRNA Gene Sequences

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Abstract.—*Bothriocephalus acheilognathi* is an introduced tapeworm in North America often reported as a serious ecological threat to native fishes. In this paper, we report the first record of *B. acheilognathi* in the Big Bend region of the Rio Grande in Texas (known as the Río Bravo del Norte in Mexico). Identification of *B. acheilognathi* was confirmed by morphologic and genetic techniques (sequences of ITS2 and V4-18S rRNA genes). Its prevalence was 27% and its intensity ranged from 1 to 5 individuals in a January 2006 collection of 115 red shiners *Cyprinella lutrensis*. In addition, it was found in the Tamaulipas shiner *Notropis braytoni*, a Rio Grande endemic and a new host record. The occurrence of *B. acheilognathi* might have negative ecological impacts on endemic fishes in the Rio Grande. Several of the fishes that could serve as definitive hosts are of conservation concern. Its occurrence also might affect the success of reintroducing the Rio Grande silvery minnow *Hybognathus amarus*, which is federally listed as endangered, in this portion of the Rio Grande.

The tapeworm *Bothriocephalus acheilognathi* infects over 100 species of fish in Africa, America, Asia, Australia, and Europe and is considered a threat to populations of endemic, commercial, and hatchery fishes (Körting 1975; Hoffman 1980; Hoffman and Schubert 1984; Salgado-Maldonado and Pineda-López 2003). It is recognized as a causative agent of detrimental infection in aquaculture operations in Asia and Europe, where it has been reported to cause 100% mortality in some hatchery ponds (Liao and Shih 1956; Körting 1975). *Bothriocephalus acheilognathi* requires as little as 2 weeks to complete its life cycle in the intermediate host and has low definitive and intermediate host specificity (Körting 1975). Eggs are passed with the feces of the fish and mobile coracidia emerge from the eggs after embryonation. The coracidia are consumed by the intermediate host, cyclopoid copepods (e.g., those of the genera *Acantocyclops*, *Macrocyclops*, *Mesocyclops*, *Tropocyclops*, *and Diacyclops*; Körting 1975; Marcogliese and Esch 1989; Díaz-Castaneda et al. 1995). The life cycle is completed when fish ingest infected copepods.

Low host specificity enables *B. acheilognathi* to rapidly colonize new drainages (Marcogliese and Esch 1989; Dove and Fletcher 2000). The natural geographic range of *B. acheilognathi* is Japan (where it was originally described by Yamaguti in 1934), China, and the Amur River basin in the Russian Far East (Bauer and Hoffman 1976; Pool and Chubb 1985; Pool 1987; Scholz 1997). One of the tapeworm's native hosts is the grass carp *Ctenopharyngodon idella* (Choudhury et al. 2006). *Bothriocephalus acheilognathi* was introduced into nonnative areas around the world, including North America, when infected grass carp were

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Received August 10, 2006; accepted February 13, 2007
Published online May 31, 2007

imported for macrophyte control (Hoffman 1980; Andrews et al. 1981). It occurs in six drainages in Mexico and in Lake Winnipeg in Canada (Salgado-Maldonado and Pineda-López 2003; Choudhury et al. 2006). In the USA, *B. acheilognathi* occurs in the Colorado River drainage in Arizona, the Virgin River in Nevada, Arizona, and Utah, Belews Lake in North Carolina, the Yampa River in Colorado, Peter Lake in Wisconsin, and the South Platte River in Nebraska (Granath and Esch 1983b; Heckmann and Deacon 1987; Brouder and Hoffnagle 1997; Ward 2005; Choudhury et al. 2006). The tapeworm also has been reported in Kentucky, Arkansas, and New Mexico (Choudhury et al. 2006). Transfer into new drainages within the USA is attributed to baitfish introductions (Heckmann et al. 1993).

Methods

In January 2006, 115 red shiners *Cyprinella lutrensis* (total length, 19–39 mm) were collected from the Rio Grande (known as the Río Bravo del Norte in Mexico) at Santa Elena Canyon near the confluence with Terlingua Creek in Big Bend National Park (Figure 1). Fish were taken with a 3-m × 1.8-m seine (mesh size, 1.8 mm) and preserved in 10% solutions of formalin. In the laboratory, the gastrointestinal tracts of the fish were removed. Tapeworms were teased from the intestinal lining and initially identified by their heart-shaped scolex with a pair of deep bothria (Scholz 1997). Tapeworms were enumerated in each fish to determine the prevalence and intensity of infection (see Margolis et al. 1982 for terminology).

Additional seine hauls made in January 2006 captured red shiners and Tamaulipas shiners *Notropis braytoni*. Five red shiners (samples 06/31–35) were preserved in 70% ethanol for genetic analysis (ITS2 and V4-18S rRNA genes) of *B. acheilognathi*. The remaining fish were kept alive in aerated containers and transported to the laboratory. Gastrointestinal tracts were removed from freshly killed red and Tamaulipas shiners and tapeworms removed from the intestinal lining. These specimens were stained with Mayer's hydrochloric carmine solution and mounted in Canada balsam as permanent preparations deposited in the U.S. National Parasite Collection, Beltsville, Maryland (collection number USNPC 98874) and the helminthological collection of the Institute of Parasitology of the Academy of Sciences of the Czech Republic (collection number C-15).

Total DNA was extracted from 0.5 cm of strobila using the DNeasy Tissue Kit (Qiagen, Sigma, St. Louis, Missouri). To amplify the sequences of ITS2 and the V4 region of the 18S rRNA gene, the primer sets Proteo1 (5′-CGG TGG ATC ACT CGG CTC-3′), Proteo2 (5′-TCC TCC GCT TAT TGA TAT GC-3′), Ces1 (5′-CCA GCA GCC GCG GTA ACT CCA-3′), and Ces2 (5′-CCC CCG CCT GTC TCT TTT GAT-3′) were used (Škeříková et al. 2001; Scholz et al. 2003). The polymerase chain reaction (PCR) program was as follows: 15 min at 95°C (Hotstar *Taq* DNA polymerase, Qiagen, Sigma); 30 cycles of 1 min denaturation at 94°C, 1 min annealing at 60°C, and 2 min extension at 72°C; and final extension for 10 min at 68°C.

The PCR products were cloned into pGEM-T Easy system 1 (Promega, Madison, Wisconsin) and sequenced in both directions using T7 and SP6 primers. DNA sequencing was performed on an ABI PRISM Model 310 automated sequencer (PE-Biosystems, Foster City, California) using the GenomeLab DTCS–Quick Start Kit (Beckman Coulter, Fullerton, California). The sequences were deposited in GenBank under the accession numbers DQ866988–DQ866997.

The sequences were sent to the Basic Local Alignment and Search Tool (BLAST) program in GenBank for comparison with other sequences in public databases. *Bothriocephalus acheilognathi* from a kawar *Leuciscus lepidus* collected by Shamall Abdullah in Iraq were used as a reference sample (GenBank accession numbers AY 340121 [ITS2 sequence], AY 340106 [V4-18S rRNA sequence]; see Škeříková et al. 2004). To assess the similarity among the sequences obtained, the Martinez–Needleman–Wunsch method, as implemented in the program MEGALIGN (DNAstar, Nevada City, California), was used.

Results

The lengths of the ITS2 and V4-18S rRNA gene sequences obtained from the five Rio Grande samples were 783–795 and 460 base pairs, respectively (Table 1). The tapeworms were identified as *B. acheilognathi* on the basis of their similarity to the reference sequences of this cestode species available in GenBank.

The ITS2 sequences of the five samples showed a similarity of 93.8–99.1%, and comparison with the sequence of the Iraqi reference sample from GenBank revealed 95.1–96.8% similarity (Table 1). Comparison with the other 27 sequences of the ITS2 gene of *B. acheilognathi* from different localities accessible in GenBank showed similarities between 94.2% and 99.9% (data not shown). The greater similarity of the ITS2 sequences between the Texas samples and previously sequenced samples than within the Texas samples themselves might indicate multiple, independent colonization of *B. acheilognathi* into the Rio Grande. However, previous studies have demonstrated

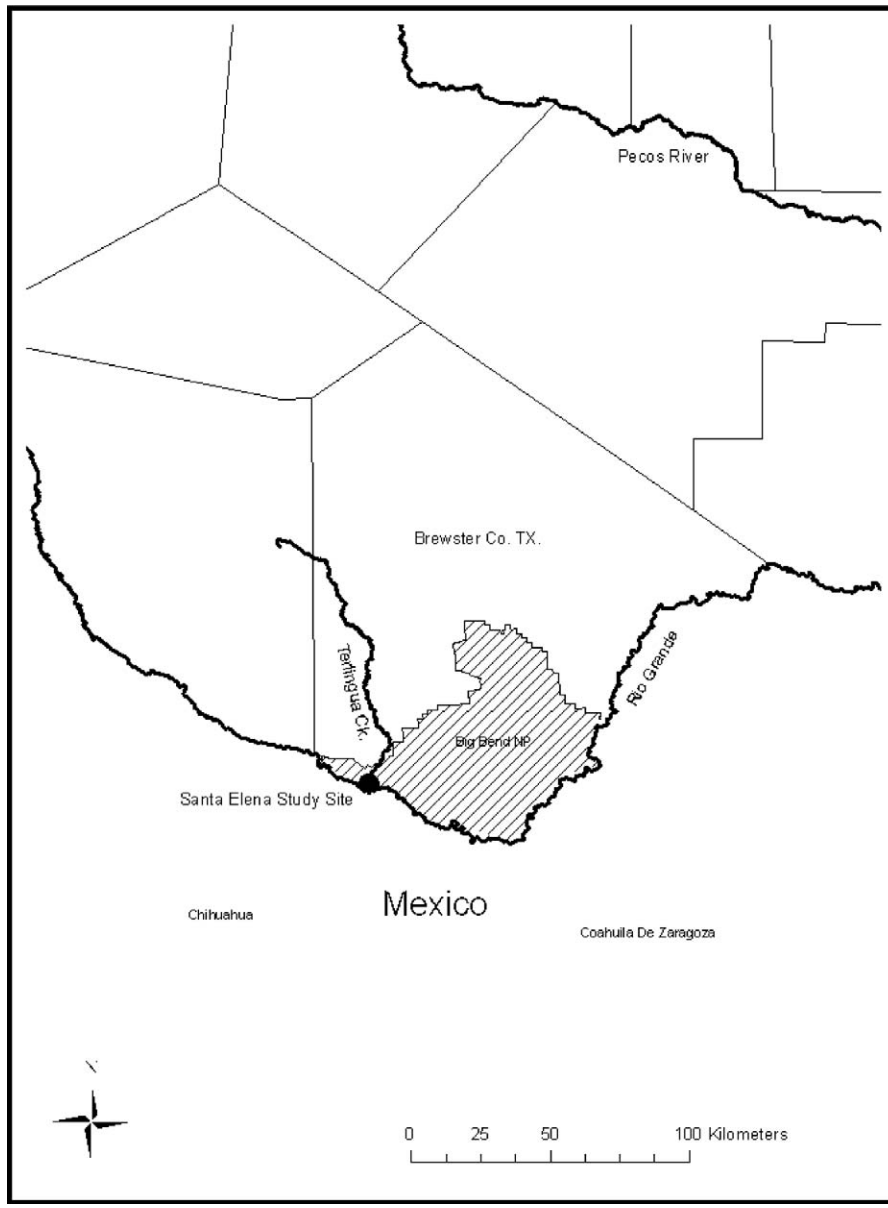

FIGURE 1.—Map of the Big Bend region, Texas, showing the location of the study site at Santa Elena Canyon.

high intraspecific variation in ITS sequences in several species of *Bothriocephalus* (Luo et al. 2002; Scholz et al. 2004; Škeříková et al. 2004).

Comparison of the V4-18S rRNA sequences demonstrated only negligible differences among the samples from the Rio Grande (similarity, 99.8–100%), indicating that the specimens are conspecific. The similarity of the Rio Grande sequences to those of the Iraqi samples in GenBank varied from 99.3% to 99.6% (Table 1).

In view of this sequence similarity and data on other species of *Bothriocephalus* (Škeříková et al. 2004), it is possible to consider all of the North American samples examined thus far to be conspecific with *B. acheilognathi*. This genetic analysis, combined with morphological comparisons, suggests that all of the tapeworms from the Rio Grande were indistinguishable from those from the wide spectrum of fish hosts and different geographical regions deposited in the helminthological collection of the Institute of Parasitology of the

TABLE 1.—Percent similarity and length of individual sequences of the tapeworm *Bothriocephalus acheilognathi* from red shiners from the Rio Grande, Texas (06/31–35) and kawar from Iraq (GenBank accession numbers AY 340106 and AY 340121). The ITS2 sequences are above the diagonal, the V4-18S rRNA gene sequences below the diagonal.

Sample[a]	06/31 (795 bp)	06/32 (790 bp)	06/33 (788 bp)	06/34 (783 bp)	06/35 (794 bp)	AY 340121 (805 bp)
06/31		94.8	94.1	93.8	94.1	96.8
06/32	100		99.0	96.4	99.1	95.7
06/33	99.8	99.8		96.2	98.6	95.1
06/34	100	100	99.8		96.9	95.2
06/35	100	100	99.8	100		96.3
AY 340106	99.6	99.6	99.3	99.6	99.6	

[a] All samples 460 base pairs (bp) except AY340106, which is 459 bp.

Academy of Sciences of the Czech Republic (Scholz and Di Cave 1993; Scholz 1997).

Having confirmed the identification of *B. acheilognathi*, we found that its prevalence in red shiners was 27% and that its intensity ranged from one to five tapeworms per fish. We report the Tamaulipas shiner as a new host record for *B. acheilognathi* and expand the range of this invasive tapeworm in North America to include the Rio Grande.

Discussion

Bothriocephalus acheilognathi is considered to be a serious threat to endemic fishes in Mexico (Salgado-Maldonado and Pineda-López 2003). Pathogenic effects can include intestinal blockage and perforation, distended abdomen, necrosis, inflammation, hemorrhaging, loss of intestinal microvilli, loss of enterocytes, reduced growth, significantly decreased survivorship, and mortality (Scott and Grizzle 1979; Hoffman 1980; Granath and Esch 1983a; Hoole and Nisan 1994; Hansen et al. 2006). Consequently, the occurrence of *B. acheilognathi* might have negative ecological impacts on native fishes in the Rio Grande. The Rio Grande drainage fish assemblage includes several endemic cyprinids that are listed as species of conservation concern because of anthropogenic modifications (Hubbs et al. 1991) that are potential hosts for the tapeworm. These include the Rio Grande silvery minnow *Hybognathus amarus*, which is listed as endangered by the Texas Parks and Wildlife Department (TPWD), the U.S. Fish and Wildlife Service (USFWS), and Mexico (CONABIO 2002); the Devils River minnow *Dionda diaboli*, listed as threatened by TPWD and USFWS and as endangered by Mexico (CONABIO 2002); the Chihuahua shiner *Notropis chihuahua*, listed as threatened by TPWD and Mexico (CONABIO 2002) and classified as threatened by Hubbs et al. (1991); the Mexican stoneroller *Campostoma ornatum*, listed as threatened by TPWD and classified as threatened by Hubbs et al. (1991); the Rio Grande shiner *Notropis jemezanus*, listed as threatened by Mexico (CONABIO 2002) and classified as threatened by Hubbs et al. (1991); and the Tamaulipas shiner, listed as threatened by Mexico (CONABIO 2002).

One fish of importance in the Big Bend region is the Rio Grande silvery minnow. This fish, the distribution of which once spanned 4,825 km of the Rio Grande from Colorado to Texas (Ikenson 2002), is now extirpated from Texas and only found in scattered locations in the Rio Grande in New Mexico (Hubbs et al. 1991; Bestgen and Platania 1991). The recovery plan for the Rio Grande silvery minnow (USDI 1999) lists the reach from the town of Presidio to Amistad Reservoir, which includes Big Bend National Park, as one of six reaches having the best reestablishment potential. With this discovery of *B. acheilognathi* in the Rio Grande, the success of reintroductions might be seriously jeopardized.

Acknowledgments

We thank the U.S. Department of Agriculture, Texas State University River Systems Institute (scientific collecting permits BIBE-2005-SCI-0071 and SPR-0601-159), the Institute of Parasitology (projects Z60220518 and LC522), and the Grant Agency of the Czech Republic (project 524/04/0342) for financial support. We also thank P. Bean and T. Heard for assistance with fish identification and field collections, T. Heard for the Santa Elena map, and three anonymous referees for helpful suggestions.

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