BioInvasions Records (2013) Volume 2, Issue 2: 99–104
doi: http://dx.doi.org/10.3391/bir.2013.2.2.02

Open Access

Research Article

The invasive Asian fish tapeworm, *Bothriocephalus acheilognathi* Yamaguti, 1934, in the Chagres River/Panama Canal drainage, Panama

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Received: 26 October 2012 / Accepted: 23 April 2013 / Published online: 25 April 2013

Handling editor: Vadim Panov

Abstract

The invasive and potentially pathogenic Asian fish tapeworm, *Bothriocephalus acheilognathi*, is reported for the first time from the vicinity of the Panama Canal zone, a region of international economic and environmental significance. The tapeworm was found in two cichlid species, *Aequidens coeruleopunctatus* and *Cryptoheros panamensis* in two tributaries of the Chagres River / Panama Canal drainage, Soberania National Park area, Panama. Sequence data from the ITS-1 region of the rRNA genome corroborate the identifications based on diagnostic morphological features. The tapeworm was not found in 201 of the other 15 species of fish belonging to 6 families that were collected during the same time. Using historical records, we argue that the tapeworm was likely introduced with the stocking of one of its principal hosts, grass carp, during early attempts to control aquatic vegetation in the Canal zone, and we predict that it is probably more widely distributed in the area, especially in Gatun Lake. The impact of this tapeworm on the native fish resources of Panama remains unknown, but is noteworthy for its presence in an already ecologically impacted region of the Neotropics.

Key words: Asian tapeworm; *Aequidens; Cryptoheros;* Cichlidae; parasites; freshwater fishes; neotropical

Introduction

The Asian fish tapeworm, *Bothriocephalus acheilognathi* Yamaguti, 1934, infects a wide variety of freshwater fishes worldwide, although it has a predilection for cyprinids (Scholz 1997; Choudhury and Cole 2011) - the grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844), could be considered a principal host. The success of this invasive, and potentially pathogenic, tapeworm (Choudhury and Cole 2011) earned it a place in the list of species selected for the recent *Handbook of Global Freshwater Invasive Species* (Francis 2011). In the Americas, it is well established as far north as southern Manitoba in Canada, as well as in the U.S. and Mexico (Choudhury et al. 2006; Bean et al. 2007; Bean 2008; Marcogliese et al. 2008; Rojas-Sánchez and García-Prieto 2008; Pérez-Ponce de León et al. 2009, 2010; Salgado-Maldonado et al. 2011, Choudhury and Cole 2011; Martínez-Aquino et al. 2012). In South America, it has been reported from Brazil (Rego et al. 1999). During a study of freshwater fishes and their parasites in tributaries of the Chagres River in Panama, we found the Asian fish tapeworm in two individuals of two native fish cichlid species. Given the history of ecological and environmental impacts associated with the Panama Canal zone, these findings are significant and we report them here.

Methods

Fishes and their parasites were collected from two tributaries of the Chagres River, Panama, near Pipeline Road (Camino Oleoducto): Quebrada Juan Grande (09°08'40.5"N, 79°43'21.1"W) was sampled January 15–17, 2010 and August 10–12,

2011, and Río Frijolito (09°08'58.1"N, 79°43'53.8"W) January 15–17, 2010. A total of 225 fish belonging to 17 species and 7 families were collected (both sampling periods combined) using seines and hoop/fyke nets, as follows: fish and numbers *Astyanax ruberrimus* Eigenmann, 1913 (4), *Brycon petrosus* Meek & Hildebrand, 1913 (9), *Bryconamericus emperador* (Eigenmann & Ogle, 1907) (32), *Bryconamericus* sp. (11), *Gephyrocharax atracaudata* (Meek & Hildebrand, 1912) (44), *Hyphessobrycon panamensis* Durbin, 1908 (15), *Roeboides guatemalensis* (Günther, 1864) (14) (all Characidae), *Piabucina panamensis* Gill, 1877 (10) (Lebiasinidae), *Hoplias microlepis* (Günther, 1864) (8) (Erythrinidae), *Brachyraphis cascajalensis* (Meek & Hildebrand, 1913) (23), *Neoheterandria tridentiger* (Garman, 1895) (2), *Poecilia gillii* (Kner, 1863) (11), (all Poeciliidae), *Aqeuidens caeruleopunctatus* (Kner, 1863) (22), *Cryptoheros panamensis* (Meek & Hildebrand, 1913) (2) (both Cichlidae), *Ancistrus chagresi* Eigenmann & Eigenmann, 1889 (1), *Hypostomus plecostomus* L. (1), *Fonchiichthys uracanthus* (Kner, 1863) (1) (all Loricariidae), and *Pimelodella chagresi* Steindachner, 1876 (16) (Pimelodidae). Fish were identified using a combination of the following sources: Smith and Birmingham (2005), Bussing (2002), Angermeier and Karr (1983), original descriptions, and an online resource (http://www.reocities.com/culumbrown/PipelineFishes/pipelinefishes.html) created by Culum Brown, Macquarie University, Australia. Nomenclature of fishes were updated using FishBase (http://www.fishbase.org) (Froese and Pauly 2013). All fish were examined fresh after killing them in a pail of aerated stream or well water with an overdose of dissolved anesthetic MS222. Gastrointestinal tracts were examined using Leica Zoom 2000 dissecting (stereo) microscopes.

Only two individual bothriocephalid tapeworms were collected, both live, one each from *A. coeruleopunctatus* and *C. panamensis* in Rio Frijolito (in 2010) and Quebrada Juan Grande (in 2011) respectively. Worms were gently washed in saline. A small portion of the strobila of the tapeworm from *A. coeruleopunctatus* was preserved in 95% ethanol for molecular studies, and the rest of the worm as well as the second worm (a small specimen) from *A. panamensis* were fixed in hot 10% formalin (3.8% aqueous formaldehyde). The specimen from *A. coeruleopunctatus* was subsequently stained in acetocarmine and mounted on a slide in Canada balsam. The small specimen from *Cryptoheros panamensis* is stored in 70% ethanol. Images were digitally captured with an Olympus DP25 camera mounted on an Olympus BX 41 microscope with DIC capabilities. The stained and mounted specimen from *A. coeruleopunctatus* has been deposited at the Coleccion Nacional Helmintos, at Universidad Nacional Autónoma de México (UNAM), Mexico, with accession number CNHE 8119.

For molecular studies, the piece of tapeworm fixed in 95% ethanol was digested, and the genomic DNA extracted and cleaned using Qiagen's DNEasy extraction kit (Qiagen Inc., Valencia, California). The18S (end) -ITS1 region of the rRNA gene was amplified with PCR (polymerase chain reaction) using primers: BD1 5' GTCGTAACAAGGTTTCCGTA 3' (forward), and BD2-A 5' TATGCTTAAGTTCAGCGGGT 3' (reverse) (Luo et al. 2002). PCR reactions were performed on a PerkinElmer GeneAmp 9700 thermocycler (PerkinElmer Inc., Wellesley, Massachusetts) using Ex-taq DNA polymerase (TaKaRa Mirus Corporation, Madison, Wisconsin) in a total reaction volume of 50 µl. The amplification protocol consisted of an initial denaturing cycle of 5 min at 94 C, 25 cycles of the following: 94 C for 30 sec, 56 C for 30 sec for primer annealing, 72 C for 1 min for replication, and a final hold for elongation at 72 C for 5 min. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen Inc). Purified products were sent to MCLab, South San Francisco, California, for automated sequencing. The sequence was manually checked and edited for accuracy using FinchTV (Geospiza Inc., Seattle, Washington). Sequences of 19 isolates of *Bothriocephalus acheilognathi* reported by Luo et al. (2002) were downloaded from Genbank (http://www.ncbi.nih.gov) and aligned with homologous sequences of the Panamanian sample in this study, using 'Muscle' alignment software in MEGA Version 5 (Molecular Evolutionary Genetic Analysis, Tamura et al. 2011; http://www.megasoftware.net) and checked by eye. Distance analysis was performed using UPGMA (Sneath and Sokal 1973) as implemented in MEGA 5. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 789 positions in the final dataset. The 814 bp sequence of the Panamanian isolate was deposited to GenBank (http://www.ncbi.nih.gov) under accession number JN632481.

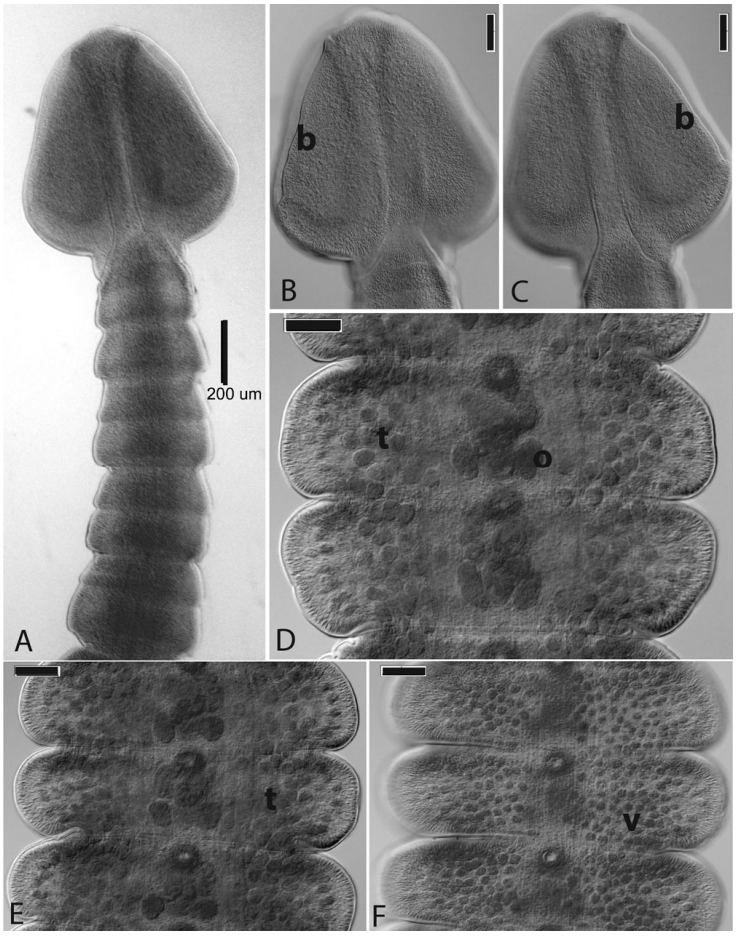

Figure 1. *Bothriocephalus acheilognathi* from *Aequidens coeruleopunctatus*. **A.** Anterior portion (scale bar = 200 um), **B & C**. Scolex showing the extent and position of the bothrial openings (b = bothrial edges), **D & E.** Mature proglottids from different regions of the mature strobila showing testicular field (t), ovary (o) and developing uterus and reproductive ducts, **F**. Proglottids showing distribution of vitelline follicles (v). B – F, scale bars = 100um. Photomicrographs by Anindo Choudhury.

Results

The tapeworms were initially identified as *B. acheilognathi* based on morphological characters. The arrowhead or heart shaped, fleshy scolex of *B. acheilognathi*, (when viewed laterally) with its anterolaterally directed narrow slit like openings (Figure 1 A-C) is unique among *Bothriocephalus* spp. (see also Scholz 1997). Furthermore, the species lacks a distinct neck (Figure 1 A) and the posterior maturing strobila is not craspedote, the proglottids having rounded edges (Figure 1 D–F). Other characteristics of the strobila are as described for the species (Scholz 1997) (Figure 1 D–F). None of the other 223 fish examined during the study were infected with *B. acheilognathi*, or with any other intestinal tapeworm for that matter.

The aligned 814 bp useable sequence from the Panamanian isolate was identical to those of 6 of the 19 isolates reported by Luo et al. (2002): AF362408 from *Cyprinus carpio*, Lugu Lake, Yunan, AF 362421 from *Hemiculter leucisculus* in Honghu Lake, Hubei, AF362425 from Culter alburnus, Baoan Lake, Hubei, AF362433 from *Culter dabryi* Baoan Lake, Hubei, all from cyprinid hosts in China, AF362409 from *Gambusia affinis*, TBGC pond and AF362424 from *Poecilia mexicana*, Waianu Stream, both in Oahu, Hawaii, U.S.A. These 7 isolates were different in one or more regions from sequences of 13 other isolates reported in that study. The distance tree generated by UPGMA (Figure 2) reflects these findings.

Discussion

No native *Bothriocephalus* species has so far been reported in the Central American region comprising Nicaragua, Costa Rica and Panama (Aguierre-Macedo et al. 2001; Sandlund et al. 2010; AC and GPPdL unpublished data from Costa Rica; this study). Farther south, Rego et al.

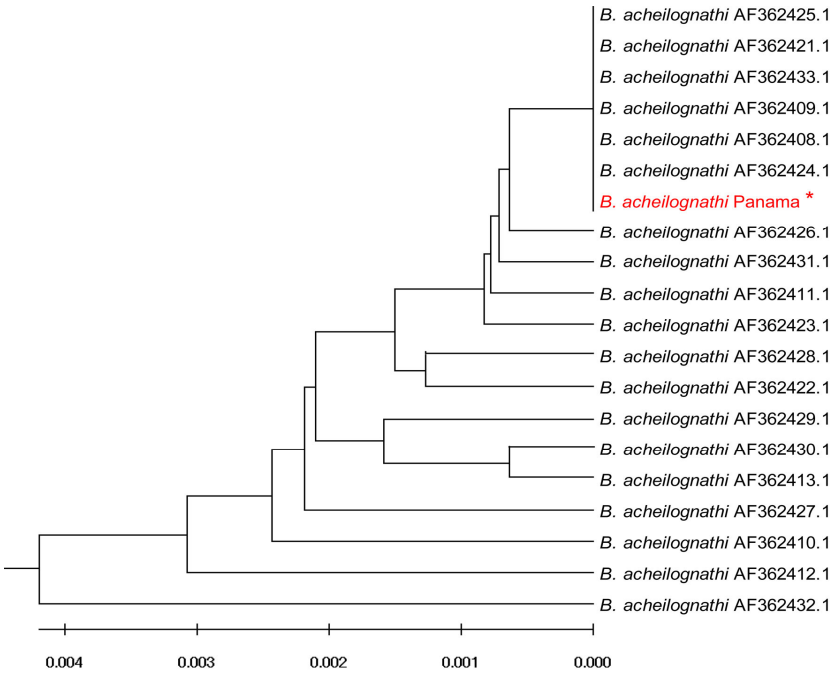

Figure 2. The distance tree from the UPGMA analysis of the sequences used in this study. The optimal tree with the sum of branch length = 0.02612602 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the distances used to infer the tree. The distances are in the units of the number of base substitutions per site. The sample from Panama in this study is labeled '*B. acheilognathi* Panama*'.

(1999) report only introduced *B. acheilognathi* from Brazil. One species, *B. pearsei* Scholz, Vargas and Moravec, 1996, is specific to cichlids, but seems to be endemic to the Yucatan Peninsula in Mexico (Scholz et al 1996; Scholz 1997). Baer (1937) described *B. musculosus* from a captive south American cichlid, *Cichlasoma biocellata* from an aquarium in Geneva, Switzerland; it was synonymized with *B. cuspidatus* Cooper, 1917 by Scholz (1997). Recently, Müller et al. (2008) reported the North American species, *B. cuspidatus* from *Cichla monoculus* in lakes (ponds?) of the Rio das Pedras farm in Campinas, São Paulo, Brazil. The scolex in Müller et al. (2008) appears contracted, but seems different from that of *B. cuspidatus* or *B. acheilognathi*. It may be a separate native species, or perhaps even *B. musculosus* of Baer (1937). The *Bothriocephalus* found in this study is morphologically clearly different from it, and from *B. pearsei* and *B. musculosus/B. cuspidatus* (Scholz 1997).

Given how distinct *B. acheilognathi* is from other *Bothriocephalus* spp. in the Americas, and the fact that we found it in an area devoid of North American congeners, we used partial sequences of the 18S-ITS1 regions of the rRNA genome, rather than other markers (e.g. Bean et al. 2007) for a more fine-tuned genetic profile of the Panamanian isolate within the *B acheilognathi* assemblage (Luo et al. 2002, 2003). Luo et al. (2002, 2003) reported genetic diversity within *B. acheilognathi* with the potential for closely related but distinct populations/ strains based on host and geography. The genetic data show that the Panamanian sample groups with a genotype characteristic of the *B. acheilognathi* found in Cultrinae (Cyprinidae) in China, but any interpretation would be premature. However, the 100% similarity in the sequences of the Panamanian and Hawaiian isolates is consistent with the U.S. being the source of this tapeworm in Panama.

The Asian fish tapeworm was likely introduced into the Panama Canal zone with the stocking of one of the parasite's principal hosts, grass carp *Ctenopharyngodon idella*. Despite physical and chemical measures to control aquatic macrophytes in the Panama Canal zone, plant biomass reached alarming levels in the 1970s (Custer et al. 1979). By that time, the grass carp had become a popular biological control agent, having been introduced in the U.S. in 1963 for the same general purpose. Grass carp became feral following their accidental escape from a hatchery in Stuttgart, Arkansas, shortly thereafter (Fuller et al. 1999). The Asian fish tapeworm likely became established in the U.S. following this event (Hoffman 1999; Choudhury et al. 2006; Choudhury and Cole 2011). In 1978, grass carp were first successfully introduced into Gatun Lake, a vast flooded extension of the Chagres River that forms roughly two-thirds of the navigational channel that is the Panama Canal. The source of the

fingerlings was a hatchery in Arkansas! Custer et al. (1978, 1979) describe, in considerable detail, these early grass carp introductions; fingerlings were transported to an acclimating facility in Paraiso, following which they were stocked into three 'grow-out' areas called 'Laguna', 'Dump 4½' and 'Calamito Lake' (Lago Calamito), the last of which is now part of the Rainforest Discovery Center in Soberania National Park, less than 2 miles from Gamboa, close to both Pipeline Road and the tributaries sampled in this study. After this grow-out phase, grass carp were stocked into Gatun Lake. These facts suggest that Calamito Lake may have been the source of the parasite in streams such as Rio Frijolito and Quebrada Juan Grande.

The Asian fish tapeworm requires a copepod intermediate host (Bauer et al. 1969; Marcogliese and Esch 1989) and large bodies of water like Gatun Lake or smaller lakes like Calamito Lake provide ideal conditions for copepods and tapeworm transmission. This could partially explain why the parasite is not more common in the streams sampled in this study since these streams are relatively less suitable for copepods. Alternatively, cichlids may not be the most suitable hosts. Outside of the cyprinids, it appears that the distantly related atherinimorphs (cyprinodontiforms and atherini-forms) make suitable hosts for this tapeworm (Salgado-Maldonado and Pineda López 2003; Choudhury and Cole 2011). The few cyprinodonti-forms examined in this study were not infected. Given the biology of this tapeworm, we predict that it will be found in higher abundance in Gatun Lake, and that the zooplanktivore atherinid *Melaniris chagresi* (Zaret 1971, 1972) would be most susceptible.

The ecology of Gatun Lake has been altered following the introduction and establishment of peacock bass, *Cichla ocellaris* Bloch & Schneider, 1801, and its predation on native fishes (Zaret and Paine 1973; Zaret 1980; Hall and Mills 2000). The Chagres River and arguably its tributaries act as a refuge for native fishes, and the presence of the Asian fish tapeworm in these streams and in Gatun Lake adds another dimension to an already impacted ecosystem. The Asian fish tapeworm is known to be pathogenic to fishes (Bauer et al. 1969; Hoffman 1999; Scholz et al. 2012) but its effects can also be more subtle (Hansen et al. 2006; Hoffnagle et al. 2006). What impact this parasite has (or has had) on the diversity and health of Panama's natural fish resources remains unknown, but further studies are needed to assess the distribution and impact of this parasite in this region.

Acknowledgements

This research was supported by the following: SNC student-faculty collaborative grants to CB, EG and AC, an endowment from the Cofrin1923 fund, and grants from the Programa de Apoyo a Proyectos de Investigación e Inovación Tecnológica (PAPIIT-UNAM) IN-220605 and Consejo Nacional de Ciencia y Tecnología (CONACyT) No. 47233 to GPPDL. Collections were made under ANAM Collecting permits SE/A 95-09 and SC/A 38-11. We wish to acknowledge the help of the following individuals: Raineldo Urriola, Marcela Paz, Lil Camacho and Orelis Arosemenas, Smithsonian Tropical Research Institute (STRI), Panama, for invaluable logistic support that made this research in Panama possible, Diomedes Quintero Arias, Museo de Invertebrados G. B. Fairchild (MIUP), Universidad de Panamá for facilitating export permits, and Francisca and Ernesto Lopez at the STRI Gamboa facility for their hospitality and help. AC, EG and CB thank Steve Dhein, Cofrin 1923 Fund for encouragement and continued support of student-faculty research partnerships at STRI. AC thanks Bob Howe, Amy Wolf, Mike Draney and Vicki Medland, Cofrin Biodiversity Center, University of Wisconsin – Green Bay, for their collaboration, help and support in the field. We thank the four anonymous reviewers for their comments and suggestions.

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