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Francisco Neptalí Morales-Serna ^{1,*}, Dania G. López-Moreno ², Juan M. Osuna-Cabanillas ²,
Saúl García-Labrador ², J. Roberto F. Vallarta-Zárate ^{3,4}, Jorge Payan-Alejo ² and Felipe Amezcua ¹

¹ Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Mazatlán 82040, Mexico

² Facultad de Ciencias del Mar, Universidad Autónoma de Sinaloa, Mazatlán 82000, Mexico

³ Dirección de Investigación Pesquera en el Atlántico, Instituto Nacional de Pesca y Acuicultura, Mazatlán 82112, Mexico

⁴ Posgrado en Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Mazatlán 82040, Mexico

* Correspondence: neptali@ola.icmyl.unam.mx

Abstract: Thread herrings (*Opisthonema* spp.) are economically important fish species in the Tropical Eastern Pacific. Knowledge of the parasitofauna of commercially exploited species is useful as it can increase our understanding of fish biology and ecology. However, our knowledge of the parasites of *Opisthonema* spp. is limited. During a fisheries exploration survey in April 2022, samples of *Opisthonema bulleri* and *O. libertate* were collected from three oceanographic stations in the Gulf of Tehuantepec in the Mexican Pacific. Parasitological analysis of these materials uncovered four parasite species: *Myosaccium ecaude* (Trematoda), *Cribromazocraes* sp. (Monogenea), *Pseudoterranova* sp. (Nematoda), and an unidentified crustacean of the family Pennellidae (Copepoda). All these species appeared rarely, except for *M. ecaude*, which reached a prevalence of 100%. The median intensity of *M. ecaude* infection was significantly higher in *O. bulleri* than in *O. libertate*. We analyzed the relationship between parasitic infection intensity and three host traits (fork length, body weight, and age). Only body weight showed a significant positive association with intensity. Future studies are required to analyze the possible effects of seasonality, locality, and host ontogeny on the occurrence of *M. ecaude* in thread herrings in the Tropical Eastern Pacific.

Keywords: Clupeidae; Trematoda; digenea; small pelagic; marine fish; *Opisthonema*



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1. Introduction

Thread herrings are commercially important small pelagic fish of the genus *Opisthonema* (family Clupeidae). These species are found forming dense shoals in tropical and subtropical marine waters of the Americas: *Opisthonema oglinum* in the Atlantic and *O. berlangai*, *O. bulleri*, *O. medirastre*, and *O. libertate* in the Pacific [1]. The latter three species, mostly *O. libertate*, contribute substantially to catch volumes of small pelagic fish in Mexico, especially in the northwestern region [2]. However, catches of these fish have shown important changes over the past decade, making it necessary to have a better knowledge of their population dynamics [2].

Marine fishes serve as hosts to a range of parasites which can be transmitted trophically or through non-trophic mechanisms. Some parasites have the potential to affect host physiology, morphology, reproduction, or behavior, and they are increasingly recognized as having significant impacts on host individuals, populations, communities, and even ecosystems. Therefore, parasitological surveys are important for assessing the fitness of fish species [3,4]. Among different groups of parasites, the trematodes, particularly digeneans of the family Hemiuridae, are commonly found in the digestive tract of marine fishes. The prevalence and intensity of infection of these parasites may be positively correlated

with host size [5]. Overall, one of the main determinants of the number of parasites is host growth; larger or older fish tend to accumulate more parasites [6]. Typically, for practical reasons, fish length is used as a proxy for age in parasite tag studies. Nonetheless, it is important to disentangle the effects of size and age on parasitic load to reduce ambiguities arising from individual differences in parasite infections, especially when comparing samples containing fish of variable size or age [7].

To the best of our knowledge, there is only one previous study of thread herring parasites in the Pacific. Pérez-Ponce de León et al. found six parasite species parasitizing *O. libertate* from Chamela Bay in the Mexican Central Pacific [8]. The reported species were the monogeneans *Kuhnia* sp. and *Polymicrocotyle manteri*, trematodes *Myosaccium ecaude* and *Parahemiurus merus*, cestode *Proteocephalidea* gen. sp., and nematode *Pseudoterranova* sp. During a recent stock assessment of small pelagic fishes from the Gulf of Tehuantepec in the Mexican South Pacific, samples of thread herrings were obtained. A parasitological examination of those samples revealed notable numbers of *M. ecaude*. The goal of the present study was twofold: first, to determine the prevalence and intensity of infection of this parasite; second, to evaluate whether fish length, weight, and age are descriptors of parasitic burden.

2. Results

A total of 121 fish specimens were collected from three sampling stations in the Gulf of Tehuantepec. Of these, 89 were identified as *O. bulleri* and 32 as *O. libertate* (Table 1). For *O. libertate* found at station S2, the mean fork length was 15.8 ± 0.77 cm, the mean body weight was 61.4 ± 6.59 g, and the mean age was 1.3 ± 0.43 years, which did not differ between the sexes (9 females and 16 males). For *O. bulleri*, the overall mean fork length was 16.5 ± 0.83 cm, the mean weight was 61.5 ± 7.41 g, and the mean age was 1.7 ± 0.58 years. These metrics did not differ significantly between sampling stations ($p > 0.05$). Sex was determined for 71 specimens of *O. bulleri* (29 females and 42 males), of which the length (17.4 ± 0.7 cm) and the weight (63.1 ± 6.2 g) of females were significantly higher than those of the males (16.4 ± 0.66 cm and 56 ± 13.6 g, respectively; $p < 0.05$) at station S3 only; whereas at stations S1 and S2, there were no differences between the sexes ($p > 0.05$). The *O. bulleri* sampled were significantly longer and older than the *O. libertate* ($p < 0.05$).

Table 1. Samples of *Opisthonema* spp. from three sampling stations in the Gulf of Tehuantepec, Mexico.

Sampling Station	Latitude, Longitude	Depth (m)	Temperature (°C)	Fish Species	n
S1	15°27'55.5" N, 93°23'55.5" W	34	30.6	<i>O. bulleri</i>	39
				<i>O. libertate</i>	1
S2	15°30'52.5" N, 93°27'59.2" W	36	30.5	<i>O. bulleri</i>	10
				<i>O. libertate</i>	31
S3	15°37'26.8" N, 93°37'32.1" W	36	30.4	<i>O. bulleri</i>	40

In total, four parasite species were found in *O. bulleri*: the trematode *M. ecaude* (Digenea: Hemiuridae) (Figure 1) in the stomach, the monogenean *Cribromazocraes* sp. (Polyopisthocotylea: Mazocraeidae) on the gills, the nematode *Pseudoterranova* sp. (Chromadorea: Anisakidae) in the mesentery, and an unidentified juvenile copepod of the family Pennelliidae (Siphonostomatoida) on the gills. For *O. bulleri*, the prevalence of *M. ecaude* was 100% in all samples and the median infection intensities were 24, 17, and 20 parasites per fish at stations S1, S2, and S3, respectively. Monogeneans, nematodes, and copepods appeared rarely (prevalence < 3% in the whole sample) and were insufficient for species identification. *Opisthonema libertate* was parasitized only by *M. ecaude*, with a prevalence of 100% and a median intensity of nine parasites per fish at station S2. The mean intensity values were close to the median (Figure 2).

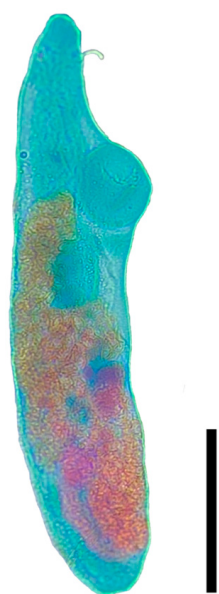


Figure 1. Microphotography of an adult specimen of the parasite *Myosaccium ecaude* found in *Opisthonema bulleri* from the Gulf of Tehuantepec. The specimen was stained with Gomori's trichrome. Scale bar = 250 μ m.

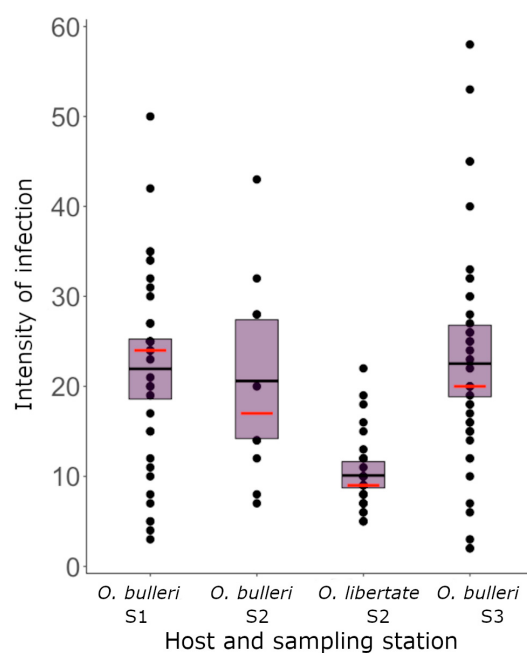


Figure 2. Intensity of *Myosaccium ecaude* infection in *Opisthonema* spp. from three sampling stations (S1, S2, and S3) in the Gulf of Tehuantepec. The boxes represent the mean with 95% confidence intervals. The shorter (red) lines inside the boxes represent the median.

There were no significant differences in the median infection intensity of *M. ecaude* among the three *O. bulleri* samples ($p > 0.05$), but the intensity was significantly lower for *O. libertate* than for *O. bulleri* ($p < 0.05$). There were no significant differences in infection intensity between the sexes ($p > 0.05$). The GLM results showed that only fish weight had a significant positive relationship with the intensity of *M. ecaude* infection (Figure 3).

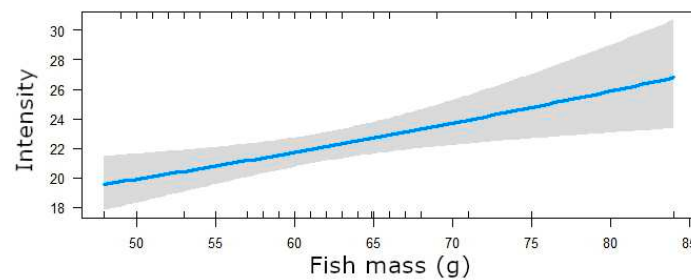


Figure 3. Relationship between the infection intensity of *M. ecaude* and the body weight of *O. bulleri* ($Z = 0.003$, $p < 0.05$).

3. Discussion

This is the first report of the presence of parasites in the slender thread herring (*O. bulleri*). To the best of our knowledge, there has only been one previous study of parasites in Pacific members of *Opisthonema*; Pérez-Ponce de León et al. reported six parasite species in *O. libertate* from Chamela Bay in the Mexican Central Pacific [8]. These authors reported *M. ecaude* with a prevalence of 7% and a mean abundance of 0.13, which are much lower infection levels than reported herein. Similarly, del-Río-Zaragoza et al. reported a low prevalence (28%) of *M. ecaude* in another clupeid, *Sardinops sagax*, from Todos Santos Bay in the Mexican North Pacific [9]. To some extent, these differences may be influenced by local variability in oceanographic conditions [10]. The Gulf of Tehuantepec has complex oceanographic patterns, with cyclonic and anticyclonic eddies that affect primary productivity and the distribution of organisms [11]. These local features could result in variations in parasitic populations at different latitudes.

The higher *M. ecaude* infection intensity of *O. bulleri* than that of *O. libertate* may be explained by the differences in the size and the age of the examined species. Although *O. bulleri* individuals showed little variability in terms of size (low intraspecific variation in body length), the GLM indicated that larger fish tended to accumulate more parasites. A possible explanation for this observation is that, in general, larger individuals ingest more food and, being older than smaller individuals, have had more time to accumulate parasites [7]. Similarly, Moreira et al. observed that the abundance of *M. ecaude* was positively correlated with the length of Brazilian sardinella (*Sardinella brasiliensis*), another clupeid [12]. However, although significant, our results showed a weak positive association between the fish weight and the parasite burden, which agrees with a previous meta-analysis that revealed a weak correlation between the fish length and the intensity of infection of adult digeneans [13].

The relatively higher infection levels in *O. bulleri* could be a reflection of host specificity, i.e., *O. bulleri* is a primary host while *O. libertate* is an auxiliary host [14,15]. Following Combes' filter concept [16], which states that encounter and compatibility filters are responsible for host specificity, we could suppose that the encounter rates between *O. bulleri* and prey (intermediate host of *M. ecaude*) are relatively higher, possibly because the feeding habits of both species of *Opisthonema* are different. While our understanding of this subject is limited, França and Severi pointed out that, despite the phylogenetic proximity of clupeids, there are differences in their ecological interactions that may facilitate their coexistence [17]. Regarding the compatibility filter, we could suppose that the molecular machinery of *M. ecaude* may be more attuned to the exploitation of *O. bulleri* than of *O. libertate*. There is no direct evidence for this hypothesis for *M. ecaude*; however, it is known that trematodes, similar to other parasites, release excretory/secretory products (ESPs) to exploit their hosts [18]. Some ESPs are involved in specific functions, such as feeding or host immune evasion. For example, Williamson et al. observed that parasite-feeding proficiency varied between host species, which depends, to some extent, on the match between the molecular structure of the food source within a host and the molecular structure of the ESP of the parasite [19]. These authors suggested that such molecular

compatibility is a contributing factor in the host specificity of parasites. Thus, we speculate that the feeding proficiency of *M. ecaude* is better in *O. bulleri* than in *O. libertate*.

The high prevalence of *M. ecaude* observed in the present study has also been observed in other clupeids, *Harengula clupeola* and *Sardinella brasiliensis*, from Brazil [12,20,21]. However, *M. ecaude* has not been found in *O. oglinum*. The digeneans reported for *O. oglinum* are *Myosaccium opisthonemae* and *Parahemiurus merus* [21,22]. According to Bray et al., 109 marine fish trematode species are found in both the Atlantic Ocean and the eastern Pacific region, some of which could be cryptic complexes or misidentifications [23]. Given the prevalence of *M. ecaude* in Pacific species of *Opisthonema*, but its absence in the only Atlantic congener, future studies should aim to confirm that the Atlantic and Pacific forms of this parasite are really conspecific.

We conclude that *M. ecaude* is a common parasite of *O. bulleri* and *O. libertate* in the Gulf of Tehuantepec. To better understand the population dynamics of this parasite, future studies should consider the possible effects of seasonality and host ontogeny, as well as comparisons with other geographical areas in the Mexican Pacific.

4. Materials and Methods

Fish samples were obtained from three fishing hauls in the Gulf of Tehuantepec (Figure 4, Table 1), throughout 22–24 April 2022, during a research cruise onboard the R/V “Dr. Jorge Carranza Fraser” of the Instituto Nacional de Pesca y Acuicultura (INAPESCA). The hauls were made with a midwater net, which consists of four equal caps (top and bottom footrope length: 48.17 m). Trawls were towed at an average speed of 6.5 km h^{−1} for 45 min, with an average depth of 20 m. The hauls were made by prior detection using echograms recorded 24 h d^{−1} with a Simrad EK60 scientific echo sounder (Simrad Kongsberg Maritime AS, Horten, Norway) equipped with five split-beam transducers (18, 38, 70, 120, and 200 kHz). Onboard, a total of 121 fish specimens were collected by hand, identified to the genus level, and frozen at −4 °C.

At the laboratory, each fish was thawed, and its fork length (cm), weight (g), and sex were recorded. Fish were identified to the species level following Berry and Barrett [24]. Sagittal otoliths were extracted, cleaned, and stored dry in microtubes. The otoliths were then used for age determination by counting opaque and hyaline bands, as described elsewhere [25]. Body surfaces, cavities, internal organs, and musculature were examined for metazoan parasites with the aid of a stereomicroscope (Motic, Richmond, BC, Canada). All metazoan parasites were counted and preserved in 4% formalin. For morphological identification, platyhelminths were stained with Gomori’s trichrome, dehydrated in a graded ethanol series, cleared in methyl salicylate, and examined as permanent mounts in Canada balsam. Nematodes and crustaceans were cleared in lactic acid. Specimens were examined using a Leica DMLB compound microscope (Leica Microsystems, Wetzlar, Germany). *Myosaccium ecaude* was identified according to the guidelines of León-Règagnon et al. [26].

The prevalence and mean and median intensity of parasitic infection were calculated following Bush et al. and Reiczigel et al. [27,28]. The mean intensity was provided because it is commonly used in quantitative parasitology, while the median intensity is suitable for describing the typical level of infection in a sample [28]. Significant differences in intensity among samples were detected using the Mood’s median test (multiple comparisons) and *a posteriori* bootstrap *t*-tests (pairwise comparisons), using the software QPweb [28].

Fish length, weight, and age were compared between samples using one-way ANOVA. Generalized linear models (GLM) with a Poisson distribution and a logarithmic link function were used to evaluate the effects of fish length, weight, and age on parasitic infection intensity. Individual models were applied for each of the three fish traits since it was assumed that they were correlated, which could lead to high variation and biased results. This type of model was well suited to this study as the response variable comes from the low probability of success count data. The model also has flexibility with data with varying error distributions [29]. GLMs were performed in the R environment within RStudio [30,31].

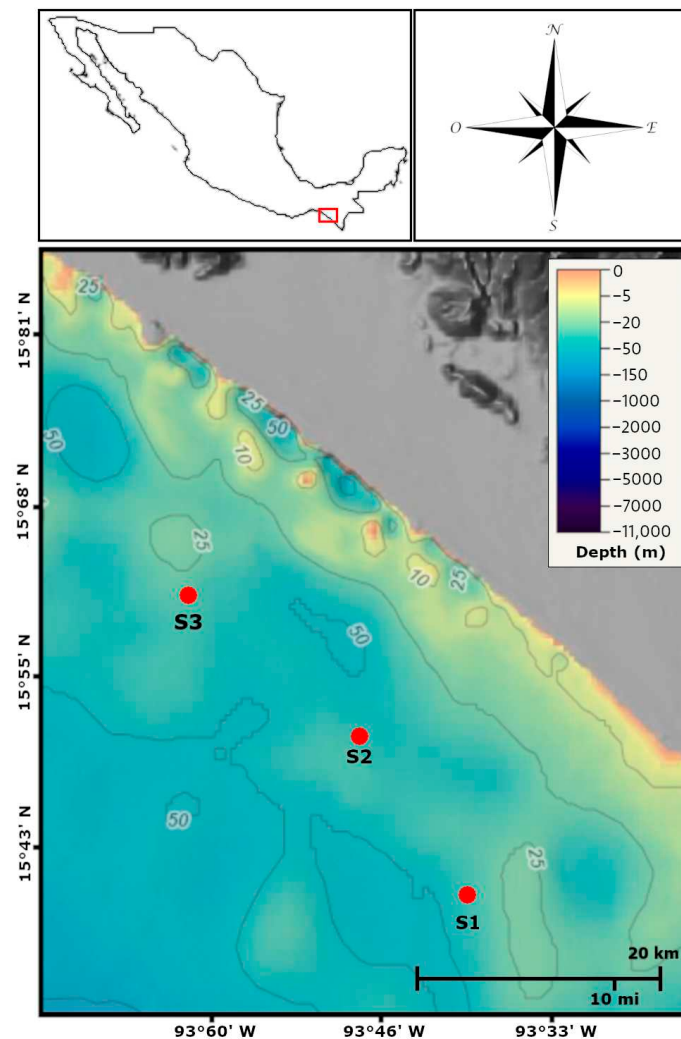


Figure 4. Study area. The red dots indicate the sampling stations (S1, S2, and S3) where samples of *Opisthonema* spp. were collected in the Gulf of Tehuantepec in the Mexican Pacific.

Author Contributions: F.N.M.-S. conceived the study, performed data analysis, wrote the manuscript, and acquired funding. D.G.L.-M., J.M.O.-C. and S.G.-L. performed field and laboratory work. J.P.-A. performed data analysis. J.R.F.V.-Z. and F.A. drafted the article and revised it critically for important intellectual content. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: No ethical approval was required. The study does not include any endangered or protected species. No live animals were caught specifically for this project. Fish examined for parasites were obtained from fishing hauls made during a research cruise focused on fisheries stock assessments. The fishing permit (PPF/DGOPA-004/22) was issued by the National Commission for Fisheries and Aquaculture.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data will be made available on reasonable request.

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