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Research Note

Helminth fauna of *Scomberomorus sierra* (Actinopterygii: Scombridae) in southeastern Gulf of California, Mexico

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Summary

From January to May 2015, a sample of 50 individuals of the Pacific sierra *Scomberomorus sierra* Jordan and Starks, 1895 captured off Mazatlán (southeastern Gulf of California, Mexico) were reviewed for helminths. A total of 6, 255 parasitic worms belonging to 11 species (9 in adult stage and 2 larvae) were obtained. Trematoda was the best represented group with 6 species of Didymozoidae Monticelli, 1888 and 1 of Bucephalidae Poche, 1907. In addition, 2 monogenean species and 2 of Nematoda were collected. In this study, the first molecular sequences for didymozoid species in Mexico were generated, and for *Glomeritrema* sp. at worldwide level. The most prevalent species were *Didymocylindrus* sp. (92 %) and *Didymocystis scomberomori* (MacCallum & MacCallum, 1916) (88 %), whereas the monogenean *Thoracocotyle crocea* MacCallum, 1913 reached the highest value of mean intensity (75.2). The coincidence between the helminthological composition established in our study and that reported for the same scombrid in 4 localities from the Mexican South Pacific (sharing 10 species) suggests that this group of species persistently parasitize *S. sierra* throughout its distribution along the Mexican Pacific coast; furthermore, due to the richness of didymozoids and the affinity of Thoracocotylidae species for *S. sierra*, both groups can be considered typical parasites of this fish.

Keywords: Fish; parasites; Pacific sierra; Gulf of California; Taxonomic composition

Introduction

Helminthological studies on members of Scombride in Mexico are scarce; until 2018, only 1 of the 27 species distributed in Mexican waters had a relatively complete helminthological record (Castillo-Sánchez *et al*., 1997). However, in the last years, this gap of knowledge has been decreasing (see Miranda-Delgado *et al*., 2019; Santos-Bustos *et al*., 2020a,b; Villar-Beltrán *et al*., 2020). In this context, the objective of this study is to establish the helminth fauna infecting *Scomberomorus sierra* Jordan and Starks, 1895 in the southeastern Gulf of California, based on morphological and / or molecular data.

Materials and Methods

From January to May 2015, 50 individuals of *S. sierra* (total length: 35.6 – 78 cm; 54 ± 53.9) caught off Mazatlán (23°14'29" N, 106°24'35" W), Sinaloa, were purchased from local fishermen.

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Fish were transported on ice to the laboratory, for immediate dissection and helminth recovery. The parasitological examination and processing followed Lamothe-Argumedo (1997). Worms were morphologically identified using taxonomic keys and specialized literature. Voucher specimens of helminths were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología (IB), Universidad Nacional Autónoma de México (UNAM), Mexico City, Mexico (Table 1). Prevalence and mean intensity values were calculated in agreement with Bush *et al*. (1997). A species accumulation curve and bootstrap richness estimator were used to determine the representativeness of the sample size. For some didymozoid species, DNA sequences were generated to compare them with those available in GenBank; these samples were fixed and preserved in 100 % alcohol. Total DNA was extracted for the nuclear marker 28 ribosomal DNA (28S), which was amplified with the same set of primers used by Mladineo *et al*. (2010). Sequences were edited using the software Genius (Kearse *et al*., 2012) and then blasted against the GenBank database to preliminary confirm the amplification of the helminth DNA. All the sequences generated in this study are available in GenBank (Table 1).

Ethical Approval and/or Informed Consent

The research related to animal use has been complied with all the relevant institutional policies for the care and use of animals in Mexico (DOF, 2001).

Results and Discussion

A total of 6,255 helminths belonging to 11 species included in 2 phyla (Platyhelminthes and Nematoda) were collected from *S. sierra* from the southeastern Gulf of California. Of these, 9 species were found in adult stage and 2 as larvae (Anacetabulum and *Hysterothylacium* sp.). More than half of the collected specimens (66.6 %) belong to Trematoda; monogeneans and nematodes represent 16.6 % each (Table 1). After 30 sampled hosts helminth species accumulation curve reached the asymptote; value of bootstrap estimator was equal to the observed richness (11 species). Didymozoidae Monticelli, 1888 was represented by 6 taxa. Four of them were not identified to species level due to the amount of eggs in their bodies, preventing the observation of some key characters for their identification (Abe *et al*., 2014). According to Pozdnyakov & Gibson (2008), Didymozoidae is the most difficult group of digeneans to deal with. Due to this and in order to achieve the finest possible level of identification, we generated the DNA sequences of the 28S for 4 of the 5 adult species collected in our sample (which coincidentally represent the first sequences obtained for these trematodes in Mexico). Even so, only the specific identity of one of them was defined, since the availability of information for the group in GenBank is scarce. Specimens of *Didymocystis scomberomori* (MacCallum & MacCallum, 1916), *Didymocystis* sp., *Didymocylindrus* sp. and *Didymozoon* sp., were found encysted in pairs and had the body divided into 2 regions. *Didymocystis scomberomori* and *Didymocystis* sp. were included in the same genus by having the anterior region attached near or at the anterior end of the flat surface of the anterior region (Pozdnyakov & Gibson, 2008); they were considered 2 separate taxa due to the different shape and size of their body, and because parasitized distinct organ (intestine and kidney, respectively) (Mladineo *et al*., 2010; Shrandt *et al*., 2016). Molecular data of 28S generated in this work, supported our identification of *D. scomberomori* that showed 99 % of identity with sequences of this species in Genbank, and recognized a second species of *Didymocystis* with 97 % of identity with *Didymocystis* sp. ex *Scomberomorus maculatus* (Mitchill, 1815) also deposited in Genbank. On the other hand, specimens assigned to *Didymocylindrus* sp. have the body with an elongated posterior region arranged perpendicular to the anterior region and joined at its center, which are diagnostic characteristics of the genus (Pozdnyakov & Gibson, 2008). Individuals of *Didymozoon* sp. were identified by having the body with the anterior region attached to the upper terminal part of the posterior region (Pozdnyakov & Gibson, 2008). The sequence of *Didymozoon* sp. obtained is only 92.5 % similar to the sequence of *Didymozoon longicolle* Ishii, 1935 deposited in GenBank (FJ628652.1); due to this, we identified our material at generic level, based on morphological characters and partially confirming it through molecular data. Specimens of *Glomeritrema* sp. were identified by having elongated and undivided body, lacking ventral sucker (Pozdnyakov & Gibson, 2008); also because were strongly entangled and entwined in a capsule with capillary net. In the present study, DNA sequences representative of this group were generated for the first time worldwide. Larval didymozoids assigned to Anacetabulum are characterized by lack acetabulum and glands surrounding the esophagus, and by the small pharynx before the intestinal caeca and after the terminal oral sucker (Pozdnyakov & Gibson, 2008). An additional species of trematode recorded in the present study was assigned to *Prosorhynchoides* Dollfus, 1929 by having simple “rhynchus”, pre-testicular ovary and vitelline glands in the anterior part of the body, where the uterus also extends (Overstreet & Curran, 2002). The only species of this genus recorded in the Pacific coast of Mexico is *Prosorhynchoides cybii* (Park, 1939), also in *S. sierra* (Santos-Bustos *et al*., 2020a). The poor condition of our material precludes assigning them accurately to the aforementioned species, and then we prefer designate as *P.* cf. *cybii*. Two Thoracocotylidae Price, 1936 monogeneans were found in this study: *Mexicotyle mexicana* (Meserve, 1938) and *Thoracocotyle crocea* MacCallum, 1913. The first one has the diagnostic features of the species (arrangement of clamps in a row, large hamuli with a simple recurved end and absence of vaginal spines) according to Rohde and Hayward (1999). *Thoracocotyle crocea* MacCallum, 1913 has a globular haptor penetrated by vitelline glands, testes and intestinal cecum as well as copulatory spines (Hayward & Rohde, 1999). Both monogeneans are common parasites of Scombridae fishes in the Atlantic and the Pacific coasts of

Table 1. Helminthological record of *Scomberomorus sierra* in southeastern Gulf of California, Mexico.

Helminth species	Infection site	Prevalence (%)	Mean Intensity	CNHE catalog number	GenBank accession number
Platyhelminthes					
Trematoda					
Didymozoidae					---
Didymocystis scomberomori	Mesentery, intestine	88	38.3	11144	MZ663671
Didymocystis sp.	Kidney	92	19.9	11148	MZ663672
Didymocylindrus sp.	Gills	58	19.1	11139	---
Didymozoon sp.	Gills	48	8.3	11142	MZ663669
Glomeritrema sp.	Gills	22	6.7	11138	MZ663670
Anacetabulum gen. sp.[Larvae]	Gills	18	9.8	11141	---
Bucephalidae					
Prosorhynchoides cf. *cybii*	Intestine	6	1	11146	---
Monogenea					
Thoracocotylidae					
Thoracocotyle crocea	Gills	56	75.2	11145	---
Mexicotyle mexicana	Gills	40	24.3	11140 11149	---
Nematoda					
Anisakiidae					
Hysterothylacium sp.[Larvae]	Intestine	4	1	---	---
Philometra sp.	Gonads	54	4.9	11159	---

Mexico (Mendoza-Garfias *et al*., 2017). The only adult nematodes found in *S. sierra* (infecting gonads), was *Philometra* sp., characterized by a filiform body, anterior and posterior ends rounded, males with posterior caudal mound, cylindrical esophagus, short, bulbous at the anterior end, with esophageal gland confined to the wall of the esophagus and females with uteri that occupy most of the body (Moravec, 1998). Their identification at the specific level was not carried out in the present study because a more detailed morphological study is required to distinguish it of the 3 *Philometra* species known in Mexican marine waters (Moravec & de Buron, 2013). Larval nematodes found in the intestine of the Pacific sierra were determined as members of *Hysterothylacium* Ward & Magath, 1917 because they have a small, almost spherical esophageal ventricle, a sac-shaped appendix with a septum that divides the appendix into 2 equal longitudinal sacs, as well as an intestinal cecum shorter than the ventricular appendix. Developmental stage of these larvae precluded its specific identification, which is based on morphology of adult worms (Pantoja *et al*., 2016). In Mexico, only *Hysterothylacium fortalezae* (Klein, 1973) is associated with scombrids from the Caribbean Sea, in the Atlantic coast (Aguirre-Macedo *et al*., 2007).

The most prevalent helminth species was *Didymocystis* sp. (92 %) followed by *D. scomberomori* (88 %); however, *T. crocea* reached the highest values for mean intensity (75.2). For the remaining helminth species, prevalence varied between 4 (*Hysterothylacium* sp.) and 58 % (*Didymocylindrus* sp.) and mean intensity between 1 (*P*. cf. *cybii*) and 38 (*D. scomberomori*) (Table 1). The high prevalence levels of both species of *Didymocystis* Ariola, 1902, could be explained by the preference of *S. sierra* to prey on sardines and anchovies (Moreno-Sánchez *et al*., 2011; Vega *et al*., 2013), since this actinopterygians are intermediate or paratenic hosts of this group of trematodes (Gómez del Prado *et al*., 2007). On the contrary, the reduced number of Anacetabulum specimens found can be attributed to the fact that juvenile stages of these didymozoids are harbored by crustaceans, mollusks and plankton (Galaktionov & Dobrovolskij, 2003), which are not the main prey in the diet of *S. sierra* (Moreno-Sánchez *et al*., 2011). *Thoracocotyle crocea* reached the highest mean intensity levels probably as effect of the anomalous warm conditions because fishes were collected during “El Niño” event (Sánchez-Velasco *et al*., 2017); according to Rohde (2005), monogeneans may be more abundant during times of prolonged heat. Species of *Philometra* require copepods as intermediate hosts and in some cases paratenic hosts (Moravec & de Buron, 2013). In the present study, prevalence levels of *Philometra* sp. suggests that its life cycle involve a paratenic host, since arthropods are not common among the food items ingested by *S. sierra* (Moreno-Sánchez *et al*., 2011). Finally, larvae of *Hysterothylacium* sp. reached the lowest levels of infection in our sample; according to Anderson (2000), nematodes of this genus developed to the third stage in invertebrates, but they can be transferred to paratenic host fish and then to the definitive, where they will mature. The helminth fauna of this fish had only been partially studied in some places in the Mexican Pacific (see Pérez-Ponce de León *et al*., 2007; Mendoza-Garfias *et al*., 2017). However, recently Santos-Bustos *et al*. (2020a) analyzed their parasite communities and concluded that the species composition of helminths in the 674 individuals studied during 10 years varied among the 4 sites sampled on the southern coast of the Mexican Pacific. Notwithstanding, this variation was not detected respecting our results; the helminth fauna of *S. sierra* off Mazatlán is also mainly constituted by didymozoids species (sharing 5 of them), and that the Pacific sierra individuals of both studies share 5 more species (2 monogeneans, 2 nematodes and 1 trematode). Moreover, eliminating the 3 accidental (*Gonocercella pacifica* Manter, 1940, *Rhadinorhynchus* cf. *pristis* (Rudolphi, 1802) Tetraphyllidea gen. sp.) and 3 generalist (*Lecithochirium microstomum* Chandler, 1935, *Anisakis* sp. and *Procamallanus* sp.) species reported by these authors, the helminth fauna of this scombrid in both studies is practically the same. This suggests that this group of helminths persistently parasitizes the Pacific sierra throughout its distribution off Mexican Pacific coast, which is supported by the detection of at least 4 of them (*M. mexicana*, *Didymocylindrus* sp., *Didymocystis* sp. and *P. cybii*) as biological tags (Santos-Bustos *et al*., 2020a). Furthermore, due to the abundance of digeneans (particularly didymozoids) and monogeneans (particularly *M. mexicana* and *T. crocea*), both groups can be considered to be typical parasites of *S. sierra* in Mexican Pacific waters. The only specialist helminth species found in *S. sierra* in the southern Pacific of Mexico [*Scomberocotyle scomberomori* (Koratha, 1955)] was not collected in our study; however, previously was reported in this fish in the Gulf of California coast (Mendoza-Garfias *et al*., 2017). When comparing the helminth fauna recorded for *S. sierra* off Mazatlán, with that of 3 other Scombridae species studied in Mexico, the species richness of *S. sierra* (11) can be placed somewhere in between; in this group of hosts, richness ranges from 8 in *Scomberomorus cavalla* (Cuvier, 1829) (Villar-Beltrán *et al*., 2020) to 27 (for *Euthynnus lineatus* Kishinouye, 1920) (Miranda-Delgado *et al*., 2019). The total abundance of helminths in the Pacific sierra is high (6,255) compared to the registered in *Sarda orientalis* (Temminck & Schlegel, 1844) (3,219 individuals in 230 fishes according to Santos-Bustos *et al*., 2020b) but located far below that reported for *E. lineatus* (more than 70,000 individuals in 496 fishes, see Miranda-Delgado *et al*., 2019).

Based on our data and those analyzed from other Scombridae species studied so far in Mexico, 2 main factors seem to determine the structuring of their helminth faunas: the food chain web and the phylogenetic affinities of certain groups of helminths (i.e., Didymozoidae at family level and Thoracocotylidae at host genus level). However, only through detailed studies that analyze the helminth fauna in Mexican scombrids throughout their distribution will it be possible to confirm these observations.

Conflict of Interest

Authors state no conflict of interest.

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