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Cover Page Footnote

We thank N. Betchel, M. Cardenas, A. Kierzek, E. Miller, J. Mulder, J. O'Brien and L. Reige for assistance with the collection of fish. We extend our gratitude to two anonymous reviewers and C. MacGregor for comments that improved the manuscript.

Asian Fish Tapeworm (*Bothriocephalus acheilognathi*) Infecting a Wild Population of Convict Cichlid (*Archocentrus nigrofasciatus*) in Southwestern California

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Abstract.—In September 2007 and May 2014, the Asian fish tapeworm, *Bothriocephalus acheilognathi* Yamaguti, 1934 (Cestoda: Bothriocephalidea), was found in populations of the non-native convict cichlid (*Archocentrus nigrofasciatus*) and mosquitofish (*Gambusia affinis*) collected from the discharge channel of a water treatment plant in Los Angeles County. Prevalence and mean intensity of infection of 450 convict cichlids and 70 mosquitofish were 55.3%/9.3 and 11%/1.4, respectively. Overall prevalence and mean intensity of infection in the convict cichlid was higher in 2007 (92%/12.3) than in 2014 (37%/5.4). In 2007, parameters of infection were size-dependent. The highest prevalence/mean intensity of infection was revealed in small fish (100%/15.5) and the lowest in large fish (66.7%/1.5). No statistically significant differences in infection parameters were found in convict cichlids of different size classes in 2014. This paper provides the first documented record of the Asian fish tapeworm infecting a wild population of the convict cichlid in the U.S.

Introduction of exotic fish into novel aquatic ecosystems is sometimes accompanied by the unintentional transmission of additional species dangerous to populations of endemic fish, commercial fish and aquaculture (Bauer et al. 1973, Hoffman and Shubert 1984, Scholz 1999, Salgado-Maldonado and Pineda-Lopez 2003). One such invader, the Asian fish tapeworm, *Bothriocephalus acheilognathi* Yamaguti, 1934 (Cestoda: Bothriocephalidea), was imported from East Asia to Europe and the Americas during the 1960s and 1970s with herbivorous cyprinids, predominantly grass carp (*Ctenopharyngodon idella*), to control growth of aquatic vegetation in freshwater ecosystems (Hoffman 1999, Williams and Jones 1994, Choudhury and Cole 2012). The Asian fish tapeworm (hereafter, Asian tapeworm) has a simple life cycle that requires only two hosts: a definitive host, a fish in which larval stages develop into adult worm producing eggs, and an intermediate host, a cyclopoid copepod, which is a transmitter of the early larval stage (Liao and Shin 1956). The entire life cycle is temperature-dependent, and under optimal temperature, 25° C, can be completed in eighteen days (Bauer et al. 1973).

Due to low specificity for both intermediate and definitive hosts, and by colonizing other cyprinid as well as poeciliid hosts, the Asian tapeworm easily became established within native fish populations in new regions and continents, eventually resulting in its

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current global distribution (Hoffman 1999, Font 2003, Choudhury and Cole 2012). Presently, it has been reported in 104 fish species in 14 families and seven orders from almost every continent except Antarctica (Salgado-Maldonado and Pineda-Lopez 2003). It is pathogenic to wild fish and aquaculture stock and may cause disease and even mortality events (Bauer et al. 1973, Scott and Grizzle 1979, Hoffman 1980, Granath and Esch 1983c, Hoole and Nissan 1994, Heckmann 2000, Hansen et al. 2006, Han et al. 2010, Britton 2011). In the U.S., after the initial discovery of the Asian tapeworm in Florida in 1975 (Hoffman 1980), it has been reported from 13 additional states (Arizona, California, Colorado, Hawai'i, Kansas, Michigan, Nevada, New Hampshire, New Mexico, North Carolina, Texas, Utah and Wisconsin), both in the wild or in fish hatcheries (Hoffman and Schubert 1984, Heckmann and Deacon 1987, Riggs and Esch 1987, Heckmann et al. 1993, Brouder and Hoffnagle 1997, Kuperman et al. 2002, Bean et al. 2007, Pullen et al. 2009, Archdeacon et al. 2010, Choudhury and Cole 2012). In California, the Asian tapeworm was first discovered in 1987 in grass carp, collected from irrigation reservoirs in Riverside and Imperial counties and in golden shiners (*Notemigonus crysoleucas*) collected from a fish farm in San Diego County (Chen 1987).

Surveys conducted in 1999-2001 revealed seven additional fish species (six cyprinid, one poecillid) in southern California infected by the Asian tapeworm (Kuperman et al. 2002). Of the six infected cyprinids, the arroyo chub (*Gila orcutti*) and Mojave tui chub (*Siphateles bicolor mohavensis*) are native, while the other four, common carp (*Cyprinus carpio*), golden shiner, goldfish (*Carassius auratus*) and fathead minnow (*Pimephales promelas*), are introduced. The single infected poecillid is the introduced mosquitofish. In June 2007, a population of convict cichlids (*Archocentrus nigrofasciatus*) was reported from the perennial discharge channel of a water treatment plant in Los Angeles County (Hovey and Swift 2012). The convict cichlid is native to Central America and is a tropical thermophilic species with a minimum temperature tolerance of 20 C (Conkel 1993, Bussing 1998). The first U.S. records of the convict cichlid were in Nevada where the fish were discovered in two natural warm springs (Deacon et al. 1964, Hubbs and Deacon 1965). In Mexico, introduced convict cichlids (as, *Cichlasoma nigrofasciatus*) were found to be infected by the Asian tapeworm (Salgado-Maldonado and Pineda-Lopez 2003), but no information on fish infection by this parasite was known for the U.S. The goal of the present study was to investigate whether the recently discovered population of the convict cichlid in California was infected by the Asian tapeworm.

Materials and Methods

Fish were collected for parasitological examination from a discharge channel with elevated water temperature 26° C [$\pm 1.5^\circ$ C]. The source of the thermally elevated water was the treated discharge from the Rio Vista Water Treatment Plant that feeds directly into the Santa Clara River, Los Angeles County (34.423806, -118.540511; WGS84). The willow riparian scrub vegetation supported by the perennial discharge channel is restricted to the southern bank of the much wider, dry sandy river bed of the Santa Clara River. The outflow travels approximately 800 m before flowing subsurface. It is believed that the established convict cichlid population at this location originated from released aquarium fish (Hovey and Swift 2012). Other fish species that occurred at the study site were the native arroyo chub, and the non-native mosquitofish, prickly sculpin (*Cottus asper*), black bullhead (*Ameiurus melas*), goldfish, and common carp (var. koi) (Hovey, unpub. field notes). Of them, only mosquitofish were available for parasitological examination.

Table 1. Prevalence and mean intensity of infection of convict cichlids (*Archocentrus nigrofasciatus*) and mosquitofish (*Gambusia affinis*) by the Asian fish tapeworm (*Bothriocephalus acheilognathi*) in 2007 and 2014.

Size class	Sample size (N)	Fish total length (TL) range, mm	Prevalence (%)	Intensity	
				Mean \pm SD	Range
Convict cichlids					
September 2007					
Entire sample	150	25 - 130	92.0	12.3 \pm 12.8	1 - 101
Class 1, small fish	100	25 - 59	100 ^{A*}	15.5 \pm 13.7 ^{B*}	1 - 101
Class 2, medium fish	35	61 - 86	80.0 ^{C*}	3.9 \pm 4.5 ^{D*}	1 - 22
Class 3, large fish	15	88 - 1303	66.6 ^{E*}	1.5 \pm 0.7 ^{F**}	1 - 3
May 2014					
Entire sample	300	39 - 112	37.0	5.4 \pm 5.2	1 - 24
Class 1, small fish	74	39 - 59	32.4 ^G	4.8 \pm 4.5 ^H	1 - 19
Class 2, medium fish	155	60 - 80	41.9 ^G	5.7 \pm 5.4 ^H	1 - 24
Class 3, large fish	71	88 - 112	25.4 ^G	3.9 \pm 3.5 ^H	1 - 14
Mosquitofish					
May 2014					
Entire sample	70	43 - 65	15.7	1.4 \pm 0.7	1 - 3

A-H: Within the category, mean values sharing the same letter are not significantly different ($P \leq 0.05$)

* p -value < 0.001

** p -value > 0.05

A total of 450 convict cichlids and 70 mosquitofish were used for this study. On 11 September 2007, only three months after the discovery of convict cichlids in the channel, 150 convict cichlids were collected to be examined for the presence of the Asian tapeworm. A second fish collection took place on 1 May 2014 and included 300 convict cichlids and 70 mosquitofish. Fish were captured by seine net and placed into 5-gallon buckets containing channel water. Within three hours of being captured, the fish were removed from the water, transferred into plastic bags and placed into a freezer. The fish were then transported while still frozen, and stored at San Diego State University in a freezer until the commencement of parasitological examinations. After being thawed, total length (TL) of each individual was measured to the nearest mm. The TL of convict cichlids collected in 2007 ranged from 25 mm to 130 mm and in 2014 ranged from 39 mm to 112 mm (Table 1). To calculate infection parameters, convict cichlids were separated into three size classes: class 1 (small), class 2 (medium) and class 3 (large) (Tables 1, 2). We arbitrarily selected the range for each of the three size classes based on the clustering of sizes. The body cavities were opened and digestive tracks removed. After a longitudinal incision of the intestine, tapeworms were carefully teased from the intestinal wall, rinsed in 0.85% saline and placed into Petri dishes with the same solution. Tapeworm identification was made using the reference keys by Bykhovskaya-Pavlovskaya et al. (1964) and Hoffman (1999). Tapeworms from each fish were enumerated to determine the prevalence, the proportion of the hosts infected, and mean intensity of infection, the mean number of parasites in the infected hosts (Bush et al. 1997). The number of fish sampled, prevalence and mean intensity are provided in Table 1. A total number of tapeworms found in fish collected in 2007 and 2014, number of tapeworms in each size class of fish and the percentage of immature and mature tapeworms are presented in Table 2. Images of immature and mature tapeworms were obtained by light microscopy (LM) and scanning electron microscopy (SEM). For LM, 10 tapeworms and several

Table 2. Number and percentage of immature and mature Asian fish tapeworms (*Bothriocephalus acheilognathi*) recovered from convict cichlids (*Archocentrus nigrofasciatus*) in 2007 and 2014.

Size class of convict cichlids	Number of tapeworms	Stage of tapeworm development, %	
		Immature	Mature
	September 2007		
Entire sample	1710	43.2	56.8
Class 1, small fish	1558	45.3	54.7
Class 2, medium fish	137	24.1	75.9
Class 3, large fish	15	13.3	86.7
	May 2014		
Entire sample	597	83.9	16.1
Class 1, small fish	133	86.5	13.5
Class 2, medium fish	393	86.1	13.9
Class 3, large fish	71	64.8	35.2

pieces of intestinal wall with tapeworms attached were examined with a Nikon Eclipse E200 microscope (Melville, NY) and photographed under magnification x40. For SEM, eight mature tapeworms fixed in 70% alcohol were rinsed in phosphate buffer saline, post-fixed in 1% osmium tetroxide, dehydrated in ascending concentrations of ethanol from 50% to 100%, critical-point dried, sputter-coated with platinum, and examined using a FEI Quanta 450 scanning electron microscope (Hillboro, OR). A series of 10 preserved Asian fish tapeworms collected from the convict cichlids was deposited into the Harold W. Manter Laboratory of Parasitology, University of Nebraska, Lincoln, Nebraska (HWML 64742).

Prevalence of infection in convict cichlids was tabulated by fish size (small, medium, large) for 2007 and 2014 separately. The resulting 2x3 contingency table was analyzed using a Pearson's chi-squared test. Mean intensity in fish from three size classes in 2007 and 2014 were estimated using the two-sample independent Mann-Whitney U test.

Results

The Asian tapeworm was the only intestinal parasite found in the 450 convict cichlids collected from the discharge channel of a water treatment plant in September 2007 and May 2014. The prevalence and mean intensity of fish infections were higher in the 2007 sample than in the 2014 sample (Table 1). In the 2007 sample, parameters of infection were different among fish from the three size classes (Table 1). The highest prevalence and mean intensity of infection was found in small fish while the lowest were found in large fish (Table 1). Intensity of infection in fish from different size classes varied widely (Table 1). The highest parasite loads in small, medium and large fish were 101, 22, and 3, respectively. Both mature and immature Asian tapeworms were recovered from fish. Mature Asian tapeworms had a heart-shaped scolex with deep long bothria, a flattened attachment disc (Fig. 1A), and a perfectly segmented strobila composed of wide proglottids containing rosette-shaped ovaries filled with eggs (Fig. 1B, C). Immature tapeworms were represented by individuals at various developmental stages, ranging from worms having a small scolex and non-segmented body, to worms with a well-shaped scolex but still poorly segmented strobila and an underdeveloped reproductive system (Figs. 1D, E). In 2007, almost 60% of tapeworms recovered from the convict cichlids were represented by mature worms (Table 2). The highest percent of mature tapeworms was found in large (class 3) fish, and small (class 1) fish contained an almost equal

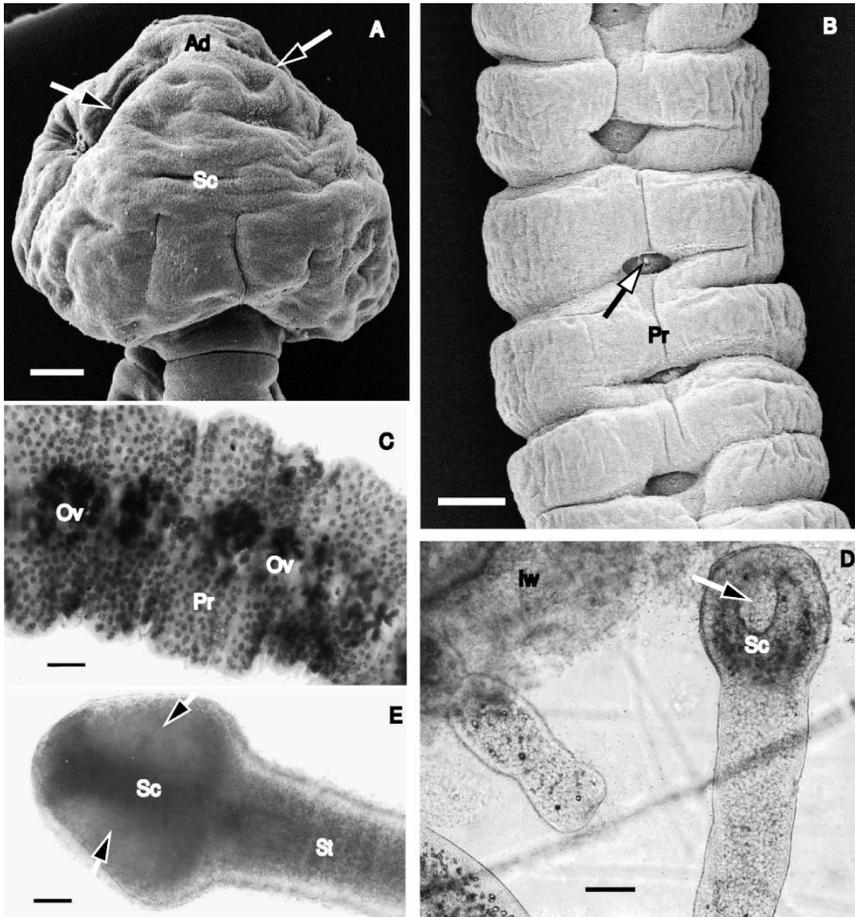


Fig. 1. Representative scanning electron microscope micrographs (A, B) and light microscope micrographs (C-E) of the Asian fish tapeworm *Bothriocephalus acheilognathi*. A) Mature worm - heart-shaped scolex with long bothria and flattened attachment disc; B) Mature worm - segmented strobila with mature proglottids and uterine pores; C) Mature worm - segmented strobila with mature proglottids and rosette-shaped ovaries; D) Immature worm - small scolex with short bothria, pre-proglottid formation of strobila; E) Immature worm - well developed scolex and early stage proglottid formation of strobilla. Ad - adhesive disk; lw - intestinal wall; Ov - ovary; Pr - proglottid; Sc - scolex; St - strobila. Black-head arrows indicate bothria, white-head arrow indicates uterine pore. Scale bars: 20µm.

number of mature and immature tapeworms (Table 2). In the 2014 sample, overall prevalence and mean intensity of infection in convict cichlids were 2.5 times and 3 times lower, respectively, than the 2007 sample (Table 1). Contrary to the 2007 results, no significant difference was found in the infection parameters of fish from the three size classes (Table 1). The highest load of Asian tapeworms at 24 individuals was found in a medium (class 2) fish. In contrast to 2007 results, about 84% of Asian tapeworms recovered from convict cichlids were immature (Table 1). The highest percent of mature Asian tapeworms was found in large (class 3) fish (Table 2). Of the 70 mosquitofish, also collected in May 2014, only eleven were infected by Asian tapeworms, with the lowest infection level being one tapeworm (Table 1). Of the fifteen Asian tapeworms found, 87% were immature (Table 2).

Discussion

The present paper documents the first record of the Asian tapeworm in a wild population of the convict cichlid in the U.S. Finding the Asian tapeworm in years 2007 and 2014 indicates the presence of a persistent reservoir of infection in the channel conveying the thermally elevated discharge from the Rio Vista Water Treatment Plant in Los Angeles County. As only convict cichlids and mosquitofish were available for parasitological examination, we have no information on the infection in five other species of fish inhabiting this channel. We cannot exclude that three fish species, arroyo chub, goldfish and common carp, all well-known for their susceptibility to Asian tapeworm (Kuperman et al. 2002), could contribute to the persistence of the parasite at this site.

The artificially elevated water temperature of 26° C [$\pm 1.5^\circ$ C] was optimal for the growth and development of the Asian tapeworm. Stimulating effect of high temperature on parasite transmission, infectivity, development and intrapopulation structure has been previously reported (Bauer et al. 1973, Sankurathri and Holmes 1976, Granath and Esch 1983a, b, c, Dobson and Carper 1992, Khan 2012). In our study, the overall infection rate in the autumn sample (2007) was higher than in the spring sample (2014). These results appear to be largely in agreement with the most common pattern of the seasonal dynamics of populations of the Asian tapeworm, in which elevation of water temperature was considered a critical factor controlling infectivity, development and intrapopulation structure (Bauer et al. 1973, Granath and Esch 1983a, b, c).

However, the water temperature at our collection site remained nearly constant throughout the year. Our sampling effort, separated by seven years, is long enough for significant changes to have occurred in the ecosystem we examined. Based on our limited sampling effort we were unable to identify alternative abiotic factors that, acting singly or synergistically with biotic factors, might affect fish infection by the Asian tapeworm. The last ones may include fluctuations in the biomass of zooplankton including cyclopoid copepod community (the intermediate host of the Asian tapeworm), shortage in biomass of phytoplankton (the food web for copepods), copepod species diversity (not all copepods are an efficient intermediate host for the Asian tapeworm) and changes in the structure of the fish community inhabiting the collection site. Water quality may also contribute to the rate of fish infection. It is possible that the chemical composition of the discharged water from the water treatment plant may affect both fish and cyclopoid copepods known for their high sensitivity to water chemistry (Ferdous and Muktedir 2009). It is also known that in the case of fish infected by the Asian tapeworm, the pattern of high prevalence of infection may be followed by low prevalence (Heckmann and Deacon 1987, Archdeacon et al. 2010), and we cannot exclude the possibility that our samplings do not fit this seasonal pattern because of the different seasons and years of sampling.

Different parameters of infection were recorded in convict cichlids in the autumn 2007 and spring 2014 samples; overall values varied among size classes. In the 2007 sample, both prevalence and mean intensity of infection were size-dependent. Prevalence of infection reached 100% in small (class 1) fish but only 66.7 % in large (class 3) fish (Table 1). There was an inverse relationship between the size class of fish and the number of worms they harbored (Table 1). The highest parasite load of 101 Asian tapeworms was carried by one of the smallest fish (TL 31 mm). Lower values of infection rate in larger fish may be associated with the elimination of heavily infected individuals, the expelling of a number of worms due to their competition for food source, or stronger immunity of large fish compared to the smaller fish. The stage of worm maturation was inverse to the

intensity of infection, and consequently to fish size (Table 2). For example, large fish carried a maximum of three Asian tapeworms, most of them mature, while in the heavily infected smaller fish, the percent of mature and immature worms were nearly equal at 45.2% and 54.7%, respectively. Based on the rate at which the Asian tapeworm developed at 26° C [$\pm 1.5^\circ$ C], the predominance of mature tapeworms infecting fish in 2007 indicates that this infection was at least one month old (Bauer et al. 1973, Williams and Jones 1994). In the spring sample (2014) we documented comparatively low infection levels in convict cichlids, regardless of fish size (Table 1). The highest parasite load of 24 Asian tapeworms was found in a medium (class 2) fish (TL 73 mm). There was an inverse relationship between the size class of fish and the number of worms they harbored (Table 1). In contrast to the fall sample (2007), the percent of mature tapeworms for all three fish size classes was lower (Table 2). Approximately 86% of the tapeworms recovered from the small (class 1) and medium (class 2) fish were immature, predominantly in the early stages of development, while 64.8% of the tapeworms from the large (class 3) fish were immature (Table 2). The predominance of immature stages of the Asian tapeworms infecting convict cichlids in the spring season (2014) indicates that the intermediate host, a cyclopoid copepod carrying infective larval stage of the procercooids, had been recently consumed. Low infection parameters and the same pattern of worm development were recorded in the mosquitofish. The seasonal patterns of infection levels and development stages of the Asian tapeworm discussed above are in agreement with previous reports of mosquitofish infections (Kuperman et al. 2002). Although we advocate for the removal of introduced and deleterious species when possible, this thermally isolated population of an infected tropical fish species in an artificially elevated and nearly constant temperature environment, provides a unique opportunity to study alternative factors influencing the seasonal population dynamics and ecological relationships of the intermediate host (cyclopoid copepods), the Asian tapeworm, and the final host, infected fish.

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