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Research Article

Genetic diversity of the Asian fish tapeworm *Schyzocotyle acheilognathi* Yamaguti, 1934 populations from introduced and native freshwater fish hosts in Mexico

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Abstract

There is a steadily growing database of host-parasite interactions between the Asian fish tapeworm and Mexican freshwater fishes, but to date, no research in Mexico has addressed the biological aspects of the invasion. There is a need for analysis to differentiate populations of the cestode found in introduced Asian carp and native freshwater fish in Mexico. In this study, we therefore analyzed the genetic diversity of the Asian fish tapeworm in populations from introduced and native freshwater fish. We explored 12 variable microsatellite loci from 65 adult *Schyzocotyle acheilognathi* recovered from freshwater fish including the introduced Asian carp *Cyprinus carpio* and native *Notropis boucardi* (Cyprinidae), *Profundulus hildebrandi* (Profundulidae), *Cichlasoma istlanum* (Cichlidae) and *Poecilia maylandi* (Poeciliidae) from four locations in two neotropical basins (Chiapas and Morelos). For the first time, we present data using a cluster analysis approach to define genetic groups. Overall, geographical isolation of the populations contributed to genetic differentiation (overall F_{ST} = 0.184), and cestode populations from cyprinids (LN and PLC) presented the highest polymorphism: 61.25% and 72.5% of total number of alleles amplified in all loci. Our results show that *S. acheilognathi* populations have invaded and established in Mexican native fish species, becoming self-sustaining populations in the invaded ecosystems. Our study offers valuable insights for developing strategies to manage cestode invasions in Mexican freshwater fish. We recommend that authorities consider including epidemiological data based on this type of evaluation as part of sanitary protocols for restocking programs of certain fish species in different localities.

Key words: invasion biology, invasion dynamics, alien helminths, aquaculture, pathogen

Introduction

The Asian fish tapeworm, *Schyzocotyle acheilognathi* Yamaguti, 1934 (= *Bothriocephalus acheilognathi*), has the largest number of recorded hosts and widest geographical distribution (found on all continents except Antarctica) of any freshwater fish helminth, making it the most successful fish parasite in the world (Kuchta et al. 2018). In the life cycle of this cestode, cyclopid copepods are intermediate hosts (~ 5 verified genera; Marcogliese and Esch 1989), and a range of more than 312 species of freshwater fish from 38 families are definitive hosts (Kuchta et al. 2018). The Asian fish tapeworm is responsible for bothriocephalosis, which is pathogenic for Asian carp (Salgado-Maldonado and Pineda-López 2003; Ahmad et al. 2014) and many other freshwater fish species (Ahmad et al. 2014). Heavy infections can cause mortality in younger life stages (fry) of carp (Hanzelová and Žitňan 1986; Bertasso and Avenant-Oldewage 2005; Han et al. 2010) and other fish species (Evans and Lester 2001).

Although the Asian fish tapeworm has been reported in 110 freshwater fish species of Mexico (López-Jiménez 1981; Salgado-Maldonado et al. 1986; Salgado-Maldonado and Osorio-Sarabia 1987; Salgado-Maldonado and Pineda-López 2003; Pérez-Ponce de León et al. 2018), the biology of its invasion in Mexico remains unknown. New host records in Mexico have been continuously added since the original recording by López-Jiménez (1981) and have continued to grow in recent years (García-López et al. 2016; Barrios-Gutiérrez et al. 2018), but little other data about the parasite in Mexican waters are available. Asian carps have been introduced in Mexico for different purposes: grass carp has been used for aquatic weed control (*Ctenopharyngodon idella*), common carp for fisheries aquaculture (*Cyprinus carpio*) and koi carp and goldfish (*Carassius auratus*), among others for ornamental aquaculture. However, it is the black carp (*Mylopharyngodon piceus*) that is presumed to have transmitted an invasive trematode parasite, *Centrocestus formosanus* (López-Jiménez 1987). Other cyprinids of North American origin have been introduced into Mexican territory as live bait for sport fishing (Contreras-Balderas 1999). High transmission of *S. acheilognathi* in introduced Asian carps and in sympatric native freshwater fish of Mexico has been documented (Salgado-Maldonado 2006), for example in Patzcuaro Lake (Salgado Maldonado et al. 2001b) and in the San Cristóbal de Las Casas endorheic basin (Velázquez-Velázquez et al. 2011). Yet critical biological and epidemiological information are still lacking. For example, there have been no analyses of the links between populations of the cestode from introduced Asian carp and native freshwater fish of Mexico, nor any data about the circulation of the parasite within and between aquaculture conditions and wild Mexican environments. Anthropogenic spread of parasites in Mexican freshwater fish populations is primarily caused by the intentional introduction of Asian carps and other exotic fish species for aquaculture purposes. This includes the production of infected Asian carps

in ponds and the subsequent release of their fry and juveniles into natural bodies of water such as dams, ponds, reservoirs, and channels (Mendoza-Alfaro et al. 2021). This highlights the need to precisely determine the infection capacity of circulating strains and the hosts they infect, along with the circumstances that promote their infection.

The analysis of the population genetic variability of parasites allows us to understand their dynamics among their hosts (de Meeûs et al. 2007). Genetic studies of populations of *S. acheilognathi* are scarce (Luo et al. 2003; Brabec et al. 2018). Since the first record of *S. acheilognathi* in Mexico (López-Jiménez 1981), there have been extensive studies focused on recording its presence in different localities, but despite significant efforts over the past 40 years, we have yet to explore the characteristics that determine the infection dynamics in specific host species. In addition, more than 20 years ago, Luo et al. (2003) raised the possibility that the Asian Fish Tapeworm is likely a species complex, based on the isolation of different gene pools. Its host specificity therefore needs to be reconsidered.

In this study, we aimed to examine the genetic diversity of *S. acheilognathi* in native and invasive freshwater fish populations from two neotropical regions of Mexico. Using 12 microsatellite markers, we analyze patterns of genetic variation to determine whether there is genetic structuring related to host species or geographic location. We also determined the unique contribution of each host species to the infection rates in different locations and described a series of morphometric features. Further, we explored the potential of native Mexican freshwater fish species as reservoirs and distributors of this parasite. Our objective was to improve the understanding of the epidemiology of *S. acheilognathi* through population differentiation by comparing data on parasites obtained from introduced carps with those from native hosts. These data are necessary to further prevent and control the transmission and dispersal of the tapeworm infection in Mexico.

Materials and methods

Study localities and sample collection

Fish were collected in four different locations. In August 2019, we collected 20 *Profundulus hildebrandi* (Profundulidae) from the San Cristobal Las Casas endorheic basin in Chiapas. In January 2020, we collected eight carps *Cyprinus carpio* (Cyprinidae) from Lago Navenchauc, Chiapas. On May 2022, we collected 10 *Poecilia maylandi* (Poeciliidae) from Las Huertas – Río Amacuzac, Morelos; 32 *Notropis boucardi* (Cyprinidae); and 6 *Cichlasoma istlanum* (Cichlidae) from Puente "La Cuera" – Tlayecac, Morelos; all of these locations are in the Balsas river basin (Figure 1). Each locality was assigned a label to facilitate the interpretation of subsequent sample analyses (see footnote text of Figure 1) The fish were caught with dip-nets and an electrofishing device. They were transported immediately after collection

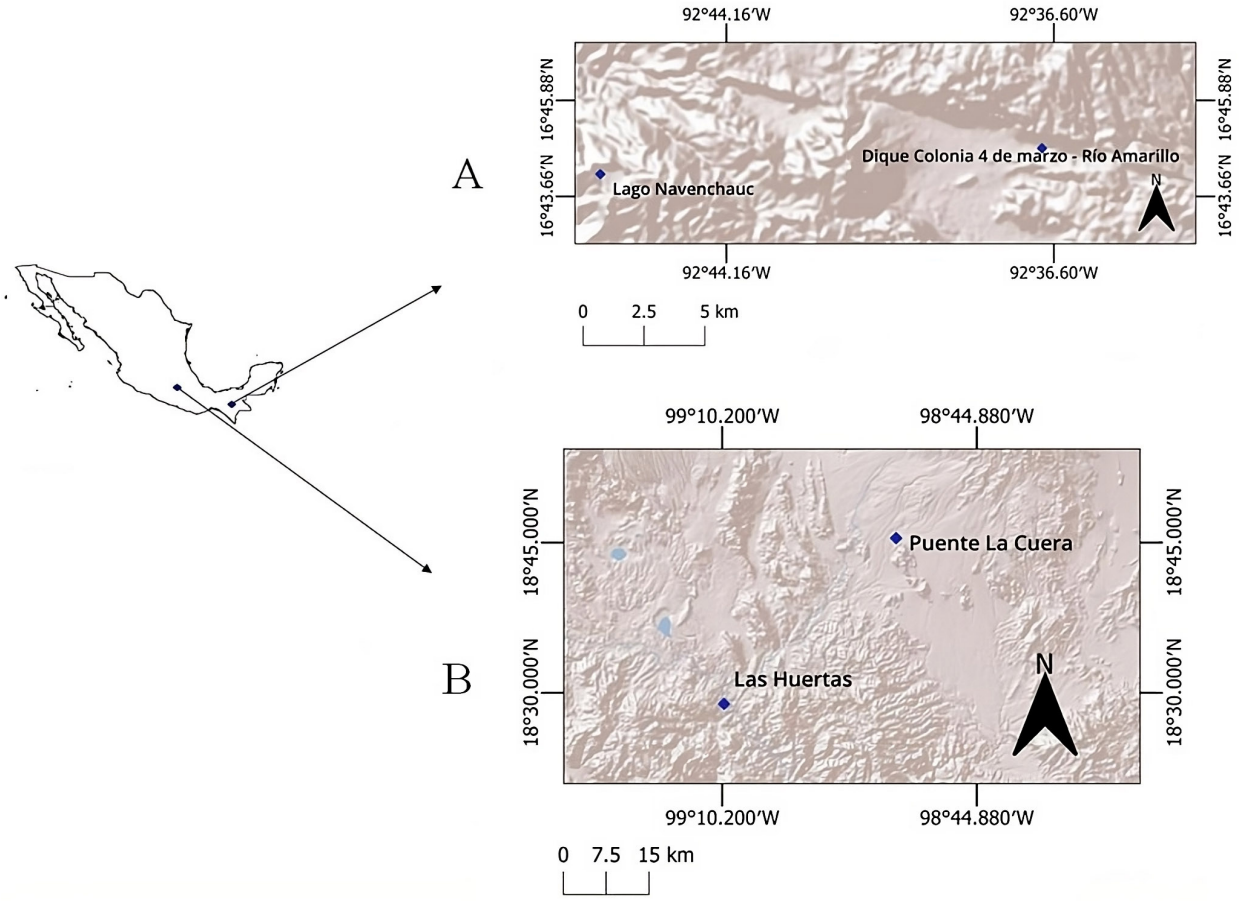

Figure 1. Locations from which *Schyzocotyle acheilognathi* were collected. A: San Cristóbal Las Casas endorheic basin, Chiapas (Dique colonia 4 de marzo – Río Amarillo, San Cristóbal de las Casas, Chiapas [RA] (16°44′46.3″N; 92°36′52.3″W) and Lago Navenchauc, Zinacantán, Chiapas [LN] (16°44′10.4″N; 92°47′04.1″W)). B: Balsas river basin ("Las Huertas" – Río Amacuzac, Tlaquiltenango, Morelos [LH] (18°28′53.5″N; 99°10′00.9″W) and Puente "La Cuera" Tlayecac, Ayala, Morelos [PLC] (18°45′26.5″N; 98°52′53.9″W)).

to the laboratory to be examined. Total length (TL), standard length (SL), and body depth (BD) of each individual host was measured. The intestinal tract of each host was examined under a stereoscopic microscope in Petri dishes with a 0.7% saline solution. The cestodes that were found were placed individually in Petri dishes filled with clean 0.7% saline. Two to six segments from the end of the strobila of each cestode were fixed directly in molecular grade ethanol for molecular analysis. We used hot 4% formalin to fix the rest of each tapeworm, including the scolex and strobila, for microscopic study.

Procedures for morphological determination of cestodes and infection data.

Specimens preserved in formalin were stained with Mayer's paracarmine or Ehlrich's hematoxylin and mounted whole with Canada balsam to make permanent preparations for the study of morphological features. Voucher specimens were deposited in the Colección Nacional de Helmintos (CNHE: 13012–13016) of the Instituto de Biología, Universidad Nacional Autónoma de México (UNAM). When specimens were obtained from more than one individual host of a particular fish species, we performed a Pearson's

correlation to determine whether helminth size increased with host size (using TL values). We calculated the prevalence (percentage of the total examined population that was infected), mean abundance (number of parasite individuals divided by the total examined population), and mean intensity (number of parasite individuals divided by the infected proportion of the examined population) according to Bush et al. (1997).

Molecular procedures

Genomic DNA was extracted from the fragments of the strobila, processing each individual separately. DNA extraction was performed using the DNAeasy Blood & Tissue Kit® (Qiagen) following the manufacturer's protocol. The final extraction was eluted in 50 µl of AE buffer and stored at −20 °C. Twelve microsatellite loci were amplified; amplification was carried out following the protocols of Luo et al. (2003) and Brabec et al. (2018). The markers used were selected based on the highest polymorphic information and detected alleles for each in the original studies by Luo et al. (2003) and Brabec et al. (2018). We analyzed the following molecular markers (followed by GenBank accession numbers in parentheses): RIBO 1, RIBO 4, RIBO 6, RIBO 7 and RIBO 8 (AF362408–AF362433), ach44832 (MG835888), ach6142 (MG835890), ach18042 (MG835891), ach63950 (MG835894), ach21425 (MG835897), ach27438 (MG835898) and ach49654 (MG835900). The PCR procedure was performed in 10 µl of Master mix with 5 µM MyTaq® Buffer 5X (Bioline©, with dNTP's included), 0.4 U – 5 µM MyTaq® DNA polymerase (Bioline©), 0.2 µM of each primer, and 5 µl of genomic DNA. PCR products were visualized by electrophoresis in 2% agarose gels in 1x TAE buffer and then sent to the Genomic Sequencing of Biodiversity and Health Laboratory at Pabellón Nacional de la Biodiversidad (UNAM), where they were run in an 96 capillary ABI 3730xl automatic sequencer (Thermo Fisher Scientific©), using the GENESCAN 500 LIZ marker for the standard size of the fragments. Genotyping files were generated in GeneMapper® v. 4.0 software (Applies Biosystems©) and allele sizes were manually reviewed and determined in GeneMarker® v. 2.6.3.

Molecular genetics data analysis

Molecular samples of *S. acheilognathi* from each population were compared between introduced carps and native hosts to determine whether genetic structuring were related to locality or host species. In order to assign individuals to their respective genetic groups, we performed a cluster analysis in STRUCTURE v. 2.3.4. (Pritchard et al. 2000) applying the Bayesian Markov Chain Monte Carlo (MCMC) method with 1,000,000 iterations with a burn-in period of 100,000 iterations. To process the outputs, we ran STRUCTURE HARVESTER (Earl 2012) and the ΔK method were used to identify the appropriate number of clusters (Evanno et al. 2005). Departure from Hardy-

Table 1. Recorded infection data of *S. acheilognathi* in host fish species (N = number of hosts examined).

Host	Locality	N	Total number of cestodes	Prevalence (%)	Mean intensity	Intensity range (min – max)
Profundulus hildebrandi	Río Amarillo – San Cristóbal de las Casas, Chiapas	20	12	35	1.7	1 – 5
Cyprinus carpio	Lago Navenchauc – Zinactnán, Chiapas.	8	37	75	6.2	2 – 14
Notropis boucardi	Puente "La Cuera" – Tlayecac, Ayala, Morelos.	32	388	78.1	15.5	1 – 81
Cichlasoma istlanum		6	32	66.7	8	1 – 25
Poecilia maylandi	Las Huertas – Tlaquiltenango, Morelos.	10	3	10	3	3

Weinberg Equilibrium (F_{IS}) was evaluated using an exact test with Bonferroni correction in Genepop v. 4.7.5 (Rousset 2008). Current population genetics parameters were calculated in FSTAT v.2.9.4. (Goudet 2003), including the allelic richness, observed (*Ho*) and expected (*He*) heterozygosities, as well as the fit of the expected value to correct for the effect of the sample size (u*He*), and pairwise differentiation between populations (F_{ST}) using the Weir & Cockerham estimator and estimate the allelic richness rarefied for sample size.

An analysis of molecular variance (AMOVA) was performed to evaluate the molecular differences between individuals from different genetic groups (PhiPT). We constructed a genetic distance matrix calculated from individual-by-individual pairwise values (see Supplementary material Table S1) and Nei genetic distances between the studied samples from each host species, and we performed pairwise distance comparisons between populations using a neighbor joining analysis. Finally, to spatially confirm the patterns of population differentiation, we performed a principal components analysis (PCA) with the adegenet R package in RStudio v.4.4.1.

Results

Schyzocotyle acheilognathi *infections in cyprinid and non-cyprinid hosts*

The infection parameters of the cestode *S. acheilognathi* in the different hosts are shown in Table 1. The highest prevalence and mean intensity values were recorded for the cyprinids *C. carpio* and *N. boucardi* and the cichlid *C. istlanum*. We recovered a remarkably high number of cestode individuals (81) from a single *N. boucardi* and up to 25 from *C. istlanum.* The profundulid *P. hildebrandi* showed intermediate levels of infection, while the poeciliid *P. maylandi* showed the lowest level of infection.

Morphometric features

A total of 57 specimens mounted on permanent slides were measured (Table S2) and identified as *Schyzocotyle acheilognathi* (= *Bothriocephalus acheilognathi*). The determination was based on the following morphological characters (see Yamaguti 1934; Scholz 1997; Kuchta et al. 2008): heart-shaped scolex with two lateral bothridia, strobila acraspedote, all proglottids with rounded edges, a genital pore in the middle part of each mature proglottid,

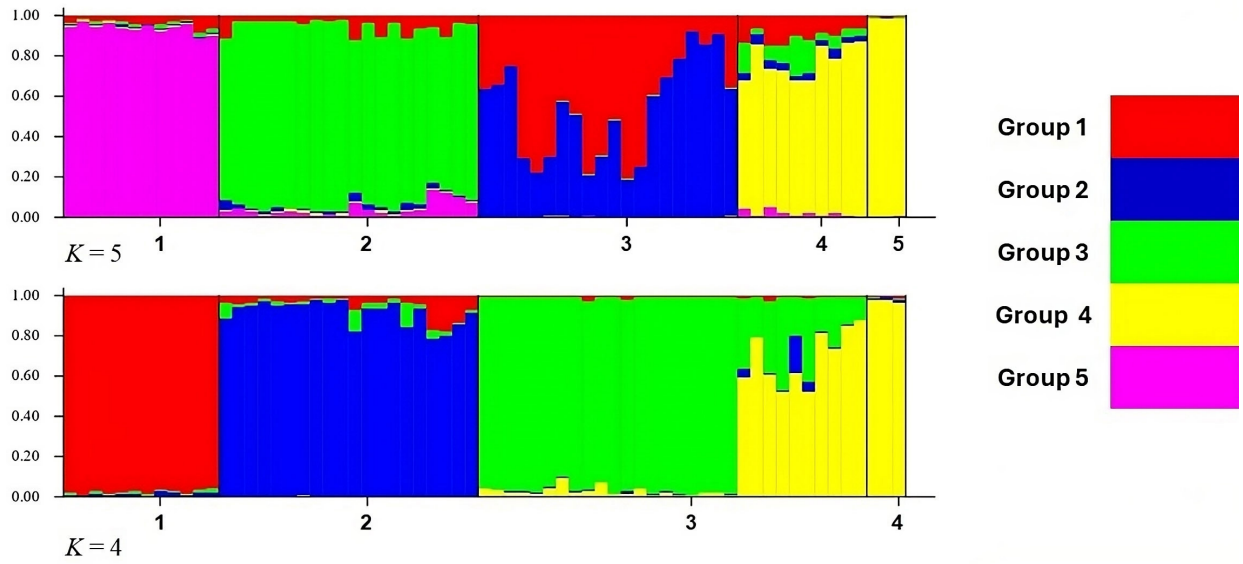

Figure 2. Clustering analysis of STRUCTURE of *S. acheilognathi* samples based on 12 microsatellite loci used in this study. Each genetic group is represented by different color ($K = 5$ clustering by host species and $K = 4$ clustering by location).

medullary placement of the testes, oval to spherical, ovary lobed, medially placed near the posterior margin of the proglottids, and gravid proglottids with eggs distributed throughout the uterus. Analyzing morphometric attributes of specimens by each host species which they belong, specimens collected from cyprinid hosts were the largest: mean total length 36.8 ± 20.5 mm in cestodes from *C. carpio* and 25.1 ± 12.4 mm in cestodes from *N. boucardi.* The highest proportion of fully developed and gravid cestodes were also collected from the cyprinid fish (13/15 specimens from *N. boucardi*, and 8/15 from the *C. carpio*). The cestodes with the highest number of gravid proglottids were also recorded from cyprinid fish; the largest number of gravid proglottids (163) was found in a cestode from a carp. Cestodes of intermediate size were found in *P. hildebrandi* (16.4 ± 10.4 mm) and *C. istlanum* (15.6 ± 6.9 mm), and there were 3 gravid specimens from each of these hosts. The smallest cestode specimens were from *P. maylandi* (7.2 ± 3.5 mm), and only one of them was gravid (with 13 gravid proglottids). There was no significant correlation between infected *N. boucardi* and *C. carpio* host size and tapeworm size ($r = 0.94$ $p > 0.05$ and $r = 0.61$ $p = 0.38$, respectively). However, in *P. hildebrandi* hosts, helminth size increased with host size ($r = 0.88$ $p = 0.007$). It was not possible estimate correlation between total length of *C. istlanum* and *P. maylandi* fishes and the size of tapeworms because they were obtained from a single host individual.

Genetic variability and population differentiation in S. acheilognathi

In individual assignments to delimit *S. acheilognathi* populations of the 4 locations studied, the *K* values inferred in STRUCTURE showed that $K = 4$ was the most likely number of genetic groups (Figure 2), providing evidence for the pattern of regional differentiation.

Table 2. Observed size ranges in base pairs (bp) and number of alleles (in parenthesis) of all *Schyzocotyle acheilognathi* populations for each microsatellite locus. The number of cestodes examined from each population is indicated in brackets below each host name.

Locus	Overall allele size range (bp)	Total Alleles per locus	Allele size range by population (alleles per locus)			
			RA [12]	LN [20]	PLC [30]	LH [3]
RIBO 1	92–130	10	119–127 (5)	120–130 (5)	92–127 (8)	92 (1)
RIBO 4	96–217	15	112–134 (5)	96–136 (7)	96–217 (14)	134 (1)
RIBO 6	93–149	10	144–145 (2)	93–149 (8)	93–149 (7)	95–145 (2)
RIBO 7	90–102	8	96–102 (4)	90–100 (6)	92–99 (4)	92–94 (2)
RIBO 8	95–163	7	95–160 (5)	95–163 (6)	95–163 (6)	95 (1)
ach44832	147–311	22	147–227 (8)	147–311 (11)	147–311 (17)	301 (1)
ach6142	237–274	8	268–274 (5)	237–274 (7)	237–274 (7)	272–273 (2)
ach18042	100–351	25	183–339 (12)	100–351 (17)	185–267 (11)	250 (1)
ach63950	233–391	14	233–362 (5)	233–362 (7)	233–391 (10)	297 (1)
ach21425	208–233	12	208–216 (6)	208–219 (8)	208–233 (10)	215–221 (2)
ach27438	178–278	18	178–272 (8)	212–278 (12)	210–278 (13)	212 (1)
ach49654	369–390	11	369–380 (7)	378–382 (4)	369–390 (9)	379–380 (2)
Total number of alleles across all loci			72	98	116	17

All 12 of the microsatellite loci used as molecular markers were successfully amplified (Table 2). Four loci had the highest number of alleles: these were ach44832, ach18042, ach27438, and RIBO 4. The cestodes of the PLC and LN populations had the highest number of amplified alleles per locus, followed by the RA population. The LH cestode population had the lowest number of amplified alleles per locus, as well as seven monomorphic loci (Table 2). The RA and PLC tapeworm populations had the highest values of allele richness (rarefied for sample size) (3.756 and 3.617, respectively). The cestodes from LH had the lowest allele richness (1.417). We also found a strong significant association between the number of cestodes analyzed and the number of alleles per locus ($r = 0.96$, $p = 0.03$).

All cestode populations showed heterozygote deficits ($Ho < He$) (Table 3). Those with the lowest deficiency of heterozygosity were from LN and PLC; this last population had the highest number of private alleles (37 for all loci). On the other hand, the populations with the highest heterozygous deficiency recorded were LH and RA. This is consistent with the Shannon information index; all populations had relatively low diversity values (less than 2), but the LH cestode population had the lowest value, below 0.5. Interestingly, PLC cestode population had an F_{IS} value that was relatively close to that of the parasites of LH population (Table 3).

Most loci showed significant differences from Hardy-Weinberg Equilibrium (HWE). Highly significant differences ($p < 0.001$) were observed at the loci RIBO 4, RIBO 8, ach63950, and ach27438 of the RA population; at loci RIBO 7, RIBO 8, ach44832, ach6142, ach18042, ach63950, ach21425, and ach27438 of the LN population; and at loci RIBO 1, RIBO 4, RIBO 6, RIBO 7, RIBO 8, ach44832, ach6142, ach63950, ach21425, ach27438 and ach49654 from PLC. However, slight deviations from HWE ($p < 0.05$) were observed

Table 3. Summary of population-genetic analysis for each *S. acheilognathi* population over all loci. The number of cestodes analyzed from each population is given in brackets below each locality label (mean value ±standard error, Na = total number of alleles, Pa = Private alleles, Ne = Number of effective alleles, I = Shannon information index, *Ho* = Observed heterozygosity, *He* = Expected heterozygosity, u*He* = unbiased expected heterozygocity, F = fixation index.

Population	Na	Pa	Ne	I	*Ho*	*He*	u*He*	F
RA	6	1.08	3.672	1.382	0.444	0.710	0.685	0.386
[12]	± 0.7	± 0.398	± 0.519	± 0.144	± 0.063	± 0.055	± 0.057	± 0.234
LN	8.2	1.67	4.947	1.698	0.613	0.776	0.778	0.213
[20]	± 1	± 0.62	± 0.624	± 0.124	± 0.080	± 0.031	± 0.032	± 0.293
PLC	9.7	3.083	4.540	1.695	0.392	0.765	0.745	0.503
[30]	± 1.1	± 0.68	± 0.681	± 0.134	± 0.066	± 0.036	± 0.037	± 0.197
LH	1.4	0.083	1.299	0.250	0.028	0.493	0.206	1
[3]	± 0.1	± 0.083	± 0.111	± 0.090	± 0.028	± 0.062	± 0.075	± 0

at the loci RIBO 6, ach44832, ach6142, ach18042, ach21425 and ach49654 of the RA population, loci RIBO 6 and ach49654 from LN, and locus ach18042 from PLC. No significant differences were found at loci from the LH population.

The LN and PLC cestode populations had the largest number of alleles (61.25% and 72.5%; N = 98 and 116). All four cestode populations had private alleles. These unique alleles reached a maximum of 15 per locus (ach18042). The results of molecular variation analysis (AMOVA) showed that 83% of the variance was within populations, while 17% was between populations. Individuals from the LH population show greater molecular variation with respect to the remaining (PhiPT = 0.458 with RA, 0.383 with LN and 0.297 with PLC), followed by individuals from the RA population (PhiPT = 0.158 with LN, 0.163 with PLC and 0.438 with LH). In turn, these populations presented strong differentiation (large F_{ST} values) which is consistent with the PhiPT value of each population (Table 4). Given the taxonomic and geographic distance between the cestode's initial host upon introduction (Asian carp) and the native Mexican species it has been documented infecting, the differences were not significant (overall F_{ST} = 0.184 ± 0.17 Sd; p = 0.9).

Comparisons of Nei's genetic distances between sample groups within host species, there is a clear regional differentiation of parasites. The parasites of three host species in the Balsas River basin were the most distant from the two populations of the San Cristóbal region (Figure 3A). This pattern was confirmed by the PCA (Figure 3B), where differentiation is appreciated at the geographical level, rather than by host species.

Discussion

Our results indicate that the *S. acheilognathi* in our sample consisted of a single species that was able to infect and mature in very different host taxa. Our data do not support the idea that *S. acheilognathi* constitutes a complex of reproductively isolated species, as Luo et al. (2003) suggested, since we found shared alleles among populations, as well as F_{ST} values without statistical differences, indicate populations of a single species. Nevertheless,

Table 4. Characterization of *S. acheilognathi* populations by locality (*F*st = effect of populations compared to the total sample of a particular locality, PhiPT = molecular variance).

Population	*F*st	PhiPT
RA	0.140	0.969
LN	0.051	1.356
PLC	0.072	1.338
LH	0.474	0.462

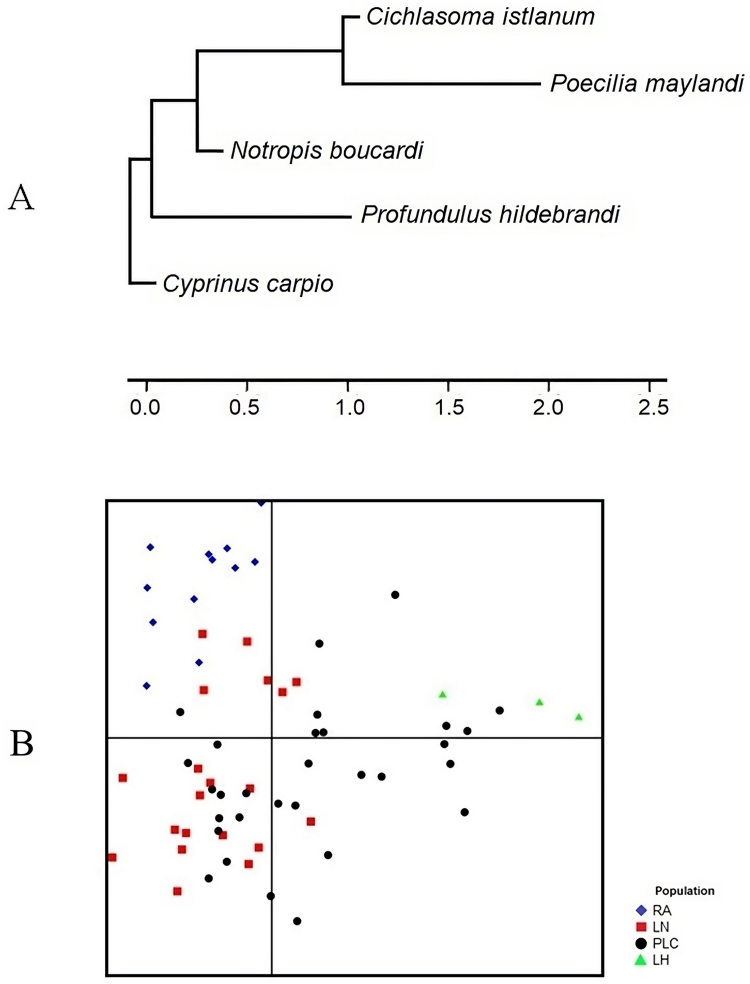

Figure 3. A: Dendrogram of Nei's genetic distance based in Neighbor Joining analysis between the studied samples of *S. acheilognathi* from each host species. B: Principal component analysis plot of cestode populations from each locality based on 12 microsatellite loci used in this study (axis 1 and axis 2 represent 24.3% and 18% of total variance).

high molecular variance among the four populations analyzed, cluster analysis and PCA demonstrated that geographical isolation of the populations contribute to genetic differentiation. Our results also show that *S. acheilognathi* populations have invaded and established in Mexican native fish species, becoming self-sustaining populations in the invaded ecosystems. The populations of the cestode in native freshwater fish in Mexican water bodies are able to promote the self-sufficiency of *S. acheilognathi* populations in water bodies, without the presence of Asian carp as a source of infectivity.

The *S. acheilognathi* population of LN had high polymorphism and cestode populations from the native endemic Mexican cyprinid *N. boucardi* and *C. istlanum* in the Balsas basin (PLC) displayed the same population parameters, indicating effective population dynamics. These two populations had the highest probability of panmixia, as well as the lowest levels of differentiation, F_{ST}, the maximum number of shared alleles and the presence of private alleles. All these data suggest populations with effective genetic dynamics (Smith and Haigh 1974; Woolhouse et al. 2002). This situation is reflected in the infection dynamics (prevalence, intensity, maturity, egg amount, etc.). Cestode population from the profundulid *P. hildebrandi* (RA) had lower genetic values, but that allows them to be self-sustaining populations. Meanwhile, the genetic values from cestodes from LH locality should be considered preliminary, given the small sample size and therefore, these results should be interpreted with caution. In this regard, Beerli (1998) mentioned the flexibility of estimation methods to analyze populations with very marked differences in their sample sizes (since it is unrealistic to assume that in nature populations are "symmetrical") and considering the aggregate distribution of parasites in an examined host population (Bush et al. 1997). The number of marker loci facilitates the representativeness of genetic data (Beerli 1998; Wilson and Rannala 2003).

The allele sizes we found are similar to those previously reported for the Asian fish tapeworm from China (Luo et al. 2003) and Africa (Brabec et al. 2018). Interestingly, we found more alleles per locus for most explored loci (except in LH), compared with those reported by Luo et al. (2003), and by Brabec et al. (2018). Nevertheless, our data show continuous HWE, suggesting the population composition by homozygous individuals in each genetic group, regardless of the number of alleles and their frequency and which evidence self-fertilization as the main reproductive strategy (de Meeûs et al. 2007).

Much of the previous research to determine population structure was based on an *a priori* definition of populations, which can cause bias (Pearse and Crandall 2004). Up until a few years ago, the cluster analysis approach had been used for genetic group delimitation for other objectives in other helminths that are invasive to wildlife, such as *Baylascaris procyonis* (Osten-Sacken et al. 2018) and *Dicrocoelium dendriticum* (van Paridon et al. 2017) with important ecological results. This approach allows for more precise population delimitation to be studied, rather than being restricted to *a priori* speculations (Pritchard et al. 2000; Pearse and Crandall 2004), which has been one of the limitations of previous population genetics studies of the Asian tapeworm carried out by Luo et al. (2003) and Brabec et al. (2018).

Field data (Velázquez-Velázquez et al. 2015; Mendoza-Alfaro et al. 2021) show that the cestode is active year-round in Mexican waters, and that populations are mixed by the copepods that act as intermediate hosts and

by fish, which are the main hosts. Fish vagility and/or passive transport of copepods (e.g., accidental introduction with stock water of origin of fry and juvenile fish in relocation campaigns and/or reconnections of streams and dams), must play a significant role in homogenizing *S. acheilognathi* metapopulations in Mexican waters. The prevalence of *S. acheilognathi* is high in native freshwater fish from both the San Cristóbal de Las Casas endorheic basin and the Balsas River basin. This is similar to most other Mexican basins where the cestode has been reported (Salgado-Maldonado and Pineda-López 2003; Salgado-Maldonado 2006). The general pattern that is apparent from all these reports is that wherever Asian carps (*Ctenopharyngodon idellus* or *Cyprinus carpio*) have been examined in Mexican bodies of fresh water, *S. acheilognathi* is recorded with a rather high prevalence (> 10%), and several sympatric native Mexican fish hosts are also positive for *S. acheilognathi*. However, the prevalence and intensity of these cestode infections, as well as the cestodes' degree of development and gravidity, differ among Mexican fish families. For instance, in the endorheic basin of San Cristóbal de Las Casas, Asian carps introduced the cestode at least 50 years ago, which has since spread widely with high prevalence. Many gravid proglottids have infected the profundulid *P. hildebrandi* (Velázquez-Velázquez et al. 2011). According to previous records, profundulid fish native to and endemic to Central America appear to be very susceptible to this infection.

Other freshwater basins in Mexico, such as the Balsas River basin (Salgado-Maldonado et al. 2001a; Caspeta-Mandujano et al. 2009), have recorded the same pattern, showing a high prevalence of *S. acheilognathi* in Asian carps. This infection is also present in other freshwater fish species, such as the native cyprinid *N. boucardi*, as well as other native and exotic sympatric cichlids and poeciliids. The case of the Lerma-Santiago river basin in Mexico is remarkable: *S. acheilognathi* parasitizes every species of Asian carp examined from the different bodies of water throughout this basin, and the sympatric endemic atherinopsids *Chirostoma* spp. and goodeids *Goodea atripinnis* recorded have very high levels of this infection (Salgado-Maldonado et al. 2001b).

The records of *S. acheilognathi* in native wild freshwater fish in Mexico reveals a failure of aquaculture procedures in the country. The introduction of infected alevins and Asian carp fry, as well as incorrect preventive treatment methods, facilitated the initial invasion and spread of the Asian cestode. However, our present results and analyses show that the high risk of infection could be supported by very high egg burden. All of this suggests that the *S. acheilognathi* infections recorded in wild native fish species at our study sites strongly contributes to the high prevalences recorded at other locations in the same hydrological basins. Our data show self-sustained, well-established

populations of the cestode in several Mexican native fish, which could contribute to the wide dispersal and high risk of infection in Mexican waters.

The infection observed in Profundulid and Cichlid fishes in southern Mexico, as well as other cichlids in the Usumacinta, Papaloapan, Coatzacoalcos, and elsewhere (Salgado-Maldonado 2006) suggests an important contribution of the infection of *S. acheilognathi* to the wide distribution and high prevalences and abundances of the cestode in freshwater basins throughout Mexico.

This study demonstrates the effectiveness of the markers created by Luo et al. (2003) and Brabec et al. (2018) and enhances our current knowledge of the population genetics of *S. acheilognathi*, as well as using the cluster analysis approach to define genetic groups. It offers valuable insights for developing strategies to manage the invasion of cestodes in freshwater fish in Mexico. We hope that the authorities responsible for aquaculture and fisheries will consider including this new framework as part of the sanitary protocol prior to the implementation of restocking programs of certain fish species in different localities in which there are epidemiological data based on this type of evaluation. This information could help control the parasite's spread to new locations and prevent its establishment in new hosts.

Author's contribution

AVO, GSM: research conceptualization; AVO, ICCG, AJM, JMCM, GSM: sample design and methodology; AVO, ICCG: investigation and data collection; AVO, ICCG, AJM, ORC: data analysis and interpretation; GSM, AVO: ethics approval; GSM: funding provision; AVO, GSM: roles/writing – original draft; writing; GSM, ICCG, AJM, ORC, YAC, JMCM: roles/writing – review and editing.

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Ethics and permits

Protocol for the use of fish in this research based on the NOM – 019 – STPS – 1993 established by Instituto de Ecología, Pesquerías y Oceanografía del Golfo de México EPOMEX. Specimens collected under the permission of Protocolo de Manejo y Cuidado Animal (SICUAE.DC-202211-4) issued by Subcomité Institucional para el Cuidado y Uso de Animales Experimentales (SICUAE – UNAM) tu AVO and GSM and Cartilla Nacional de Colector Científico (FAUT-0105) issued by Secretaría del Medio Ambiente y Recursos Naturales (SEMARNAT) to GSM.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Genetic distance matrix calculated from individual-by-individual pairwise values.
Table S2. General morphometric features of *Schyzocotyle acheilognathi* specimens of each host population.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2025/Supplements/BIR_2025_Villa-ODogherty_etal_SupplementaryMaterial.xlsx