First record of *Paronatrema vaginicola* (Dollfus 1937) parasite in the western coast of Baja California Sur, Mexico

April 12, 2023

RESEARCH NOTE

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Published 12 April 2023 • www.doi.org/10.51492/cfwj.109.2

Key words: Baja California Sur, infesting, México, *Prionace glauca*, parasite, trematode

Citation: Ochoa, M. R., J. Rodriguez, R. Inohuye, and V. Gracia López. 2023. First record of *Paronatrema vaginicola* (Dollfus 1937) parasite in the western coast of Baja California Sur, Mexico. California Fish and Wildlife Journal 109:e2.

Editor: Kristen Elsmore, Marine Region

Submitted: 23 February 2022; **Accepted:** 13 July 2022

Competing Interests: The authors have not declared any competing interests.

Sharks, rays, and manta rays are natural hosts for a great variety of parasites, particularly helminths and epibionts since they offer a diversity of microhabitats. In Mexican waters, about 132 species of helminth parasites have been documented in sharks (Carrier et al. 2004; Merlo-Serna and García-Prieto 2016), which can be found infesting them in two ways. Firstly, endoparasites can be located in internal organs of sharks, such as the guts, stomach, intestine, liver, oviducts and the vascular system. Secondly, ectoparasites use the external parts of the body infecting the eyes, fins and cloaca, (Dollfus 1937; Curran and Overstreet 2000; Bray and Cribb 2003; Méndez and Galván-Magaña 2016; Penadés-Suay et al. 2017).

Blue sharks (*Prionace glauca*) are distributed in tropical and temperate waters (12-20°C), with vertical movements over 1000 m in depth (McMillan et al. 2011). The maximum recorded size has been 400 cm total length (TL) with sexual segregation (Nakano and Stevens 2008), which is a pelagic shark with an opportunistic feeding strategy that depends on prey abundance with a trophic level of 4.05 (Hernández-Aguilar et al. 2016). The blue shark is an incidental species in the catch of larger pelagic fish (tuna, marlin, swordfish, mahi mahi) caught on longline hooks (Galeana-Villaseñor et al. 2009). The reports about parasitic fauna of blue sharks found for the northwest Atlantic Ocean were composed of the following species: three species of copepods (*Kroyeria lineata, Phyllothereus cornutus* and *Echthrogaleus coleoptratus*); four species of cestodes (*Prosobothrium armigerum, Anthobothrium laciniatum, Hepatoxylon trichiuri* and *Platybothrium auriculatum*); and the nematode *Anisakis simplex* (Henderson et al. 2002). These parasites were found in the gut, spiral valve stomach, gills, and skin (Henderson et al. 2002). In Papua, New Guinea, the trematode parasite *Paronatrema vaginicola* (Dollfus 1937) was first described infesting the shark *Squalus* sp. Other records of this parasite have been from the blue shark (*P. glauca*) and thresher shark (*Alopias pelagicus*) at Punta Arena, Boca Alamo, San Isidro, and Santa Maria in the Gulf of California (Curran and Overstreet 2000).

We obtained 114 specimens of *P. glauca* from those caught by the industrial fishing vessel Damasta, which targets pelagic fish, such as tuna, mahi-mahi, oily fish, marlin, swordfish and sharks with longline fishing gear along the Baja California Sur western coast (BCSWC) in Mexico. Their fishing area ranged from Cabo San Lucas area (22º 53 N, 109º 54 W) to San Juanico (26º 55 N, 112º 47 W), at 50 km off the coast from November 2013 to March 2014 (**Fig. 1**). Currently, a ban on elasmobranch fishing has been established (NOM-029-PESC-2006, Mexico), which aims to regulate and conserve sharks and rays. This ban applies from 31 May to 31 July of each year in Baja California Sur (BCS), since the approval of the standard has established that circle hooks must be used to reduce bycatch (DOF 2007). The samples obtained were part of the ongoing research study on trophic ecology, which consisted only of internal organs (stomachs and gonads) and the external part of the pelvic fins where the reproductive apparatus in males is located (claspers and anus). Size separation into juveniles and adults was performed based on logbook data. We indirectly estimated shark maturity stage by the degree of calcification of shark claspers distinguishing them between juveniles and adults (**Table 1**) by using the morphological criteria proposed by Carrera-Fernández et al.(2010). The samples were frozen in the cold room until the ship returned to the port of San Carlos in BCS and then transferred for analyses to the Fish Ecology Laboratory at the Centro de Investigaciones Biológicas del Noroeste (CIBNOR) in the city of La Paz, BCS.

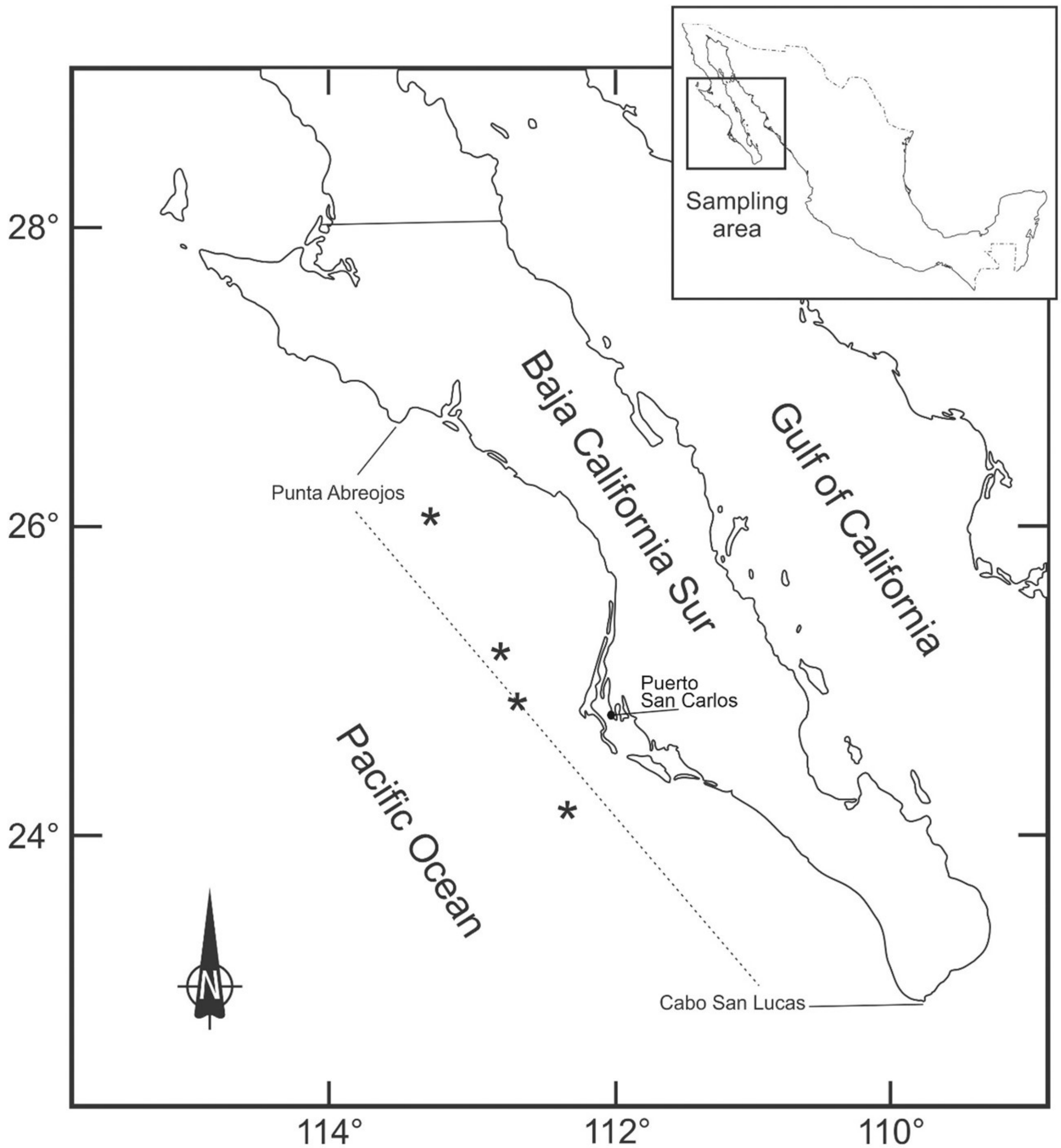

Figure 1. Fishing area of the commercial fishing fleet Damasta for blue shark *Prionace glauca* in the western coast of Baja California Sur, México from November 2013 to March 2014. * indicate shark catching

Table 1. Specimens of the blue shark *Prionace glauca* parasitized by *Paronatrema vagincola* caught off the western coast of Baja California Sur, Mexico from November 2013 to March 2014.

Date	Clasper length	Group size	Number of Parasites
Nov 2013	16 cm	Adult	4
Jan 2014	19.8 cm	Adult	2

Date	Clasper length	Group size	Number of Parasites
Jan 2014	12.5 cm	Juvenile	1
Mar 2014	20 cm	Adult	2
Mar 2014	11.4 cm	Juvenile	1

Parasites are visible to the naked eye (**Fig. 2**); they were stored in vials with 70% ethanol and then taken to the Parasitological Diagnostic Laboratory at CIBNOR. Subsequently, we slightly flattened the parasites between slides and coverslips in 10% formalin solution for 24 h. After that, they were preserved in 70% ethanol, stained with Gomori's trichrome, cleared with methyl salicylate and mounted on synthetic resin for microscopic observation (**Fig. 3**). We used taxonomic keys and references (Dollfus 1937; Curran and Overstreet 2000; Gibson et al. 2002) for identification. Measurements were made with a graduated eyepiece, and microphotographs were taken with a Stemi508 Zeiss stereomicroscope (Oberkochen, DE) with a digital camera. We found ten *P. vaginicola* parasites in two juvenile and three adult *P. glauca* sharks (all organisms were male) positioned on the external part of the stomach and pelvic fins with a prevalence of 4% and an intensity of 1–4 parasites per host.

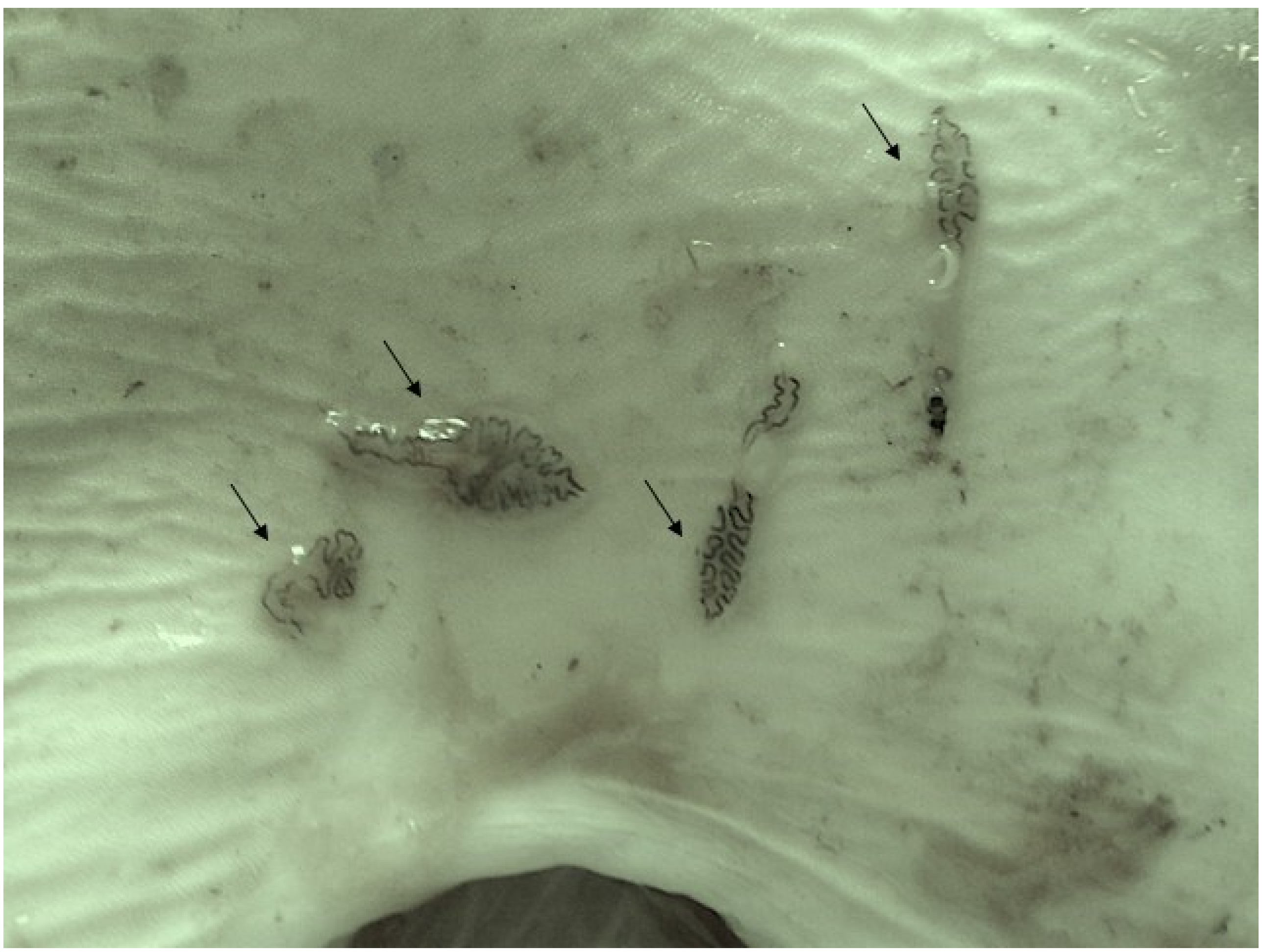

Figure 2. *Paronatrema vaginicola* located in the ventral side of the pelvic fins of the blue shark *Prionace glauca* caught in the western coast of Baja California Sur, México from November 2013 to March 2014.

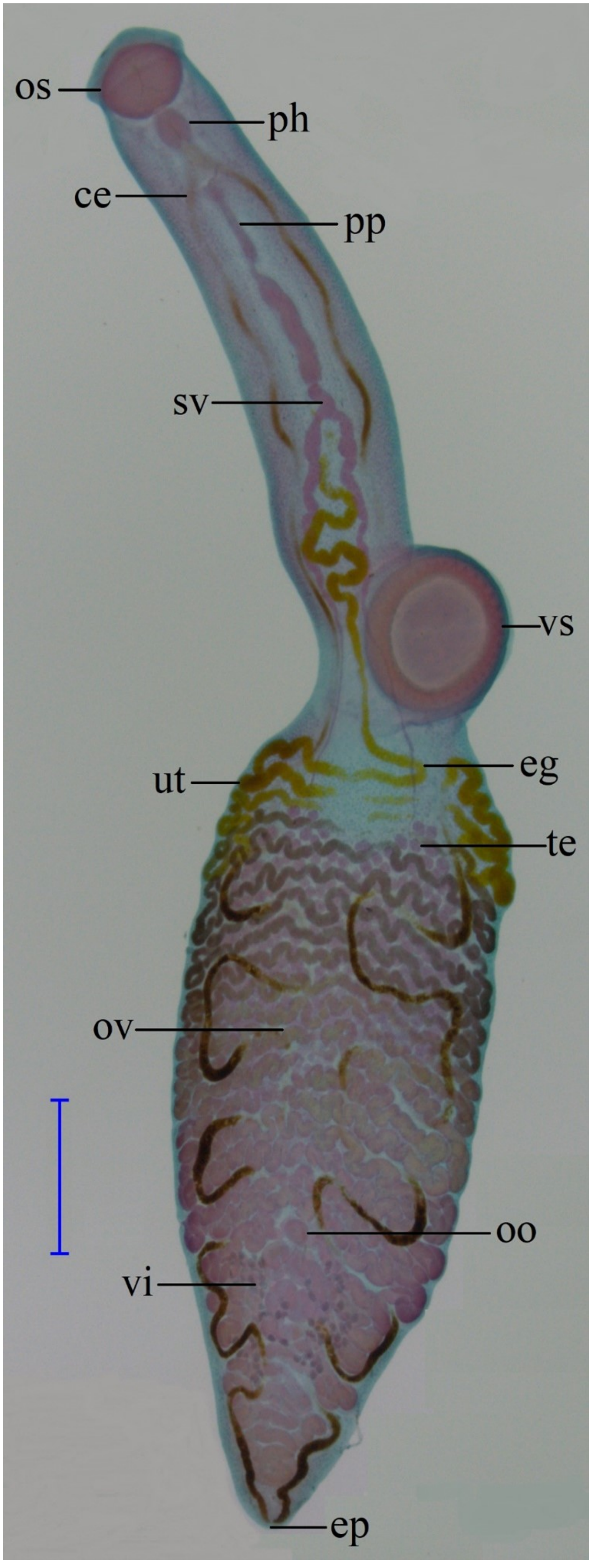
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Figure 3. Ventral view of Gomori's trichrome stain of *Paronatrema vaginicola* parasitizing blue shark *Prionace glauca*. Scale bar: 2 mm. Abbreviatures ce: ceca, eg: egg, ep: excretory pore, ov: ovary, oo: ootype, os: oral sucker, ph: pharynx, pp: pars prostatica, sv: seminal vesicle, te: testes, ut: uterus, vi: vitellaria, vs: ventral sucker.

We identified the parasites as *Paronatrema* based on diagnostic characteristics of the family Syncoeliidae (Gibson et al. 2002). The genus *Paronatrema* is poorly known. The most recent generic diagnosis for the genus *Paronatrema* (Dollfus 1937) was included in the keys to Trematoda by Gibson et al. 2002. The genus *Paronatrema* is characterized by having a cylindrical anterior body and flattened and oval posterior body, small accessory suckers on the oral and/or ventral suckers, and a large ventral sucker that is apparently not pedunculate. The genus is also characterized by having a sinuous ceca, apparently with closed end (or forming a uroproct or cyclocoel interpretation uncertain). Testes and vitellaria consist of rows of segmented follicles or tubules. Ovaries are composed of irregular follicles, and the uterus has numerous loops in the posterior region. The genus *Paronatrema* is a parasite of skin or oviduct or stomach of sharks and rays. Type species *P. vaginicola* Dollfus 1937 (Gibson et al. 2002; Table 2).

Table 2. Comparison of measurements and biological data of *Paronatrema vaginicola*. Length (L), width (W), all expressed in millimeters parasitizing *Prionace glauca* caught off the western coast of Baja California Sur, Mexico, from November 2013 to March 2014.

Reference	**Dollfus 1937**	**Curran and Overstreet 2000**	**This study**
Host	*Squalus* spp.	*Prionace glauca Alopias pelagicus*	*Prionace glauca*
Locality	New Guinea	Punta Arena, Boca del Alamo, and San Isidro Boca del Alamo and Santa Maria	Cabo San Lucas to San Juanico
Locality	México	Gulf of California BCS, México	western coast of Baja California Sur, Mexico
Site	Oviduct	Cloaca, gill arches, and buccal cavity	Pelvic fins, and external part of the stomach
Specimens	2	5	5
Total body (L)	8.4–9.32	8.0–20.5	13.1–26.2
Forebody (L)	3.461–3.577	3.004–6.512	6.4–11.4
Hind body (W)	2.906[a]	2.0–3.5	1.9–2.4
Forebody (W)	3.06–5.3	4.4–6.5	2.8–6.0
Oral sucker (L)	0.923–1.077	0.932–1.179	0.950–1.275
Oral sucker (W)	1.030–1.154	1.022–1.135	1.125–1475
Ventral sucker (L)	2.308–2.499	1.824–2.807	2.000–2.725
Ventral sucker (W)	2.384–2.577	1.824–2.610	1.800–2.875
Accessory suckers	27	35–38	34–38

Reference	Dollfus 1937	Curran and Overstreet 2000	This study
Egg (L) Egg (W)	Immature	0.025–0.030 0.020–0.022	0.026–0.029 0.022–0.023

[a] Measurement from Fig. 5 in Dollfus (1937)

The specimens found in this study were in agreement with the morphological description of *Paronatrema vaginicola* by Curran and Overstreet 2000; the differences observed were in terms of total length (larger in this study) and width (thinner in this study), probably due to fixation and flattening of the specimens (**Table 2**). In relation to the original description by Dollfus 1937—based on two immature and not flattened specimens—it stands out that they have a lower number of accessory suckers (27) compared to those observed by Curran and Overstreet and in this study (34–38 suckers). The results in this study demonstrate that prevalence and intensity of *P. vaginicola* parasitizing blue sharks was high, with a significant sample size obtained from commercial fisheries (n = 114) off the coast of state of Baja California Sur, Mexico, compared to other studies where only three parasites were found in five *P. glauca* and two parasites in eight *A. pelagicus* sharks of those analyzed (Curran and Overstreet 2000). The importance of knowing parasite species and rate of infestation in an organism (in this case the blue shark) can provide information on parasite-host relationships to allow determining to some extent the state of health of the ecosystem, since they are excellent samplers of environmental conditions. (Overstreet 1997).

Studies of parasites in sharks have reported they tend to be harbored within the pharynx, esophagus, buccal cavity, gill arches, and anal opening or cloaca (Dollfus 1937; Shvetsova 1994; Curran and Overstreet 2000; Pérez-Ponce de León et al. 2007). For the western coast of BCS, Méndez (2005) analyzed 27 blue shark stomachs, finding 247 helminths and 4996 in the intestines, with a total of six helminths, three cestode genera, and two other species. Cadwallader et al. (2014) have documented that the symbiotic association of cleaner fish with the thresher shark species *A. pelagicus* can be beneficial. In this case, the authors observed how the species *Labroides dimidiatus* and *Thalassoma lunare* prey on digenean ectoparasites found on the shark pelvic region where the genus *Paranotrema* was also found. The life cycle of *P. vaginicola* is poorly known because only records of parasites have been found in adult sharks in pelagic areas and feeding on squid and fish.

Morales-Ávila et al. (2015) reported a larval stage of the trematode *Paranotrema mantae* parasitizing the euphausiid *Nycthiphanes simplex* in the Gulf of California. The type of diet has been associated with parasite infestation. The blue shark is a top predator and displays a wide trophic spectrum of prey on euphausiids, such as lobster (*Pleuroncodes planipes*), mackerel (*Scomber japonicus*) and several squid species (e.g., *Dosidicus gigas, Sthenoteuthis oualaniensis*) along the BCSWC (Hernández-Aguilar et al. 2016). The epibenthic habitat of *N. simplex* may facilitate the transmission of benthic parasites to nekton hosts and likewise from them to pelagic predators. Future studies may be able to determine whether syncoelid trematodes actively seek out their host or passively infect nekton and their predators through prey-predator interactions. In Mexico, records of the helminthic fauna of elasmobranchs have been scarce and fragmentary during the last 20 years, which is understandable, considering the difficulty of obtaining access to organ or tissue samples of the organisms. To date, 81.7% of sharks and 67.4% of rays distributed in Mexican waters lack helminthological studies (Merlo-Serna and Garcia-Prieto 2016).

In conclusion, observing that the parasites in this study were larger compared to those in Curran and Overstreet (2000) study, it could be a host effect since they reported that *P. glauca* parasites were larger than those of *A. pelagicus*. Moreover, as a new contribution, this study extends *P. vaginicola* parasite distribution to the BCSWC, which was only recorded for the Gulf of California, and provides information on its morphometrics and robustness of prevalence and intensity. In addition, the blue shark is one of the most commonly caught shark species for human consumption, but more knowledge of the *P. vaginicola* parasite is still lacking. The findings of this parasite species (*P. vaginicola*) in Pacific waters provides a reference of a possible migration of blue sharks between the coasts of the Baja California Peninsula. Assuming this possibility, satellite tagging studies are needed in this area to confirm their movements. Furthermore, a study on the parasite *P. vaginicola* should be considered to determine the life span of the adult stage on its hosts and life cycle in general, as well as the conditions that cause these parasites to have been found only in male *P. glauca* sharks and not in females. Finally, the interpretation of this study is that by observing the parasites near the pelvic fins, they may transit out of the shark's body when the shark dies.

Acknowledgements

This study was conducted under the Project Food Habits and Structure of Fishes within the Centro de Investigaciones Biológicas del Noroeste. The authors would like to thank the crew in Damasta ship and fisherman Antonio Simeón for sampling the collection, Jésica Pérez Inohuye for image editing, and Diana Fischer for English edition.

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