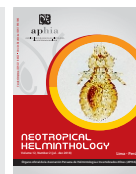




## Neotropical Helminthology



ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

### INTESTINAL HELMINTH PARASITES OF BLACKTIP SHARK *CARCHARHINUS LIMBATUS* (MÜLLER & HENLE, 1839) (CARCHARHINIFORMES: CARCHARHINIDAE), IN CHACHALACAS BEACH, VERACRUZ, MÉXICO

### HELMINTOS PARÁSITOS INTESTINALES DEL TIBURÓN PUNTAS NEGRAS *CARCHARHINUS LIMBATUS* (MÜLLER & HENLE, 1839) (CARCHARHINIFORMES: CARCHARHINIDAE), EN PLAYA CHACHALACAS, VERACRUZ, MÉXICO

Miguel Angel Dorantes-González<sup>1</sup> & Oscar Méndez<sup>2\*</sup>

<sup>1</sup>Centro Interdisciplinario de Ciencias Marinas, Instituto Politécnico Nacional. Av. IPN S/N Col. Playa Palo de Santa Rita CP 23096, La Paz, Baja California Sur, México

<sup>2</sup>Laboratorio de Hidrobiología, Facultad de Biología, Campus Xalapa, Universidad Veracruzana. Circuito Gonzalo Aguirre Beltrán S/N Zona Universitaria CP 91090 Xalapa, Veracruz, México

\*Corresponding author: [spiroxys@hotmail.com](mailto:spiroxys@hotmail.com)

## ABSTRACT

Thirty-nine spiral valves of blacktip shark *Carcharhinus limbatus* (Müller & Henle, 1839) caught by artisanal fisheries in Chachalacas Beach, Veracruz were examined for helminth parasites. A total of 7029 helminth was found, of which 99% were cestodes and 1% was a nematode. We recorded 20 species of cestodes and one nematode. The cestodes *Paraorygmatobothrium* cf *sinuspersicense* (Malek, Caira & Haseli, 2010) and *Nybelinia* sp. 1 (Poche, 1926), registered the mayor values of prevalence and mean intensity of infection. *C. limbatus* is recorded as new host for *P.* cf *sinuspersicense*; *Anthobothrium laciniatum* (Linton, 1890); *Rhinebothrium* sp. (Linton, 1889); *Thysanocephalum thysanocephalum* (Linton, 1899); *Cathetocephalus thatcheri* (Daily & Overstreet, 1973); *Oncomegas* cf *wageneri* (Palm, 1995); *Disculiceps pileatus* (Linton, 1891); *Poecilancistrum caryophyllum* (Diesing, 1850) and *Hysterotylacium* sp. Ward & Magath, 1917. All helminths found in blacktip shark *C. limbatus* represent new records for the coastal zone of the state of Veracruz, Mexico.

**Keywords:** cestodes - Gulf of Mexico - nematodes - parasites - sharks - spiral valves

## RESUMEN

Se examinaron 39 válvulas espirales de tiburón puntas negras *Carcharhinus limbatus* (Müller & Henle, 1839) capturados por la pesca artesanal en la localidad de Playa Chachalacas, Veracruz. Se encontró un total de 7029 helmintos, de los cuales el 99% pertenecieron a los cestodos y 1% a los nematodos. Cada helminto colectado fue procesado mediante las técnicas específicas para cada grupo. Registramos 21 especies de helmintos, de las cuales 20 fueron céstodos y una de nemátodo. Los céstodos *Paraorygmatobothrium* cf *sinuspersicense* (Malek, Caira & Haseli, 2010) y *Nybelinia* sp. 1 (Poche, 1926), presentaron los mayores valores de prevalencia e intensidad promedio de infección. Se registra a *C. limbatus* como nuevo hospedero para *P.* cf *sinuspersicense*; *Anthobothrium laciniatum* (Linton, 1890); *Rhinebothrium* sp. (Linton, 1889); *Thysanocephalum thysanocephalum* (Linton, 1899); *Cathetocephalus thatcheri* (Daily & Overstreet, 1973); *Oncomegas* cf *wagneri* (Palm, 1995); *Disculiceps pileatus* (Linton, 1891); *Poecilancistrum caryophyllum* (Diesing, 1850) y *Hysterotylacium* sp. Ward & Magath, 1917. Todos los helmintos encontrados en el tiburón puntas negras *C. limbatus* representan nuevos registros para la zona costera del estado de Veracruz, México.

**Palabras clave:** cestodos - Golfo de México - nematodos - parásitos - tiburones - válvulas espirales

## INTRODUCTION

The Blacktip shark *Carcharhinus limbatus* (Müller & Henle, 1839) is one of the most valuable species for the artisanal fishery of the Gulf of Mexico (Tovar-Ávila, 1995; Castillo-Géniz *et al.*, 1998; Tovar-Ávila *et al.*, 2009). The sharks act as final host of a wide range of intestinal parasitic helminths (Vankara *et al.*, 2007; Haseli *et al.*, 2010; Palm, 2011), mainly cestodes (Caira & Healy, 2004; Vankara *et al.*, 2007; Randhawa & Poulin, 2010; Palm, 2011). Multiple taxonomic studies about helminths of sharks have been made of all around the world (Caira *et al.*, 1999) covering large regions, however, the pattern of distribution of the parasites may vary within the host with a wide distribution. Thus, the presence of certain parasites in cosmopolitan sharks may be common in multiple areas or be restricted locally. In Mexico, the knowledge about the helminths infecting this fishing resource is scarce (Méndez & Galván-Magaña, 2016; Merlo-Serna & García-Prieto, 2016), therefore, the objective of this research is to describe the helminth fauna of the spiral valve of blacktip shark *C. limbatus* in the locality of Chachalacas, Veracruz, México, contributing to the knowledge of the diversity of parasitic helminths of sharks in our country.

## MATERIAL AND METHODS

Thirty-nine spiral valves of blacktip shark *C. limbatus* was collected by the artisanal fisheries in Chachalacas Beach (19°25'03" N y 96°19'29"O), Veracruz, México, at a distance of 20 km from the coast, between January 2012 and November 2014. Data of total length, sex, date and place of capture were taken from each host. The spiral valves were obtained by the fishermen according to their routine. Each intestine was sealed with rubber bands at each end, placed in plastic bags with formaldehyde 10% and tagged with the data of each shark (number of host, scientific name, date and locality). In the laboratory, each spiral valve was washed with normal water to remove the excess of formaldehyde. These was cut longitudinally and extended in a dissection tray. Spiral valve cuts of approximately 10 cm<sup>2</sup> were made to facilitate the search for helminths. The collect of helminths was made under a stereoscopic microscope. Each helminth was counted and transferred to vial jar with 70% ethanol. The nematodes were placed in glycerin vials at concentrations of 50%, 75% and 100% for their transparency. For the taxonomic determination, 10 specimens of each species were dehydrated in ethanol 70%, 96% and 100%, stained with Mayer carmine, clarified with methyl salicylate and permanent preparations were made with Canada

balsam. The nematodes once cleared and identified were returned to the vials glycerin vials. The identification of the helminths was done to the largest possible taxon using specialized literature for cestodes (Khalil *et al.*, 1994; Palm, 2004; Ruhnke *et al.*, 2006; Ruhnke & Caira, 2009) and nematodes (Anderson, 2000). Some samples were prepared for examination with scanning electron microscopy (SEM). For scanning electron microscopy (SEM) studies, the specimens were washed for 30 min with sodium cacodylate buffer 0.1 M, and are post - fixed with osmium tetroxide 1% for 30 min; Subsequently the samples were washed again for 30 min in sodium cacodylate and then were dehydrated in ethanol gradients from 30% to 100% for 10 min in every step, at the end were dried in a critical point dryer. The sample was subsequently recovered with a film of typically one evaporator in high vacuum (sputter/coater Polaron SC7640). Some micrographs were obtained using a Philips XL-30 environmental SEM. The specimens were deposited in the Colección Nacional de Helminthos (CNHE) from Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), CNHE: 9353–9356. Prevalence and mean intensity ( $\pm$  standard deviation) following Bush *et al.* (1997) were used to describe the infection of each cestode species.

## RESULTS

A total of 7029 individuals from 21 species (Table 1) of helminths were collected. The Cestoda class was the most representative with 20 species and the Phylum Nematoda with one species. The intestinal cestodes of *C. limbatus* are mainly of species belonging to the orders Trypanorhyncha, Tetraphyllidea and Onchoproteocephalidea.

All the host were parasitized by at least two species of helminths. Only three sharks (10%) recorded the highest number of species (12 species) and four sharks (13%) the lowest number of species (two species). The mean number of helminths by shark, regardless of species, for the 39 hosts was  $180 \pm 225$  individuals, with a range of 3 - 1177 helminths. Of the 7029 helminths found, 64% correspond to *Paraorygmatobothrium cf sinuspersicense* (Malek, Caira & Haseli, 2010), followed by *Nybelinia sp.1* (Poche, 1926) (12%) and

*Paraorygmatobothrium sp.1* (Ruhnke, 1994) (6%) (Table 1).

The values of prevalence and mean intensity are shown in Table 1; where the species of cestodes with higher prevalence and mean intensity were *Paraorygmatobothrium cf sinuspersicense* and *Nybelinia sp.1*. (Fig. 1A). The remaining species showed low prevalence values, parasitizing less than 50% of the hosts, similarly their abundances and mean infection intensity were low (Table 1).

All the helminths collected are new records for the locality and the cestodes *Oncomegas cf wagneri* (Palm, 1995); *Paraorygmatobothrium cf sinuspersicense* (Fig. 1D); *Anthobothrium laciniatum* (Linton, 1890); *Rhinebothrium sp.* (Linton, 1889); *Thysanocephalum thysanocephalum* (Linton, 1899); *Cathetocephalus thatcheri* (Daily & Overstreet, 1973) (Fig. 1G); *Poecilancistrum caryophyllum* (Diesing, 1850) (Fig. 1C) *Disculiceps pileatus* (Linton, 1891) (Fig. 1F), as well as the nematode *Hysterothylacium sp.* Ward & Magath, 1917, are new records for *C. limbatus*.

## DISCUSSION

This is the first study on intestinal helminths of the blacktip sharks *C. limbatus* in Mexican waters of the Gulf of Mexico, which consists of 21 species: 20 cestodes and 1 nematode. This high composition of cestode species shows that *C. limbatus* acts as the final host of a wide variety of cestodes, indicating the presence of multiple prey that act as intermediary hosts and that are part of the diet of *C. limbatus*. Owens (2008) recorded 15 species of cestodes in 20 black tip sharks, *C. limbatus* on the shores of Mississippi and Florida in the Gulf of Mexico, while Méndez & Dorantes-González (2013) recorded eight species of tapeworms in bull shark *Carcharhinus leucas* (Müller & Henle, 1839) in the Gulf of Mexico, confirming the richness of helminths as predators.

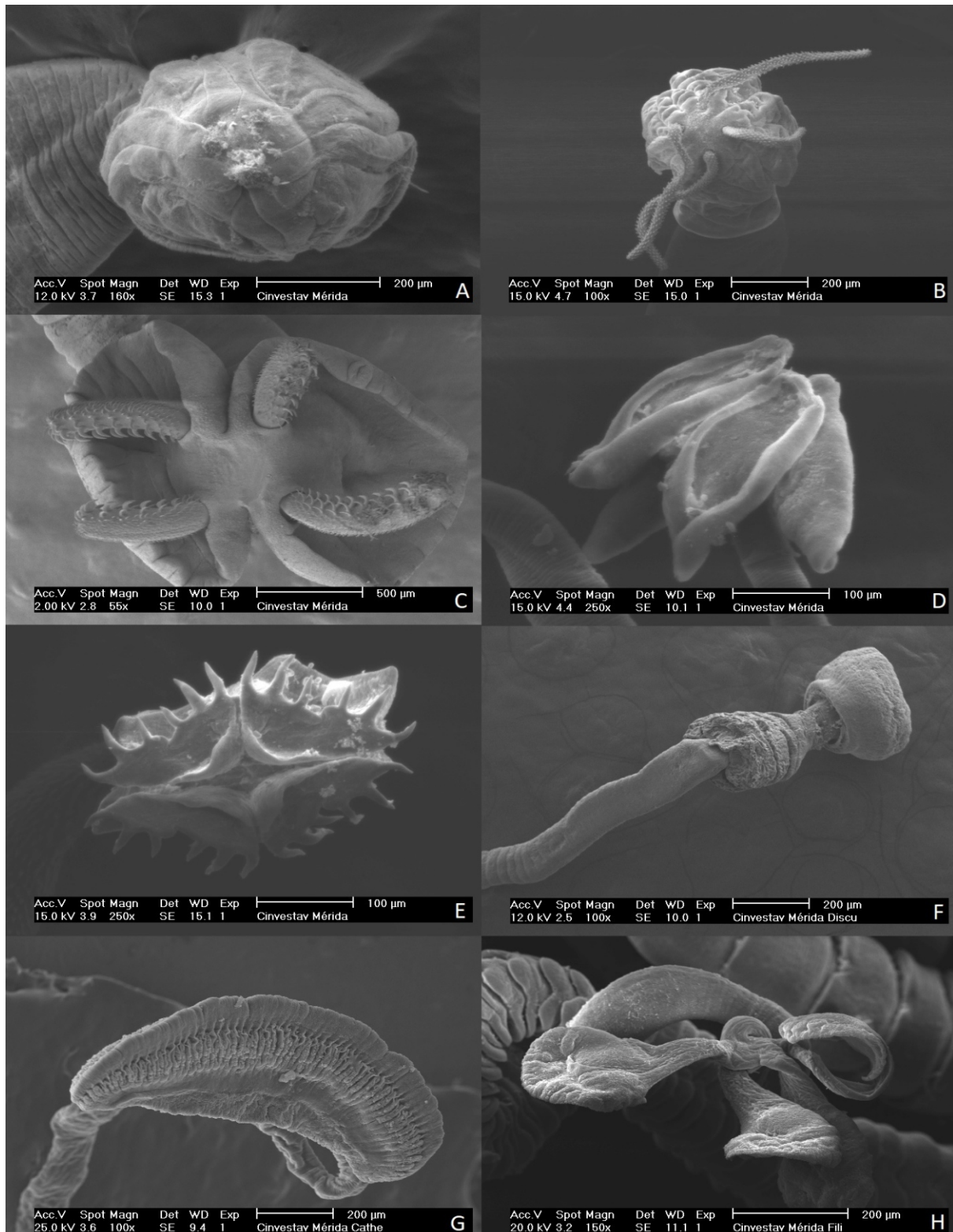
The blacktip shark *C. limbatus* feeds on 99% teleost fishes (de Silva *et al.* 2001; Barry, 2002), that act as intermediate host of the four orders of registered cestodes. The genera *Paraorygmatobothrium* and *Anthobothrium*

**Table 1.** Helminth parasites collected in 39 spiral valves of *Carcharhinus limbatus* in Playa Chachalacas, Veracruz, México. The acronyms were the following: N = number of individuals; SD = standard deviation.

Helminths	N	Prevalence (%)	Mean Intensity ± SD	Range
<b>Order Tetrathyridida Carus, 1863</b>				
<i>Paraorygmatobothrium</i> cf. <i>simuspersicense</i> (Malek, Caira & Haseli, 2010)	4516	89.74	129.03 ± 187.71	...
<i>Paraorygmatobothrium</i> sp. (Ruhnke, 1994)	405	43.59	23.82 ± 44.09	1–187
<i>Anthobothrium laciniatum</i> (Linton, 1890)	45	28.21	4.09 ± 7.33	1–26
<i>Anthobothrium</i> sp. 1 (Van Beneden, 1850)	96	38.46	6.4 ± 7.52	1–25
<i>Anthobothrium</i> sp. 2 (Van Beneden, 1850)	17	2.56	17	17
<i>Rhinebothrium</i> sp. (Linton, 1889)	2	5.13	1	1–1
<i>Thysanocephalum thysanocephalum</i> (Linton, 1899)	2	2.56	2	2
<b>Order Cathetocephalida Schmidt &amp; Beveridge, 1990</b>				
<i>Cathetocephalus thatcheri</i> (Daily & Overstreet, 1973)	36	7.69	12 ± 12.17	4–26
<i>Disculiceps pileatus</i> (Linton, 1891)	4	7.69	1.33	1–2
<b>Order Onchoprotocephalida Caira, Jensen, Waeschenbach, Olson &amp; Littlewood, 2014</b>				
<i>Phoreiobothrium</i> sp. 1 (Ruhnke, 1994)	255	38.46	17 ± 37.33	1–137
<i>Phoreiobothrium</i> sp. 2 (Ruhnke, 1994)	211	48.72	11.11 ± 19.75	1–89
<i>Phoreiobothrium</i> sp. 3 (Ruhnke, 1994)	30	10.26	7.5 ± 6.35	2–13
<b>Order Trypanorhyncha Diesing, 1863</b>				
<i>Nybelinia</i> sp. 1 (Poche, 1926)	868	66.67	33.38 ± 33.63	1–136
<i>Nybelinia</i> sp. 2 (Poche, 1926)	204	48.72	10.74 ± 14.47	1–49
<i>Nybelinia</i> sp. 3 (Poche, 1926)	78	28.21	7.09 ± 7.06	2–25

Continued Table 1

<i>Eutetrarhynchidae</i> sp. 1 (Guiart, 1927)	136	48.72	7.16 ± 9.9	1–34
<i>Eutetrarhynchidae</i> sp. 2 (Guiart, 1927)	26	12.82	5.2 ± 1.3	1–4
<i>Oncomegas cf wagneri</i> (Palm, 1995)	1	2.56	1	1
<i>Poecilancistrum caryophyllum</i> (Diesing, 1850)	5	5.13	2.5	2–3
<i>Otobothrium australe</i> (Palm, 2004)	6	10.26	1.5 ± 1	1–3
<b>Order Ascaridida</b>				
<i>Hysterothylacium</i> sp. Ward & Magath, 1917	86	48.72	4.53 ± 5.99	1–26



**Figure 1.** Some cestodes presented in the blacktip shark *Carcharhinus limbatus*: *Nybelinia* sp.1. (A); *Nybelinia* sp. 2 (B); *Poecilancistrum caryophyllum* (C); *Paraorygmatobothrium* cf. *sinuspersicense* (D); *Phoreiobothrium* sp. 1 (E); *Disculiceps pileatus* (F); *Cathetocephalus thatcheri* (G); genus *Anthobothrium* (H).

(Tetraphyllidea) (Fig. 1H), as well as *Phoreiobothrium* (Onchoproteocephalidea) mature in large sharks (Carcharhiniforms) and their larval stages only infect teleost fishes (Chambers *et al.*, 2000; Jensen & Bullard, 2010), suggesting that teleosts occupy a place emphasized in the life cycles of the species of these genera. However, a diversity of other carcharhinid and sphyrid sharks have been reported as hosts for species of these genera in the Gulf of Mexico (Jensen, 2009), indicating that the web of trophic connections among intermediate teleost hosts and elasmobranch definitive hosts involved in the life-cycles of species in these genera is likely much more complex.

The cestodes *Paraorygmatobothrium cf sinuspersicense* and *Nybelinia* sp. 1 were numerically dominant species in our study, followed by *Paraorygmatobothrium* sp.; *Phoreiobothrium* sp. 1 (Ruhnke, 1994) (Fig. 1E); *Phoreiobothrium* sp. 2 (Ruhnke, 1994) and *Nybelinia* sp. 2 (Poche, 1926) (Fig. 1B) (Table 1). A similar pattern was found previously by Méndez & Dorantes-González (2013) for the cestodes of bull shark from the Chachalacas region, who reported the trypanorhynchid cestodes *Callitetrarhynchus gracilis* (Rudolphi, 1819) Pintner, 1931, *Otobothrium* sp. 1 Linton, 1891 and *Otobothrium* sp. 2 Linton, 1891 as numerically dominant species. In contrast, Owens (2008) record the order Tetraphyllidea with the highest composition of species, according to Caira & Jensen (2014) these species of cestodes belong to the orders Tetraphyllidea (four species), to the order Onchoproteocephalidea (six species), to the order Cathetocephalidea (one species), and to the order Trypanorhyncha (four species). In our study, three species belonging to the order Onchoproteocephalidae are recorded, however, the number of species may increase with a more exhaustive review of the genus *Phoreiobothrium*.

The record of *Oncomegas cf wageneri* and *Rhinebothrium* sp., extend its geographic distribution range and register new hosts. Several authors have reported the larva of *O. wageneri* from host teleost intermediaries. Thatcher (1961) records a plerocercoid of *O. wageneri* in *Lutjanus aya* (Bloch, 1790) (Lutjanidae) collected in the Gulf of Mexico, near Isla Grande, Louisiana, USA. Vidal-Martínez *et al.* (2004) record *O. wageneri* in

the sole *Syacium gunteri* Ginsburg, 1933 in Campeche, south of the Gulf of Mexico. *O. wageneri* adults are recorded in the *Dasyatis centroura* (Mitchill, 1815) (Rajiformes: Dasyatidae) as a definite host for the Gulf of Mexico (Toth *et al.*, 1992). The presence of *O. wageneri* in the blacktip shark *C. limbatus* indicates that this shark is possibly feeding on benthic fish such as *S. gunteri* and *Paralichthys* sp. Girard, 1858. The rhinebothrideans parasitize elasmobranchs (sharks and rays) as adults and their larvae infect both teleosts and molluscs. In the Gulf of México, the larval stages have been recorded in the southern flounder, *Paralichthys lethostigma* Jordan & Gilbert, 1884 (Paralichthyidae) (Jensen & Bullard, 2010), although the consumption of squid from the Loliginidae family is not ruled out as part of the diet of *C. limbatus* (Barry, 2002).

The presence of *Hysterothylacium* sp., in *C. limbatus* represent the first record of nematodes as parasites of this shark. Méndez (2005) report nematodes of the family Anisakidae in the blue shark *Prionace glauca* (Linnaeus, 1758) for the coast of Baja California Sur (Mexico). Moravec & Justine (2006) report to *Terranova scoliodontis* (Baylis, 1931) Johnston & Mawson (1945) in the tiger shark *Galeocerdo cuvier* (Péron & Lesueur, 1822) in New Caledonia. The adult worms of *Hysterothylacium* mostly parasitize the digestive tract of fishes (Deardorff & Overstreet, 1980) whereas the larval stages live in different tissues of numerous fish species, which act as intermediate and/or paratenic hosts (Køie, 1993). The present finding of this species represent new host.

*Paraorygmatobothrium* sp. 1 together with *Nybelinia* sp. 1 showed the highest values of prevalence and intensity of infection. Malek *et al.* (2010) mention that gender *Paraorygmatobothrium* is the most common parasite in carcharhinid sharks, mainly the genus *Carcharhinus* (*C. limbatus*; *Carcharhinus acronotus* (Poey, 1861); *Carcharhinus amblyrhynchoide* (Whitley, 1934); *Carcharhinus amboinensis* (Müller & Henle, 1839); *Carcharhinus falciformis* (Müller & Henle, 1839); *Carcharhinus melanopterus* (Quoy & Gaimard, 1824); *Carcharhinus obscurus* (Lesueur, 1818); *Carcharhinus plumbeus* (Nardo, 1827); *Carcharhinus sorrah* (Müller & Henle, 1839) and *Carcharhinus leucas*) (Ruhnke & Thompson,

2006; Méndez & Dorantes-González, 2013).

This study reports *C. limbatus* as a new host for eight species of cestodes (*P. cf sinuspersicense*; *A. laciniatum*; *Rhinebothrium* sp.; *T. thysanocephalum*; *C. thatcheri*; *O. cf wagneri*; *D. pileatus*; *P. caryophyllum*) and one species of nematode (*Hysterotylacium* sp.). All the helminths found in the blacktip shark *C. limbatus* represent new records for the coastal zone of the state of Veracruz, expanding their ranges of distribution and contributing to the registration of helminth fauna of elasmobranchs for the Gulf of Mexico.

### ACKNOWLEDGMENTS

To the laboratory of Hydrobiology, Facultad de Biología, Universidad Veracruzana Campus Xalapa (UV). To José Luis Recio Silva, Israel Lozano, Jonathan Gómez and Yitzendi López Serrano for their help in the field work. To the fishermen of the locality of Playa Chachalacas and to the staff of the fish shop "Carillo". To Ana Ruth Cristobal by SEM (CINVESTAV—Mérida). Part of this project was funded by the postdoctoral CONACyT scholarship #205133.

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Received November 27, 2018.  
Accepted December 25, 2018.