Metazoan Parasites of the Pacific Silverstripe Halfbeak, *Hyporhamphus naos* (Osteichthyes: Hemiramphidae) in Mazatlán Bay, Mexico[1]

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Abstract: The Pacific silverstripe halfbeak, *Hyporhamphus naos* is one of the most commercially important fish species in Mazatlán Bay, north-western Mexico. Irrespective of its relatively small size, it is highly appreciated in the local gastronomy. Every year between April and May adults congregate to spawn in Mazatlán Bay; however, information about their parasite fauna is lacking. This study reports on the metazoan parasite fauna of *H. naos*. Thirty individuals were sampled during the 2022 fishing season. Nine parasite taxa were collected, including five species of ectoparasites, that is, the monogeneans *Axinoides* sp. and *Kuhnia* sp., the copepods *Ergasilus* sp. and *Lepeophtheirus* sp., and the isopod *Mothocya gilli* Bruce, 1986, and four endoparasites, the nematode *Philometra* sp., and three metacercariae including the heterophyid *Opisthometra planicollis* (Rudolphi, 1819), the strigeid *Cardiocephaloides medioconiger* (Dubois and Pérez-Vigueras, 1949), and the cyathocotylid *Mesostephanus microbursa* Caballero, Grocott and Zerecero, 1953. Parasites were identified using morphology, or a combination of morphology and DNA sequences. This study includes new host records for all metazoan parasites collected, except for *Philometra* sp. which has been previously documented infecting the gonads of *H. naos*. *Philometra* sp. was the species with the lowest prevalence (<10%), whereas *O. planicollis* and *C. medioconiger* reached the highest prevalence values with 63.3% and 50%, respectively. We discuss the factors that potentially determine the infection parameters of the metazoan parasites in the area, and the potential zoonotic importance of the cyathocotylid *M. microbursa*.

Keywords: *Hyporhamphus naos*, Sinaloa, helminth seabird parasites

Fishes in the family Hemiramphidae (halfbeaks) are distributed in the Atlantic, Indian, and Pacific oceans (Banford and Collete 2001). Species of the genus *Hyporhamphus* are widely distributed along the Eastern Pacific coast. The Pacific silverstripe halfbeak, *Hyporhamphus naos* is distributed from San Diego, California, USA, to Paita, Peru, and off the Galapagos Islands. This species is usually found in schools at the surface (Banford and Collette 2001). They play an important role in the fisheries of the state of Sinaloa, particularly in the bay area of Mazatlán where they are known as as "pajarito." Every year, between April and May, adult fish congregate to spawn in the warm waters of Mazatlán, providing the

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opportunity for local fisherman to capture and sell these fish locally (Santos-Guzmán and Ramírez-Zavala 2018, Grano-Maldonado and Mendieta-Vega 2019). Despite the importance of the Pacific silverstripe halfbeak, information on their parasitic fauna is scarce; a single study recorded the presence of a philometrid nematode infecting the gonads of female halfbeaks (Rivera-Toscano et al. 2022). The main objective of this study was to report the metazoan parasites of *H. naos* using morphological and molecular characters and to discuss the potential risk for zoonotic diseases given the human consumption of the fish in the area.

MATERIALS AND METHODS

Thirty specimens of the "pajarito," *Hyporhamphus naos* were obtained from commercial capture from April to May 2022 in Mazatlán Bay (23° 14′ 03″ N, 106° 27′ 40″ W) within the Natural Protected Area "Las Islas del Golfo de California." Total length (cm) was recorded for each fish. The parasitological survey included examination under a stereoscopic microscope of the external surface (eyes, fins, skin, and gills) and internal organs (stomach, intestinal caeca, intestine, spleen, gallbladder, liver, kidneys, brain, and heart). Muscle was separated and subsequently examined by compression between two 10 × 10 × 1.0 cm flat glasses to search for encysted parasites. Recovered parasites were relaxed with near-boiling saline and preserved in 96% ethanol for morphological and 100% for molecular analyses. For morphological analyses, platyhelminths were stained with Mayer's paracarmine or Harri's hematoxylin and mounted on permanent slides with Canada balsam; nematodes and crustaceans were cleared with 50% glycerol. Voucher specimens of some helminths and crustaceans were deposited in the Colección Nacional de Helmintos (CNHE), or the Colección Nacional de Crustáceos (CNCR), Instituto de Biología, Universidad Nacional Autónoma de México (México), with the following accession numbers: *Kuhnia* sp., one specimen, CNHE 11885; *Axinoides* sp., one specimen, CNHE 11886; *Philometra* sp., one specimen, CNHE 11887; *Ergasilus* sp., seven specimens, CNCR 37004; *Mothocya gilli*, one specimen, CNCR 37005; *Lepeophtheirus* sp., one specimen, CNCR 37006. Ecological parameters of the infection, that is, prevalence, mean intensity, and mean abundance were estimated following Bush et al. (1997).

Some specimens were processed for molecular analyses, to aid in species identification. Genomic DNA was isolated using DNAzol Reagent (Invitrogen) according to the manufacturer's protocol. The large subunit of the ribosomal DNA was amplified using the primers 391 (5′-AGCGGAGGAAAAGAA ACTAA-3′) and 536 (5′-CAGCTATCCT GAGGGAAAC-3′) (García-Varela and Nadler 2005) for the D1-D3 domains of the 28S. Amplification and sequencing protocols followed those used by Solórzano-García and Pérez-Ponce de León (2017). Sequences were assembled and edited using Geneious v7 (Kearse et al. 2012). Three datasets were constructed separately, one for the superfamily Diplostomoidea, one for Heterophyidae, and one for Philometridae. Each dataset included the newly obtained sequences plus sequences available in GenBank. The best-fit model of molecular evolution for each dataset was calculated with jModel Test version 0.1.1 program (Posada 2008) using the Akaike information criterion (AIC). The phylogenetic analyses were performed using maximum likelihood (ML) and Bayesian inference (BI) methods and the (CIPRES) Science Gateway v3.3 (Miller et al. 2010). The ML was carried out with the RAxML version 7.0.4 (Silvestro and Michalak 2011), using 10,000 bootstrap replicates. BI analyses were inferred with MrBayes version 3.2.7 (Huelsenbeck and Ronquist 2012), including two simultaneous MCMC runs for 10 million generations, sampling every 1,000 generations, a heating parameter value of 0.2, and a "burn-in" of 25%. Trees were drawn using FigTree v.1.3.1 (Rambaut 2012). Genetic divergence between taxa was estimated using uncorrected "p" distances in MEGA v 6 (Tamura et al. 2013).

RESULTS

Of the 30 specimens of *H. naos* examined (total length 19.5 ± 0.9 cm), 576 parasites belonging to nine taxa were collected, namely *Axinoides* sp. and *Kuhnia* sp. (Monogenea), *Opisthometra planicollis*, *Cardiocephaloides medioconiger*, and *Mesostephanus microbursa* (Digenea), *Philometra* sp. (Nematoda), *Ergasilus* sp. and *Lepeophtheirus* sp. (Copepoda), and *Mothocya gilli* (Isopoda) (Figure 1). All individual fish were infected at least with one parasite species. The gills were the most infested organ, with four parasite species (two monogeneans, one trematode, and one isopod).

The BLASTn search of the 28S rRNA gene for the trematode metacercariae indicated 99% identity with *Opisthometra planicollis* (OR482645–OR482647), *Mesostephanus microbursa* (OR482650–OR482655), and *Cardiocephaloides medioconiger* (OR482648–OR482649). Phylogenetic analyses containing members of the families Strigeidae and Cyathocotylidae in one case, and species of the family Heterophyidae in the other case, corroborate the identity of these trematode

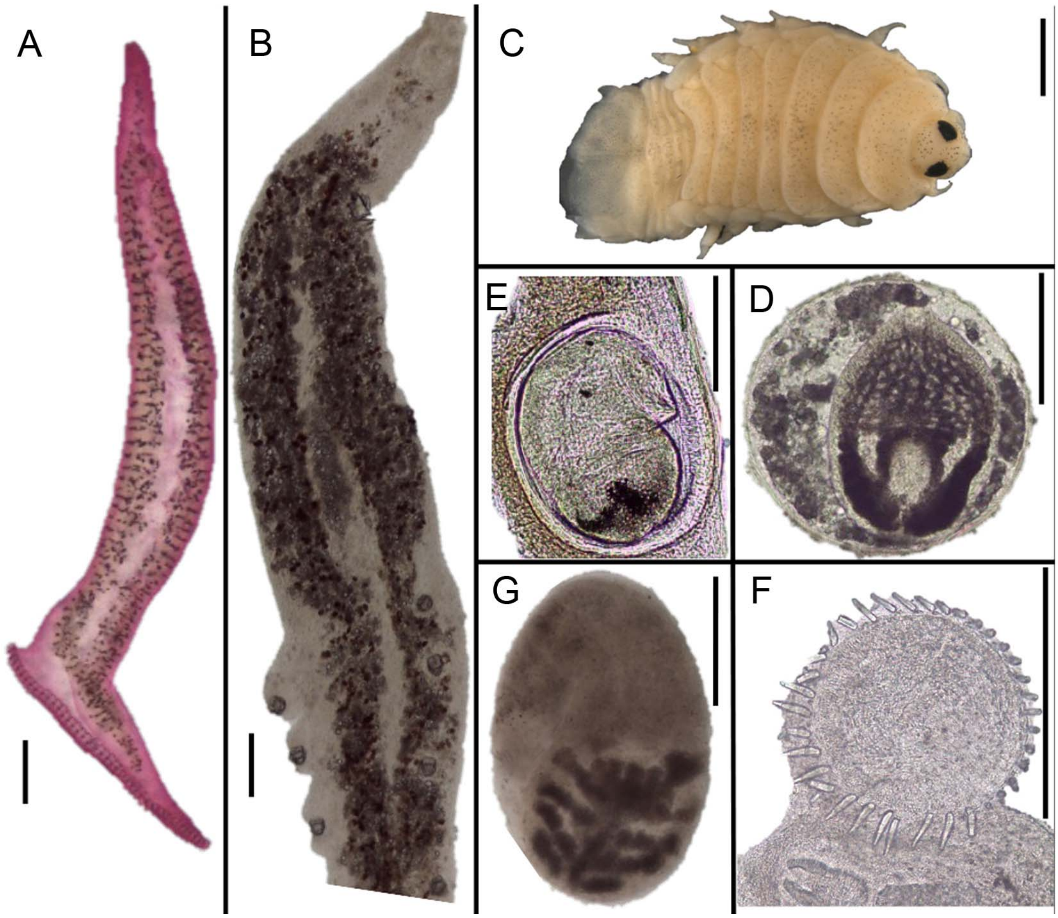

FIGURE 1. Photomicrographs of some representative metazoan parasites of *H. naos* off the coast of Mazatlán Bay. (*A*) *Axinoides* sp.; (*B*) *Kuhnia* sp.; (*C*) *Mothocya gilli* ♀, dorsal view, (*D*) metacercariae of *Mesostephanus microbursa* (encysted in muscle); (*E*) metacercariae of *Opisthometra planicollis* from the gills (encysted); (*F*) anterior end of *O. planicollis* showing the ring of 37 spines around oral sucker; (*G*) metacercariae of *O. planicollis* (free from the cyst). Scale bars, *A*, *B*, and *D*–*F* = 200 µm; *C* = 2 mm.

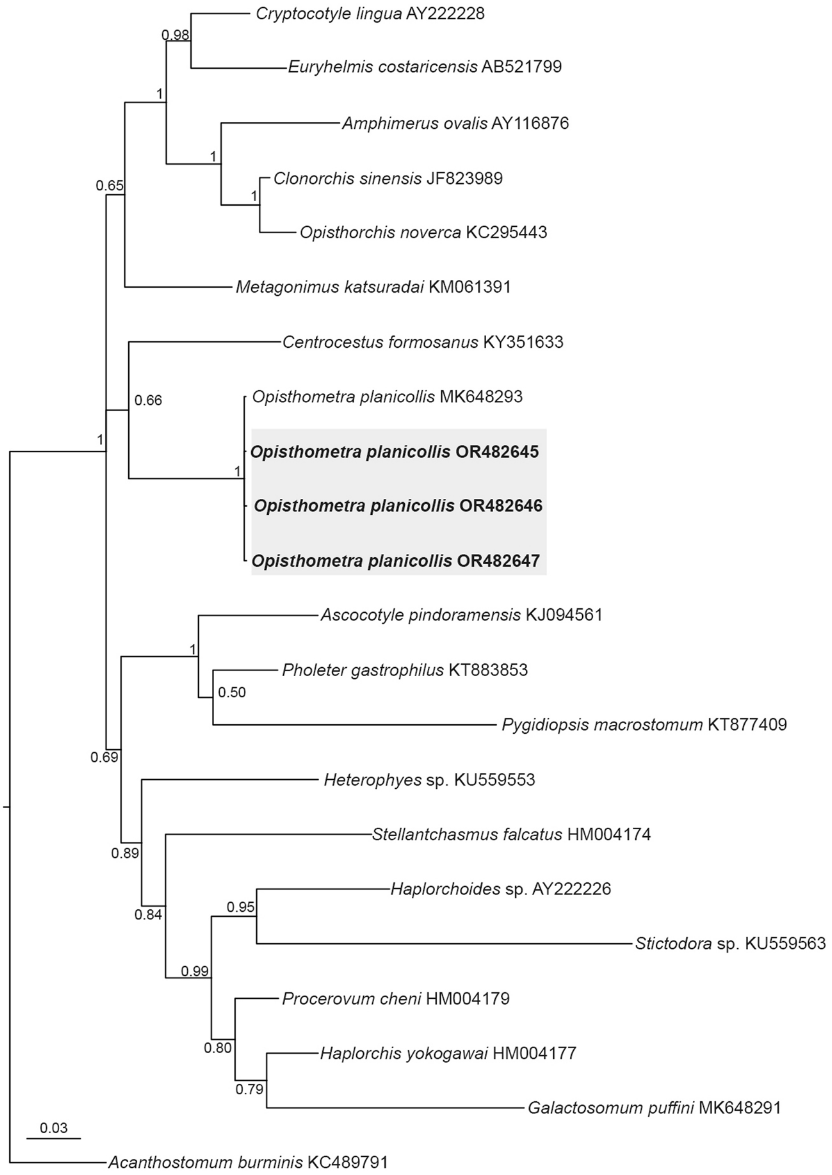

FIGURE 2. Consensus Bayesian inference and maximum likelihood trees inferred with 28S rRNA data set for species of diplostomoid metacercariae recovered from *H. naos* off the coast of Mazatlán Bay. Numbers near nodes indicate posterior probabilities (BI) and ML bootstrap support values.

species (Figures 2 and 3, respectively). Sequences of specimens sampled in this study were nested within each of the clades corresponding to a particular species in the phylogenetic trees, forming reciprocally monophyletic clades. For example, two novel sequences nest with GenBank sequences (MN820664 and MH521247) of *C. medioconiger* and form a highly supported monophyletic clade. The nematode was identified to genus level using 28S rDNA sequences; BLASTn search showed 92% identity with

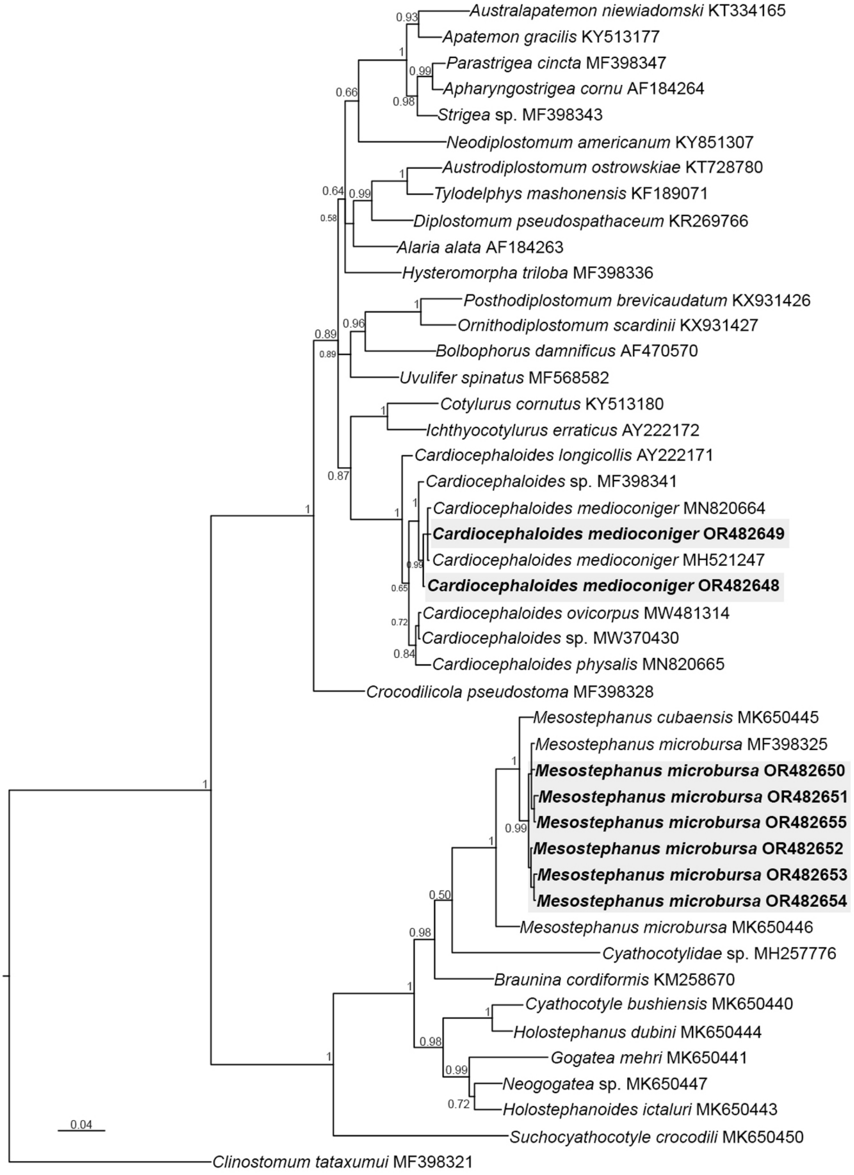

FIGURE 3. Consensus Bayesian inference and maximum likelihood trees inferred with 28S rRNA data set for species of heterophyid metacercariae recovered from *H. naos* off the coast of Mazatlán Bay. Numbers near nodes indicate posterior probabilities (BI) and ML bootstrap support values.

Philometra spp. but given the paraphyletic relationship between *Philometra* Costa, 1845 and *Philometroides* Yamaguti, 1935, it was recorded as *Philometra* sp. (OR482656) (see "Discussion" section) (Figure 4). All the other parasite taxa were solely identified on morphological grounds. Unfortunately, some of the recovered specimens were in poor condition preventing their identification to species level.

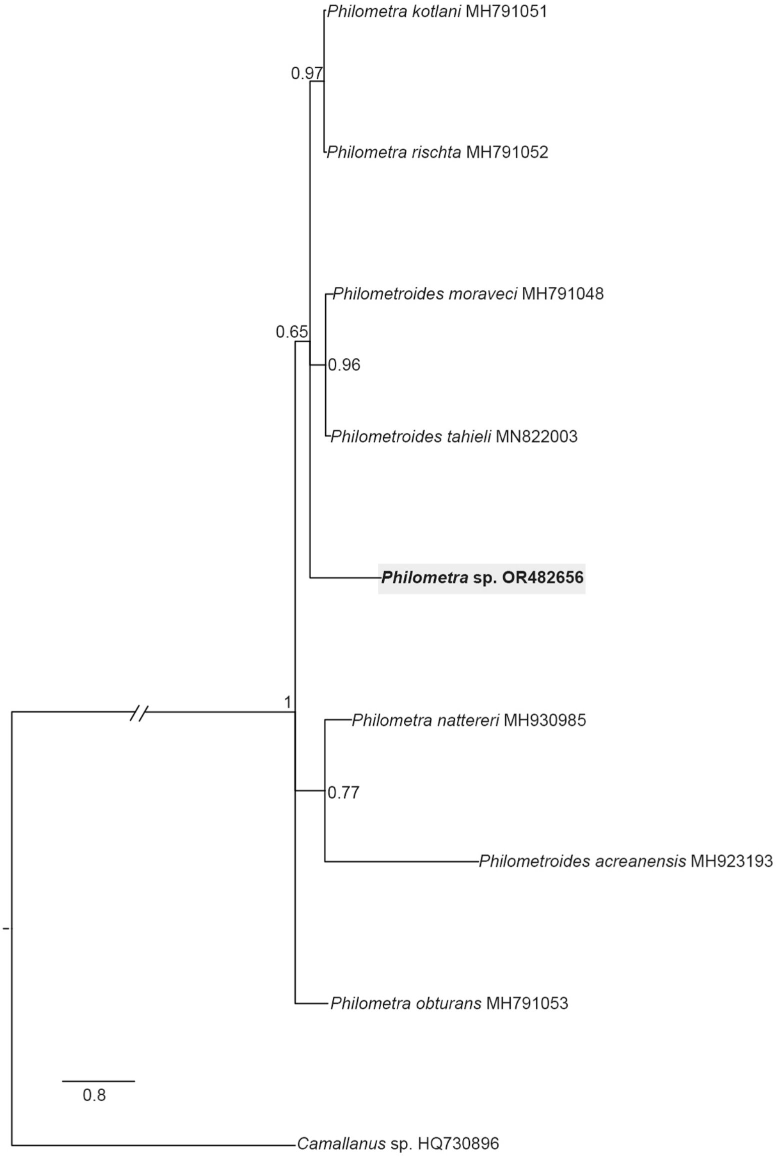

FIGURE 4. Consensus Bayesian inference and maximum likelihood trees inferred with 28S rRNA data set for species of philometrid nematodes recovered from *H. naos* off the coast of Mazatlán Bay. Numbers near nodes indicate posterior probabilities (BI) and ML bootstrap support values.

The parasite community consisted of nine metazoan parasites. Ecological parameters are shown in Table 1. The most prevalent parasites were the metacercariae of three trematode species, that is, *Opisthometra planicollis* (63.3%), *Cardiocephaloides medioconiger* (50%), and *Mesostephanus microbursa* (23.3%) infecting the gills, brain, and muscle, respectively, and the crustacean *Ergasilus* sp. (23.3%) parasitizing the skin of their hosts (Table 1). As a group, crustaceans and trematodes were the most diverse, with three species each. The

TABLE 1

Parasite Taxa Found in *Hyporhamphus naos* in Mazatlán Bay

	Parasites	Family	Site of Infection	NP	HI	%	MI	MA	II
Monogenea	*Axinoides* sp.	Axinidae	Gills	2	1	3.33	2	0.06	–
	Kuhnia sp.	Mazocraeidae	Gills	1	1	3.33	1	0.03	–
Digenea	*Opisthometra planicollis*[a]	Heterophyidae	Gills	140	19	63.33	7.37	4.6	(1–26)
	Cardiocephaloides medioconiger[a]	Strigeidae	Brain	152	15	50.00	10.13	5.06	(1–35)
	Mesostephanus microbursa[a]	Cyathocotylidae	Muscle	253	7	23.33	36.14	8.43	(1–131)
Nematoda	*Philometra* sp.	Philometridae	Gonads	2	2	6.67	1.00	0.06	–
Crustacea	*Ergasilus* sp.	Ergasilidae	Skin	24	7	23.33	3.43	0.8	(1–10)
	Mothocya gilli	Cymothoidae	Gills	1	1	3.33	1	0.03	–
	Lepeophtheirus sp.	Caligidae	Skin	1	1	3.33	1	0.03	–

NP = number of parasites, HI = number of hosts infected, % = prevalence, MI = mean intensity, MA = mean abundance, II = intensity range.

[a] Metacercariae.

most abundant parasite was the metacercariae of *M. microbursa* infecting the muscle of their hosts.

DISCUSSION

Parasites provide valuable information about their hosts and the environment and are considered important components of biological diversity in aquatic ecosystems (Holmes 1990). Despite its importance, parasite diversity is still poorly known in some regions of Mexico, especially fish parasites in the Northwest. This is the first study that describes the parasite community of the Pacific silverstripe halfbeak, *Hyporhamphus naos*.

Mazatlán Bay represents a new locality record for seven of the nine taxa of metazoan parasites (excepting the isopod *Mothocya gilli*, and the nematode *Philometra* sp.), whereas *H. naos* represent a new host record for eight of them (excepting for the nematode *Philometra* sp.). It is noteworthy the absence of intestinal parasites in our sample of *H. naos*.

One of the identified monogeneans belongs to the genus *Kuhnia* for which at least three species have been reported in the Mexican Pacific coast (Pérez-Ponce de León et al. 1999, Mendoza-Garfias et al. 2017). In the present study, we report for the first time the genus *Kuhnia* Sproston, 1945 in a hemiramphid off the Mexican Pacific coast. The second species of monogenean belongs to the genus *Axinoides*. *Axinoides raphidoma* Hargis, 1956 was described from *Tylosurus pacificus* in Chamela Bay, on the Pacific coast of Mexico (Pérez-Ponce de León et al. 1999). Unfortunately, the two sampled specimens in our study were in poor condition to be compared with the specimens from Chamela Bay and determine if they are conspecific.

The three species of trematode metacercariae were identified using molecular characters. Metacercariae from the brain of *H. naos* corresponded with *C. medioconiger* due to high sequence overlap with specimens reported from the Royal Tern, *Thalasseus maximus* from Mississippi, USA (Achatz et al. 2020). Metacercariae encysted in the gills of *H. naos* corresponded with *O. planicollis*. Sequence identity was established with adult specimens sampled from the blue-footed booby, *Sula nebouxii* from Isla Isabel, Mexico (Hernández-Mena et al. 2017). The third metacercariae, sampled from the muscle of *H. naos* was identified as *M. microbursa* since 28S rDNA sequences were almost identical to those reported from adults from migrant gannets *Morus bassanus* off Mississippi, USA (Achatz et al. 2020), and from blue-footed boobies *S. nebouxii* from Isla Isabel, off the Mexican Pacific coast (Hernández-Mena et al. 2017). Interestingly, this trematode species was originally described from the brown pelican, *Pelecanus occidentalis* from the Pacific coast of Panama and from Baja California, Mexico (Caballero et al. 1953); it is possible then to postulate that the definitive host of *M. microbursa* found in the *H. naos* in Mazatlán Bay is either the brown pelican or the blue-footed bobby.

The 28S BLASTn search of sequences of the nematode from the gonads of *H. naos* was first identified as belonging to the genus *Philometroides*. However, the phylogenetic analysis demonstrated that species of this genus are nested in the same clade with species of *Philometra* rendering the genus paraphyletic. This pattern has been previously documented, suggesting uncertainty in the separation of these two genera (Negreiros et al. 2019). Given the taxonomic instability, and that *Philometra* sp. was previously reported from the same host and locality (Rivera-Toscano et al. 2022) as in the present study, we took a conservative position and considered that our specimens correspond to the genus *Philometra*, waiting to obtain more individuals for a detailed morphological and molecular study.

Finally, three species of crustacean ectoparasites were found on *H. naos* and were identified by their morphological characters. We were unable to identify the species of the genus *Lepeophtheirus* von Nordman, 1832 within the copepod family Caligidae since only one specimen was obtained. The second species of copepod was identified as belonging to the genus *Ergasilus* because they possess an antenna with a single claw, reduced maxillae, and maxillipeds absent in females (see Suárez-Morales and Santana-Piñeros 2008 and references therein). The isopod was identified as *Mothocya gilli* based on the size of the organism (ovigerous female 12.0 mm long), and on the presence of dispersed chromatophores over dorsum, antennules, antennae, and pereopods. According to Bruce (1986) and Smit et al. (2014), this species occurs along the Pacific coast of Mexico, from the central Gulf of California to Manzanillo Bay, Colima, and the southern oceanic side of Baja California. *M. gilli* was previously reported in Mazatlán from the gills of *Hyporhamphus gilli* and *H. unifasciatus* along the Eastern Pacific coast (Bruce 1986). However, this is the first report in *H. naos* in this zone of the Mexican Pacific Coast.

Ecological parameters of infection, that is, prevalence and mean intensity are relatively low for most parasite taxa found in *H. naos*. The fact that trematode metacercariae reached their highest levels of prevalence and mean intensities may indicate that the fish is widely consumed by marine birds in the area. The islands within Mazatlán Bay are considered a breeding and migration zone for a large diversity of marine birds (Piña-Ortiz et al. 2017).

The importance of these hosts in the life-cycle of trematodes has been demonstrated in several studies (see Violante-González et al. 2015 and references therein). Even though the intensity of infection and prevalence found in *H. naos* was low, the parasite richness was similar to that observed in other marine and estuarine fishes along the eastern Pacific coast of Mexico (6–24) (Pérez-Ponce de León et al. 2000, Violante-González et al. 2008, 2009, 2016, Villalba-Vasquez et al. 2022). Most of these studies suggested that the factors determining parasite species richness, as in most marine fishes, are related to host feeding habits. The results of our study concur with the pattern for marine fish parasite communities characterized as having overall low species richness, low diversity, and dominance by a single species (see Holmes 1990).

Cyathocotylid trematodes, such as *Mesostephanus* spp. are of medical importance. One species, *M. appendiculatus* (Ciurea, 1916) is a cosmopolitan intestinal fluke considered a potential zoonotic fish-borne parasite transmitted to humans by the consumption of raw or undercooked fish (see El-Bahy et al. 2017 and references therein), and it has been also reported infecting stray cats (El-Azazy et al. 2015). To date, there are no reports of *M. microbursa* infecting humans in Mexico (Rojas-Hernández et al. 2014, Garrido-Olvera et al. 2022). Although *H. naos* is highly appreciated in the local cuisine (Grano-Maldonado and Mendieta-Vega 2019), it is mainly consumed as a deep-oil-fried dish, lowering the risk of accidental human infection

In conclusion, molecular markers such as the 28S ribosomal gene have proven to be very useful for the identification of trematode species. Since larval stages lack the needed morphological diagnostic traits, as they reach the adult form when completing their life cycle in fish-eating birds, molecular markers were used as DNA barcodes for identification, allowing us to establish a link with adults morphologically identified and for which DNA sequences are also available. Furthermore, we report for the first time the metazoan parasite fauna of a fish species of high cultural and economic value, since it is culturally important as a fishery target and diet item for regional fishermen and consumers who annually await for its arrival in the Mazatlán Bay. Nonetheless, the effects of these parasites on host body condition and long-term persistence are still poorly known and warrant further investigations.

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