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Identification of the metazoan parasite fauna of the Yellowfin tuna, *Thunnus albacares* (Bonnaterre, 1788) (Scombridae) from off the coast of Sinaloa, in northwestern Mexico

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Abstract

The yellowfin, *Thunnus albacares* (Bonaterre), is one of the most important resources for commercial fisheries along the Mexican Pacific coast. The parasite fauna of this important marine resource has been documented in different regions across its global distributional range. However, few studies have been conducted on parasites of tuna populations in the Cortezian Marine Ecoregion (CME) in northwestern Mexico, despite the development of the fresh and frozen sushi/sashimi-grade tuna market in recent decades. Our study aimed at identifying the metazoan parasite fauna of *T. albacares* in the CME. Between 2023 and 2024, 17 individuals were studied for parasites before being processed. Parasites were collected and identified using morphological and molecular data. One hundred twenty-five metazoan parasites were collected, representing 10 species, including the monogeneans *Hexostoma thynni* (Delaroche, 1811) Rafinesque, 1815, *Neobenedenia girellae* (Hargis, 1955) Yamaguti, 1963, and *Capsala* sp., the digenean *Hirudinella ahi* (Pallas, 1774) Baird, 1853, the larval cestode *Heterosphyriocephalus tergestinus* (Pintner, 1913) Dallarés, Carrassón and Schaeffner, 2016, larval forms of the nematode *Anisakis typica* (Diesing, 1860) Baylis, 1920, the acanthocephalan *Rhadinorhynchus laterospinosus* Amin, Heckmann, and Ha, 2011, and three species of copepods, *Euryphorus brachypterus* (Gerstaecker, 1853), *Pseudocycnus appendiculatus* Heller, 1865 and *Brachiella thynni* Cuvier, 1830. *Anisakis typica* reached the highest prevalence of infection in our samples, and along with *R. laterospinosus*, it reached the highest mean intensity values. This study reports a species that represents a zoonotic risk (*A. typica*) and contributes to the understanding of the parasite diversity of commercially important fish in the CME.

Introduction

Scombrid fish of the genus *Thunnus* are top predators in pelagic marine ecosystems and support extensive fisheries in tropical and subtropical oceans across the world (Moore *et al.* 2019). Stocks have been reduced due to unregulated harvesting, which has led to governmental agencies to impose stringent quotas for particular species. Meanwhile, aquaculture practices for some species have been developed in certain countries (Lee 1998). The information regarding the diseases of these commercially important fish has been reviewed by Munday *et al.* (2003) and Aiken *et al.* (2007). Populations of *Thunnus* spp. kept in captivity have also been surveyed for parasites in the Mediterranean Sea (Mladineo and Tudor 2004; Nowak *et al.* 2006) and in Australia (Munday *et al.* 2003; Deveney *et al.* 2005; Nowak *et al.* 2006). Information about the parasite fauna of *Thunnus* spp. in Mexico is very scarce; only three studies have reported the presence of some parasite species in these hosts. For instance, Sánchez-Serrano and Cáceres-Martínez (2011) analysed 30 specimens of the Pacific bluefin tuna, *T. orientalis* Temminck and Schlegel, from off the coast of Ensenada, Baja California. Four helminth taxa were reported in that study, including two didymozoid trematodes, one acanthocephalan, and one nematode; however, they were not identified to species level. Aiken *et al.* (2007) reported molecular data for two species of platyhelminths, *Cardicola* sp. and *Capsala* sp., as parasites of *T. orientalis* from Isla Coronado on the northern Mexican Pacific coast. More recently, Román-Reyes *et al.* (2019) reported the presence of the copepod *Pennella filosa* (Linnaeus, 1758) on the skin of *T. albacares* (Bonaterre) in the northwestern coast of Mexico.

The yellowfin tuna, *T. albacares*, is a commercially important fish species that represents a significant source of government revenue in many countries, as it is one of the most heavily harvested fish by weight in the tropical waters of the Pacific and Indian oceans (see Moore *et al.* 2019, and

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references therein). *Tunnus albacares* populations are widely distributed in Mexican waters of both the Atlantic and Pacific coasts. In the coastal waters of Sinaloa, on the Pacific coast, yellowfin tuna is a high-value fish for commercial and recreational fishing. The tuna fleet of Sinaloa lands 77,761 tons of yellowfin tuna annually (SIPESCA 2023). Although several studies have documented the metazoan parasite biodiversity of marine fishes off the coast of Sinaloa (see Grano-Maldonado and Pérez-Ponce de León 2023), information about the parasite fauna of the yellowfin tuna is very scarce. The main objective of this study was to identify the metazoan parasite fauna of *T. albacares* captured off the coast of Sinaloa and landed in Mazatlán Bay for processing, using a combination of morphological and molecular data.

Materials and methods

Sample collection

During the 2023 and 2024 fishing seasons of the tuna fleet along the coast of Sinaloa, a subsample of 17 individuals was analysed for parasites. The coast of Sinaloa is included in the Cortezian Marine Ecoregion (CME) (Spalding *et al.* 2007). Fish were frozen after capture and subsequently landed for processing in Mazatlán. Once in the food processing plant, fish were thawed, the surface was screened for ectoparasites, and the viscera and the gills were separated before filleting. The viscera and the gills were kept in plastic bags and placed on ice. The screening for parasites was conducted at the Facultad de Ciencias del Mar, Autonomous University of Sinaloa. The gills were screened for ectoparasites by a gill wash with 0.85% saline solution prepared with NaCl; the supernatant was removed, and the sediment was poured into a Petri dish for observation under a stereomicroscope (Olympus SZ40). The internal organs (stomach, intestine, liver, spleen, and gonads) were dissected, placed in Petri dishes with 0.85% saline, and observed under the stereomicroscope. As expected, all sampled parasites were dead; the specimens were washed in 0.85% saline and preserved in 96% ethanol for morphological and molecular analyses. For morphological studies, platyhelminths and acanthocephalans were stained with Mayer's paracarmine and mounted on permanent slides with Canada balsam, while 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 in the Colección Nacional de Crustáceos (CNCR), Instituto de Biología, Universidad Nacional Autónoma de México (Mexico), with the accession numbers: CNHE 12101-12104, and the CNCR: 37725-37726. The prevalence and mean intensity of infection were estimated following Bush *et al.* (1997).

Molecular analyses

Some individual helminths were processed for molecular analyses. Genomic DNA was isolated using DNAzol Reagent (Invitrogen) according to the manufacturer's protocol. The D1-D3 domains of the large subunit of the ribosomal DNA (28S) were amplified using the primers 391 (5-AGCGGAGGAAAAGAAACTAA-3) and 536 (5-CAGCTATCCTGAGGGAAAC-3) (García-Varela and Nadler 2005). The Cytochrome c oxidase subunit 1 (COI) was amplified using the primers LCO1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′) and HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′) (Folmer *et al.* 1994). The amplification and sequencing protocols followed those previously described in Grano-Maldonado *et al.* (2024a, b). Sequences were assembled and edited using Geneious v7 (Kearse *et al.* 2012). Sequences of individual parasite taxa were assessed by their percentage identity, conducted through a BLAST search in the NCBI database. Arbitrarily, a sequence identity value ≥99% for the 28S rRNA gene and ≥95% for the COI mitochondrial gene was considered valid in this study to achieve a species-level designation. To further corroborate the species identified molecularly, phylogenetic trees were built to test the position of the newly sequenced individuals in relation to those deposited in the GenBank dataset. Four datasets were constructed separately to assess the family level relationships, three of them for 28S rDNA, i.e., Anisakidae Railliet and Henry, 1912; Sphyriocephalidae Pintner, 1913 and Hirudinellidae Dollfus, 1932; and one for COI mtDNA to asses relationships of Rhadinorhynchidae Lühe, 1912. The phylogenetic analyses were performed using maximum likelihood (ML) on CIPRES Science Gateway v3.3 (Miller *et al.* 2010). The nucleotide substitution models (AIC criterion in JmodelTest2) were the following: *Rhadinorhynchus* GTR+I+G; *Hirudinella* and *Heterosphyriocephalus* GTR+G; *Anisakis* HKY+G. The ML was carried out with the RAxML-HPC2 on ACCESS (8.2.12) (Stamatakis 2014), using 1000 bootstrap replicates. Trees were drawn using FigTree v.1.3.1 (Rambaut 2012).

Results

A total of 17 yellowfin tuna were sampled with an overall range size between 63 and 185 cm; 10 males (Mean TL: 117.3.6 ± 45.1 cm; weight: 44.7 ± 27.5 kg), and 7 females (Mean TL: 120.9 ± 45.1 cm; weight: 46.7 ± 26.0 kg) were sampled. One hundred twenty-five parasites were collected. All hosts were infected with at least one parasite species (1–6 species). Ten parasite taxa were identified, comprising three monogeneans (*Capsala* sp., *Hexostoma thynni*, and *Neobenedenia girellae*), one trematode (*Hirudinella ahi*), one cestode (*Heterosphyriocephalus tergestinus*), one acanthocephalan (*Rhadinorhynchus laterospinosus*), one nematode (*Anisakis typica*), and three copepods (*Euryphorus brachypterus*, *Pseudocycnus appendiculatus*, and *Brachiella thynni*) (Table 1). Parasite identification was accomplished either by using morphological characters solely, as in the case of the three parasitic copepods, *Capsala* sp. (identified up to genus level, see discussion), and *H. thynni*, or by using molecular data. In all cases, BLAST search allowed us to identify the taxa to species level. Five parasite taxa were successfully sequenced. The 28S rDNA gene was targeted for *N. girellae*, *H. ahi*, *H. tergestinus*, and *A. typica* reaching sequence identity values of 99%, 99.6%, 99.6%, and 99.8%, respectively; moreover, the mitochondrial cytochrome oxidase subunit 1 gene (COI) was targeted for *R. laterospinosus*, reaching a sequence identity of 98%. For additional validation, phylogenetic analyses were conducted to confirm interrelationships between the newly sequenced individuals and sequences deposited in GenBank (Figures 1 and 2).

Six of the 10 parasite taxa were recovered as ectoparasites (three monogeneans and three copepods). The gills were the infection site with the highest parasite species richness since one species of monogenean and two species of copepods were collected. Only two of the parasite taxa were found as larval stages, including the third-stage larvae of the nematode *A. typica* and the plerocercoid of *H. tergestinus*; all the other parasites were adult forms. The capsalid monogenean was the only taxa not identified to species level (Table 1). The larval forms of *A. typica* reached the highest prevalence of infection (76.5%); these larval forms and the acanthocephalan *R. laterospinosus* reached the highest mean intensity values with 3.4 and 3.8 parasites per infected host, respectively.

Table 1. Metazoan parasites of the yellowfin tuna, *Thunnus albacares*, off the coast of Sinaloa, northwestern Mexico

Parasite group	Parasite species	Infection site	Total	Prevalence (%)	Mean Intensity
MONOGENEA					
Hexostomidae	*Hexostoma thynni* (Delaroche, 1811) Rafinesque, 1815	Gills	3	11.8	1.5
Capsalidae	*Capsala* sp.	Skin	8	17.6	2.7
Capsalidae	*Neobenedenia girellae* (Hargis, 1955) Yamaguti, 1963	Skin	2	11.8	1.0
DIGENEA					
Hirudinellidae	*Hirudinella ahi* Yamaguti, 1970	Stomach	9	29.4	1.8
CESTODA					
Sphyriocephalidae	*Heterosphyriocephalus tergestinus* (Pintner, 1913) Dallarés, Carrassón, and Schaeffner, 2016	Stomach	3	11.8	1.5
ACANTOCEPHALA					
Rhadinorhynchidae	*Rhadinorhynchus laterospinosus* Amin, Heckmann, and Ha, 2011	Intestine	19	29.4	3.8
NEMATODA					
Anisakidae	*Anisakis typica* (Diesing, 1860) Baylis, 1920	Intestine, body cavity, muscle	44	76.5	3.4
COPEPODA					
Caligidae	*Euryphorus brachypterus* (Gerstaecker, 1853)	Gills	2	5.8	2.0
Pseudocycnidae	*Pseudocycnus appendiculatus* Heller, 1865	Gills	24	47.1	3.0
Lernaeopodidae	*Brachiella thynni* Cuvier, 1830	Pectoral fin	11	41.2	1.6

Discussion

This study contributes novel information about the parasite fauna of marine fish on the northwestern Pacific coast of Mexico; only one parasite taxon had been previously reported as a parasite of the yellowfin tuna, the copepod *Penella filosa* L. (Román-Reyes *et al.* 2019). All the metazoan parasite species recovered from the yellowfin tuna in this survey represent new geographical records, since they are reported for the first time as parasites of this commercially important fish in waters off the coast of Sinaloa in the Cortezian Marine Ecoregion. In addition, four species of metazoan parasites represent new host records, as they are reported for the first time as parasites of *T. albacares*, namely the monogenean *N. girellae*, the cestode *H. tergestinus*, the acanthocephalan *R. laterospinosus*, and the copepod *B. thynni*. Supplementary Table S1 lists the species of metazoan parasites of *T. albacares* across its global distributional range. The list, compiled from at least 35 bibliographical sources, includes 57 species (15 monogeneans, 28 digeneans, 2 cestodes, 2 acanthocephalans, 2 nematodes, and 8 copepods). Interestingly, most papers are isolated reports about the presence of a particular species or group of species of *T. albacares* in a specific area (e.g., Calhoun *et al.* 2013; Kohn *et al.* 2004; Purivirojkul *et al.* 2011). Few studies have reported the entire parasite fauna of *T. albacares* in a particular area (see Bane 1969; Fernandes *et al.* 2002; Aiken *et al.* 2007), and some records are presented while describing the lesions caused by particular parasite species on the yellowfin tuna (e.g., Justo *et al.* 2008, 2009; Bullard *et al.* 2015). Although the sample size in our study is relatively small, 10 parasite species were identified, and four of them were added as new host records to the host-parasite list of this commercially important fish species, which indicates that more studies are necessary to gather a complete list of the parasites that may infect this host species across its distributional range.

Species identity using morphology and DNA

The identification of five of the parasite taxa was accomplished using DNA sequences (*N. girellae*, *H. ahi*, *H. tergestinus, A. typica*, and *R. laterospinosus*). The first four species reached a percentage identity through the BLAST search higher than 99% for the 28S rRNA gene, whereas for the acanthocephalan, the percentage identity for COI was 98%. The remaining five species were identified solely on morphological grounds; only one of them was not identified to species level, the monogenean *Capsala* sp. Species of *Capsala* are characteristically large monogeneans and common ectoparasites of marine fish (Bullard *et al.* 2015); they are clearly distinguished from the other capsalid reported in this study, *N. girellae*, by having a septate haptor. The genus *Capsala* currently comprises 25 species, according to WoRMS (2025) (accessed at https://www.marinespecies.org/aphia.php?p=taxdetails&id=119263 on 5 June 2025). Of these species, three have been reported parasitizing marine fishes in the Mexican Pacific coast, i.e., *Capsala laevis* (Verril, 1857), and *C. pricei* as parasites of the striped marlin, *Kajikia audax*, and *Capsala caballeroi* Winter, 1955 parasitising the scombrid *Sarda orientalis* (see Mendoza-Garfias *et al.* 2017 and references therein). Particularly in tuna fish, Aiken *et al.* (2007) reported *Capsala* sp. as a parasite of *T. orientalis* in Isla Coronado, off the coast of Baja California, in the northeastern Pacific coast of Mexico. Bullard *et al.* (2015) reported *Capsala biparasiticum* (Goto, 1894) from the buccal cavity of the yellowfin tuna, *T. albacares*; however, the record was made in tuna captured in the Gulf of Mexico, on the Atlantic coast. Unfortunately, very few individuals of *Capsala* were sampled, and they were in poor condition, making it difficult to identify them and confirm whether or not the specimens corresponded to any of the previously reported *Capsala* species parasitising marine fishes from the Mexican Pacific.

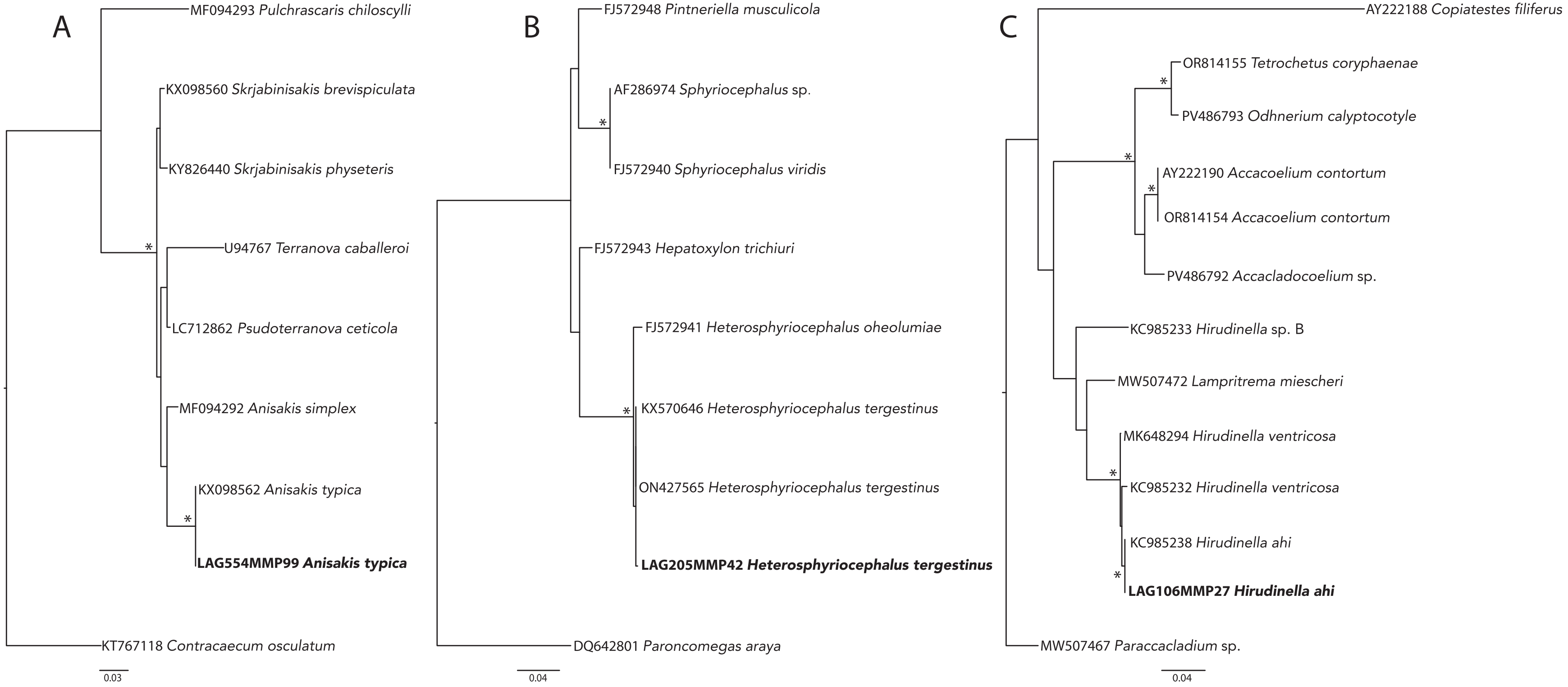

Figure 1. Maximum Likelihood trees inferred with 28S rDNA showing the phylogenetic position of parasites of *T. albacares* from off the coast of Sinaloa. A) *Anisakis typica*; B) *Heterosphyriocephalus tergestinus*; and C) *Hirudinella ahi*. Asterisks in nodes indicate bootstrap support values higher than 80

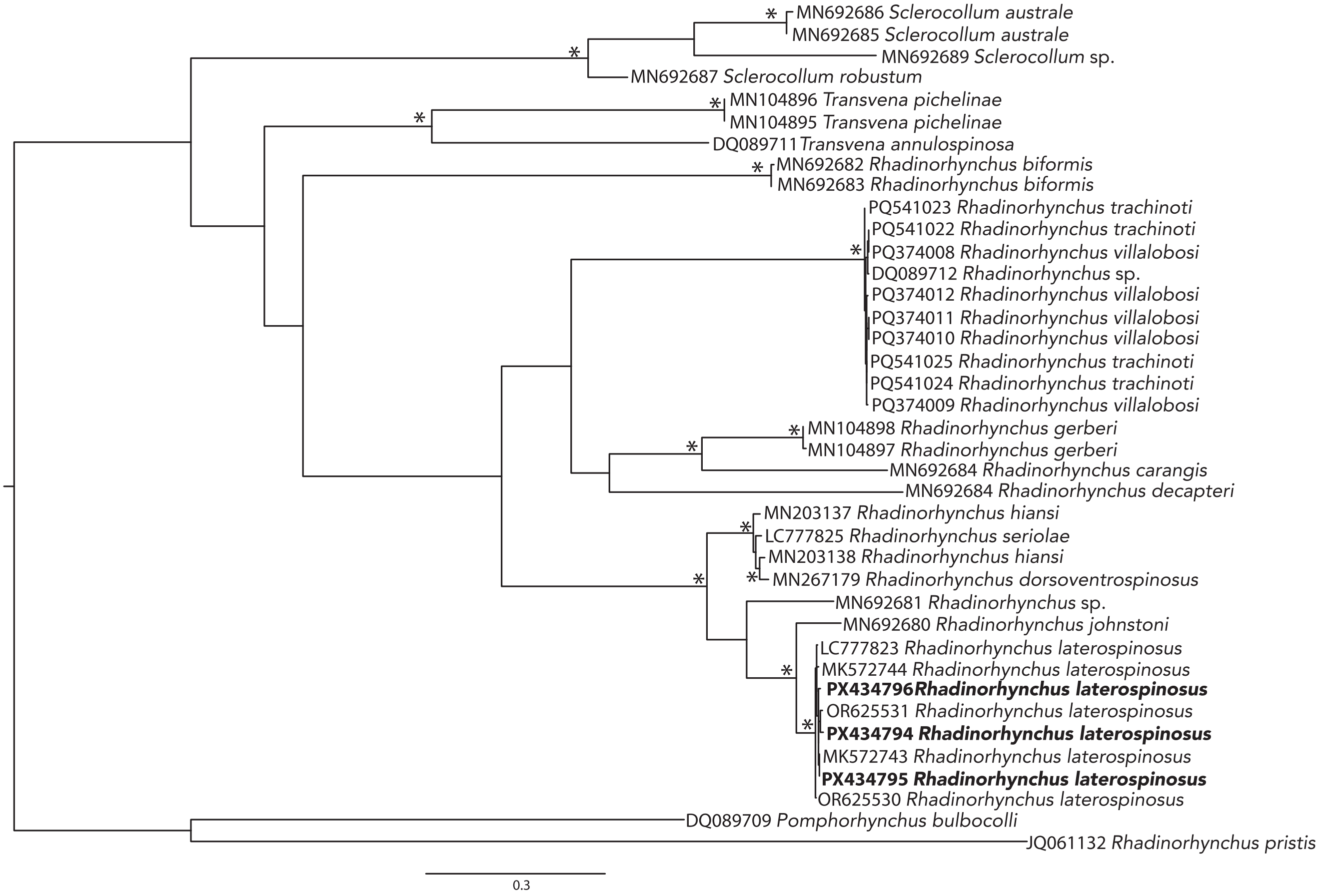

Figure 2. Maximum Likelihood trees inferred with COI mtDNA showing the phylogenetic position of *Rhadinorhynchus laterospinosus* from *T. albacares* from off the coast of Sinaloa. Asterisks in nodes indicate bootstrap support values higher than 80.

Most of the parasite taxa found in *T. albacares* in this study were adults. Only two of them were larval forms. One of them was the third-stage larvae of *A. typica*. This nematode, like many other anisakids, completes its life cycle in marine mammals (Mattiucci *et al.* 2017; Shamsi *et al.* 2017; Mostafa *et al.* 2023). An important aspect of *A. typica*, which is found in the yellowfin tuna, is its zoonotic potential. Considering that fresh yellowfin tuna is preferably consumed raw in the form of sushi or sashimi, the presence of anisakids may raise food safety concerns. The presence of anisakids such as *Anisakis simplex* and *A. pegreffi* has been reported in bluefin tuna (*Thunnus thynnus*) in the Mediterranean and in the North East Atlantic (Mladineo and Poljak 2014; Bao *et al.* 2025), as well as *A. pegreffi* and *A. typica* in bluefin tuna caught off Brazil (Mattiucci *et al.* 2002). Bao *et al.* (2025) suggested that the detection of larvae in the caeca and intestines of the bluefin tuna populations they studied may indicate that there is no need to continue investigating the potential food safety concerns associated with raw tuna consumption because anisakis larvae could not be found in the muscle. However, in this study, two of the 44 larvae of *A. typica* were collected from the muscle, although it is acknowledged that the mean intensity of infection of anisakids larvae in the yellowfin tuna is very low, and the possibility that the larvae migrated to the flesh post-mortem cannot be ruled out.

The second larval form obtained in this study was the plerocercoid of the cestode *H. tergestinus*, which completes its life cycle in elasmobranchs (Dallarés *et al.* 2017). This indicates that a shark should feed upon the yellowfin tuna for the cestode to complete its life cycle. It is not clear if yellowfin tuna represent a dead-end host for the cestode, since the trophic spectrum of thresher sharks such as *A. pelagicus* is narrow and includes mostly cephalopods and teleosts as hakes and they are considered specialist predators (Calle-Morán and Galván-Magaña 2020). The tapeworm was originally described (as *Sphyriocephalus tergestinus*) from the Thresher shark, *Alopias vulpinus* (Bonnaterre), in the Mediterranean Sea (Dallarés *et al.* 2017). In the same paper, the authors provided evidence to re-allocate the species in the genus *Heterosphyriocephalus* Palm, 2004; additionally, they described a new species of the genus, *H. encarnae* Dallarés, Carrassón and Schaeffner, 2017, from the stomach of *Alopias pelagicus* Nakamura, collected in the Gulf of California, off Boca del Alamo, on the Pacific coast of Mexico. The new species was easily separated from other congeners mainly by its small size, small number of proglottids, a long velum with an irregular and folded margin, and the absence of a pars post-bulbosa (Dallarés *et al.* 2017). Unfortunately, DNA sequences for the new species were not provided, although the wide geographical distribution range of the plerocercoid of *H. tergestinus* was reported; the report included their presence in teleost fishes from localities of the Mediterranean Sea, the North Atlantic, and the Indian Pacific Oceans, and also that from the western coastline of Mexico by Dollfus (1967). Based on geographical distribution, it is expected that the specimens sampled in this study correspond to *H. encarnae* since the yellowfin tuna were captured off the coast of Sinaloa, which is very close to the type locality of *H. encarnae*, within the

CME. The lack of sequence data for *H. encarnae* precludes at the moment, testing the hypothesis that the specimens from this study may correspond to that species. Still, the sequence identity value obtained for these samples was 99.6% similar to that of *H. tergestinus*, clearly indicating that they belong to the same species.

This study further corroborates the usefulness of incorporating DNA sequence data into the taxonomic identification of fish parasites, as succinctly demonstrated by Aiken *et al.* (2007), while providing molecular evidence for the cosmopolitan distribution of platyhelminth parasites of tunas (*Thunnus* spp.) worldwide. The genetic library of parasite species affecting economically important fish is increasing steadily, generating baseline genetic data for studies like the one presented herein. This is the first study aimed at describing the parasite fauna of yellowfin tuna in the CME. The results of this study provide a key basis for identifying parasites in the northwest Mexican Pacific. They are complementary to the list of parasite species that infect yellowfin tuna worldwide (see Supplementary Table S1). This information is also essential for the development of aquacultural practices on this fish species. Still, more studies are necessary to fully understand the parasite fauna of fish populations of commercially important fish in the wild, especially those of pelagic species such as *T. albacares.* Generally, it is assumed that the parasite fauna of these fish is well understood. However, new parasite species, host records, and geographical records are published regularly across all continents. Moreover, this type of study may also provide data on species of parasites with potential to be used as biological tags in stock identification (MacKenzie and Hemmingsen 2015; Irigoitia *et al.* 2017), and studies on host migrations (Binning *et al.* 2022). Precise taxonomic identification of parasites is fundamental to address questions in these areas.

Supplementary material. The supplementary material for this article can be found at http://doi.org/10.1017/S0022149X25100795.

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Competing interests. The authors declare no competing interests.

Data availability. All study data are included in the article and in the Supplementary material. DNA sequence data was deposited in GenBank, and voucher specimens were deposited at the National Collections of Helminths and Crustaceans.

Study permits. Not applicable.

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