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Rapid Communication

The invasive tapeworm *Bothriocephalus acheilognathi* Yamaguti, 1934 in the endangered killifish *Profundulus candalarius* Hubbs, 1924 in Chiapas, Mexico

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Abstract

The non-native Asian fish tapeworm *Bothriocephalus acheilognathi* was registered for the first time in 2008–2009 in a threatened Mexican fish *Profundulus candalarius*. From June 2008 to April 2009 the overall prevalence of the cestode in the fish intestines was around 35% (N = 242 *P. candalarius* examined). Unstrobilized and inmature cestodes formed the greatest proportion (91.07%) of the parasite population. Neither the prevalence, nor the mean intensity varied significantly with host sex. Smaller fish (10–29 mm) were parasitized more frequently than medium size fish (30–59 mm), prevalence 43.5% vs 27.3%. Although, the cestodes' monthly prevalence and mean intensity per host varied from 18 to 70 percent and from 2.75 to 9.27 respectively, no significant seasonal difference was found. There was a continuous recruitment of cestodes within the host population, as well as a nearly continuous growth and maturation of the parasites to adults. Most cestodes are lost from the host as development of parasites proceeds, and only 12 of the 437 collected tapeworms reached gravidity. The most important features of the data presented here include the confirmation that *B. acheilognathi* can reproduce in Mexico.

Key words: freshwater ecosystems, invasions, helminths, cestode, prevalence, intensity

Introduction

The antropogenic introduction of pathogens and parasites to freshwater fish populations is a matter of increasing concern (Salgado-Maldonado and Rubio-Godoy 2014). Parasites play an important role concerning host population densities and geographical distribution (Dove 1998; Font 2007). Native species are expected to be more susceptible to the non-native pathogens as they have never been previously exposed and thus have limited immune response (Dove 1998; Britton et al. 2011; Peeler et al. 2011).

The Asian fish tapeworm *Bothriocephalus acheilognathi* Yamaguti, 1934, has been introduced widely outside its native distribution, and is now considered of special concern worldwide (Scholz et al. 2012; Salgado-Maldonado and Rubio-Godoy 2014). It is one of the most severe cestodes for cultured carp in Europe (Bauer et al. 1973; Scholz et al. 2012), and a potential threat for native freshwater fish populations worldwide (Heckmann et al. 1987; Font and Tate 1994; Dove 1998; Salgado-Maldonado and Pineda-López 2003; Font 2007; Archdeacon et al. 2010). Currently the cestode is widely distributed across the world, with about ≈ 235 host species belonging to 19 families, of freswater fishes (Scholz et al. 2012). In the Americas it ranges from Canada to Panama (Choudhury et al. 2006; Choudhury et al. 2013; Marcogliese 2008), with a peak of abundance in Mexico where it has been reported in 77 freshwater fish species (Salgado-Maldonado and Pineda-López 2003; Salgado-Maldonado 2006; Salgado-Maldonado and Rubio-Godoy 2014). Until now in South America it has only been recorded in an imported contingent of carp *Cyprinus carpio* at a fish farm in Northern Brazil (Rego 2000). The main pathway for the introduction of *B. acheilognathi* has been identified as the fish farming of Chinese carps (Bauer et al. 1973; Andrews et al. 1981; López-Jiménez 1981; Font and Tate 1994; Dove et al. 1997; Dove and Fletcher 2000; Choudhury and Cole 2012; Scholz et al. 2012), but then secondary pathways involving novel hosts are likely to take place (Font 2007; Scholz et al. 2012). However, the introduction of the cestode at a location does not necessarily means that it has established due to the role of several hosts in its life cycle, as hosts are not always present.

In this study, we aimed at characterizing the population dynamic of *B. acheilognathi* in an endangered Mexican killifish *Profundulus candalarius* Hubbs, 1924. There is currently a lack of understanding about the changes among host populations after *B. acheilognathi*'s introduction (Scholz et al. 2012).

Methods

Fish *Profundulus candalarius* were caught using a gill net every two months between June 2008 to May 2009 at a stream belonging to the endorrheic basin of Teopisca (16°33′14″N, 92°28′45″W, altitude 1791 m), which lies in the highlands of Chiapas, Mexico. The entire alimentary tract was removed and intestines were examined for parasites using a stereomicroscope. All cestodes found were counted, collected, and preserved in formalin 4% until stained in Mayer's paracarmine, dehydrated, and cleared in methyl salicilate, to make whole mounts. Cestodes were identified following Scholz et al. 2012, and Salgado-Maldonado et al. 2015. Cestodes were distinguished as: 1) unstrobilized, inmature cestodes; 2) mature, when mature proglottids were seen; and 3) gravid, with ripen eggs in uterus. Infection parameters, prevalence and mean intensity, were calculated as described by Bush et al. (1997). Voucher specimens of *B. acheilognathi* were deposited at Colección Nacional de Helmintos (CNHE: 9780), Instituto de Biología, Universidad Nacional Autónoma de México.

Results

Sex related prevalence

Population levels of the cestode are high: a total of 242 *P. candalarius* were examined, 85 of which (29♂, 24♀, 32 juveniles) wereparasitized by the cestode (prevalence 35.1%). Fish were parasitized with one to 35 (mean intensity 5.1) cestodes per parasitized host; a total of 437 individual tapeworms were recovered from the intestines, 91.07% of which were inmature, juvenile worms, i. e. unstrobilized and inmature cestodes forms the largest proportion of the population during sampling period. Pooling the data of all adult hosts (54 juvenile fish were discharged from this analysis) in order to compare the parameters of infection between 100 male *P. candalarius* versus 88 females, neither the prevalence (♂ 29%, ♀ 27.3%, F = 0.014, P = 0.90), nor the mean intensity (♂ 4.1, ♀ 4.9, F = 0.11, P = 0.73) varied significantly with host sex.

Size related prevalence

Fish of 10 – 60 mm standard length, SL, were caught during the sampling period. The cestodes were not distributed evenly throughout host size groups. Smaller fish (10 – 29 mm SL, length size class I), weremore frequently parasitized (N = 108, prevalence 43.5%), than medium size fish (30 – 59 mm Length size class II) (N = 128, prevalence 27.3%); however, intensity values did not significantly differ between these size classes (Length size class I, intensity range 1 – 25, mean intensity 5.2; length size class II, intensity range 1 – 35, mean intensity 4.7 cestodes per infected fish). The small number of larger than 60 mm SL fish (N = 6) prevent any meaningful comparison with the smaller ones, however prevalence of infection in these larger fish was 50%, and intensity ranged from 1 to 23, and mean intensity was 8.7.

Temporal variation in prevalence

The cestode showed a broad range of temporal distribution since it was recorded from all months studied; however, changes in prevalence and mean seasonal intensity were not statistically significant (Table 1). The prevalence varied smoothly, it rose over June to October to peak in December (dry season), and then declined sharply. Mean intensity fluctuated irregularly amongst sampling months, but despite erratic peaks there was no significant seasonal pattern changes (Table 1). Moreover, data show a continuous recruitment of new generation of the cestode into killifish, as well as a nearly continuous growth and maturation to adults; gravid worms however, appeared to occur at a relatively low rate, and only a very small proportion of cestodes became gravid. Unstrobilized and inmature cestodes form the largest proportion (91.07%, 398 of 437 individual tapeworms) of the cestodes recovered during all sampling dates. Mature cestodes were also recovered almost continuously, except in February 2009, but in significantly less proportions (6%, 27/437) than juveniles, gravid cestodes were

Table 1. Changes in the prevalence, mean intensity, and in stages of development of *Bothriocephalus acheilognathi* in *Profundulus candalarius* in a creek in Teopisca, Chiapas, 2008 – 2009.

	No. hosts examined	No. hosts parasitized (prevalence %)	Mean intensity (range)	No. of cestodes by stage of development / month		
				Juveniles	Adults	Gravids
Jun 08	44	8 (18.2%)	2.7 (1-7)	20	2	0
Aug	40	15 (37.5%)	9.3 (1-35)	89	8	5
Oct	31	11 (35.5%)	2.5 (1-9)	27	1	0
Dec	40	28 (70.0%)	5.0 (1-25)	124	11	5
Feb 09	40	11 (27.5%)	8.5 (1-22)	93	0	0
Apr	47	12 (25.5%)	4.3 (1-23)	45	5	2

recovered only in three out of six samples, and in a very low proportion (August 5, December 5, April 2).

Discussion

After its introduction in 1965 (López-Jiménez 1981), the cestode has rapidly expanded its distribution to most of the political states of Mexico and into many river drainages within the country, remarkably infecting many native freshwater fish populations (Salgado-Maldonado and Pineda-López 2003; Salgado-Maldonado 2006; Salgado-Maldonado and Rubio-Godoy 2014). These facts underlie the ability of the cestode to complete all stages of its life history, because of its well known lack of host specificity, and its rapid rate of spread. However, it is noteworthy that in *P. candalarius* the cestode grows to maturity but very few reach the gravid state, making unlikely that the parasite could persist in the native host without the presence of a reservoir of infection in carp, or a continuously supply of infected carps.

The very wide host range and geographic distribution acquired by the cestode in Mexico is striking. Some freshwater fish families inhabiting Mexico are very susceptible to the infection, as the Profundulidae. *B. acheilognathi* was introduced into the highlands of Chiapas, Mexico, in 1995, along with the introduced cyprinid *Cyprinus carpio*.These fishes were evidently and highly parasitized (Velazquez-Velazquez Pers. Obs.) when introduced into two fish farms in the area, the “Centro Acuícola San Cristóbal” at San Cristóbal Las Casas, and the “Centro Acuícola Ixtapa” at Francisco Romo Serrano, Ixtapa, Chiapas. After initial introduction to Chiapas it can be assumed, *B. acheilognathi* was able to reach native hosts populations including the endangered *P. hildebrandi* (see Velázquez-Velázquez and Schmitter-Soto 2004; Velazquez-Velazquez et al. 2009, 2011) and *P. candalarius* (present work). Our data show the cestode has been able to establish breeding populations on *P. candalarius* at a Teopisca stream, acquiring then a new host. This is the third record of *B. acheilognathi* for the fish family Profundulidae, the cestode has been previously found also in *P. portillorum* (Salgado-Maldonado et al. 2015).

Despite the paucity of data, the data presented here allow us to explore several important aspects of the biology of the cestode in one of the new areas where this worm has been introduced. The recorded changes tend to support the interpretation of a continuous recruitment, growth and maturation, however, few cestodes develop to maturity and most are lost from host as development proceeds, and fewer of them reach to a gravid state.

The low proportion of cestodes that became gravid was expected, as common for a great majority of highly fecund species. Clearly, from high numbers of recruits only a fraction of them mature, but the greatest loss of parasites occurred before maturation, with only a few reaching being gravid, a pattern of changes similar to those reported by Granath and Esch (1983b). No explanation can be advanced to account for this observation in the present study, perhaps competition for space or nutrients could have occurred amongst inmature cestodes. The host immune response or perhaps a temperature related rejection could also be the cause of parasite mortality (see Granath and Esch 1983b; Marcogliese and Esch 1989; Nie and Kennedy 1992).

The cestode *B. acheilognathi* is considered to be a potential threat to endemic fishes in Mexico due to their pathogenic effects (Salgado-Maldonado and Pineda-López 2003). The occurrence of *B. acheilognathi* might have negative ecological impacts on native fishes; for example, it is well documented that heavy infection by this cestode species leads to great mortalities of fry under culture conditions (Bauer et al. 1973).

The most important features of the data presented here include the confirmation that once introduced into Mexico, the parasite reproduces, and that tropical warm climate favours a pattern of continuous growth and transmission under natural conditions involving native fish species.

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