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Research note

First record of *Multicalyx cristata* (Aspidocotylea) in *Sphyrna lewini* (Elasmobranchii) in the southern Gulf of Mexico

Primer registro de Multicalyx cristata (Aspidocotylea) en Sphyrna lewini (Elasmobranchii) en el sur del golfo de México

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Abstract

Three individuals of *Multicalyx cristata* (Aspidocotylea) were identified in the gallbladder of a hammerhead shark, *Sphyrna lewini* (Elasmobranchii), in Playa Chachalacas, Veracruz, Mexico. This report makes a significant contribution to our understanding of the biogeography of the Aspidocotylea species that parasitize sharks, expanding their documented range into the Mexican waters of the Gulf of Mexico and in the Atlantic Ocean.

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Keywords: Sharks; Parasites; Trematoda; Aspidogastrea; Gallbladder

Resumen

Se determinaron 3 individuos de *Multicalyx cristata* (Aspidocotylea) de la vesícula biliar de un tiburón martillo *Sphyrna lewini* (Elasmobranchii) en playa Chachalacas, Veracruz, México. Este registro es una contribución significativa en términos de la biogeografía de las especies de Aspidocotylea que parasitan tiburones, incrementando su distribución en aguas mexicanas del golfo de México y océano Atlántico.

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Palabras clave: Tiburones; Parásitos; Trematoda; Aspidogastrea; Vesícula biliar

Multicalyx cristata (Faust & Tang, 1936) (Fig. 1) was originally described in the spiral valve (intestine) of a cownose ray, *Rhinoptera bonasus* (Mitchill, 1815), near Biloxi Bay, Mississippi, USA. Since then, *M. cristata* has been reported in the gallbladders of various elasmobranch species (Bray, 1984; Dollfus, 1958; Hendrix & Overstreet, 1977; Stunkard, 1962; Thoney & Bureson, 1987) in both the Atlantic and the Pacific

Oceans (Table 1). Although the species has been reported in the spiral valves of some hosts (*R. bonasus* and *Sphyrna lewini*), *M. cristata* typically target the gallbladders of adult hosts (Dollfus, 1958; Stunkard, 1962) and the bile ducts of juvenile hosts (Hendrix & Overstreet, 1977). Manter (1954) and Hendrix and Overstreet (1977) reported juvenile *M. cristata* in *Menticirrhus americanus* (Linnaeus, 1758) (Sciaenidae) and *Sphoeroides testudineus* (Linnaeus, 1758) (Tetraodontidae), indicating that teleost fishes can serve as paratenic or transport hosts; this also may be a route of infection for neritic predators, like sharks. The intermediate hosts of *M. cristata* are currently unknown; however, Gibson and Chinabut (1984) mention that

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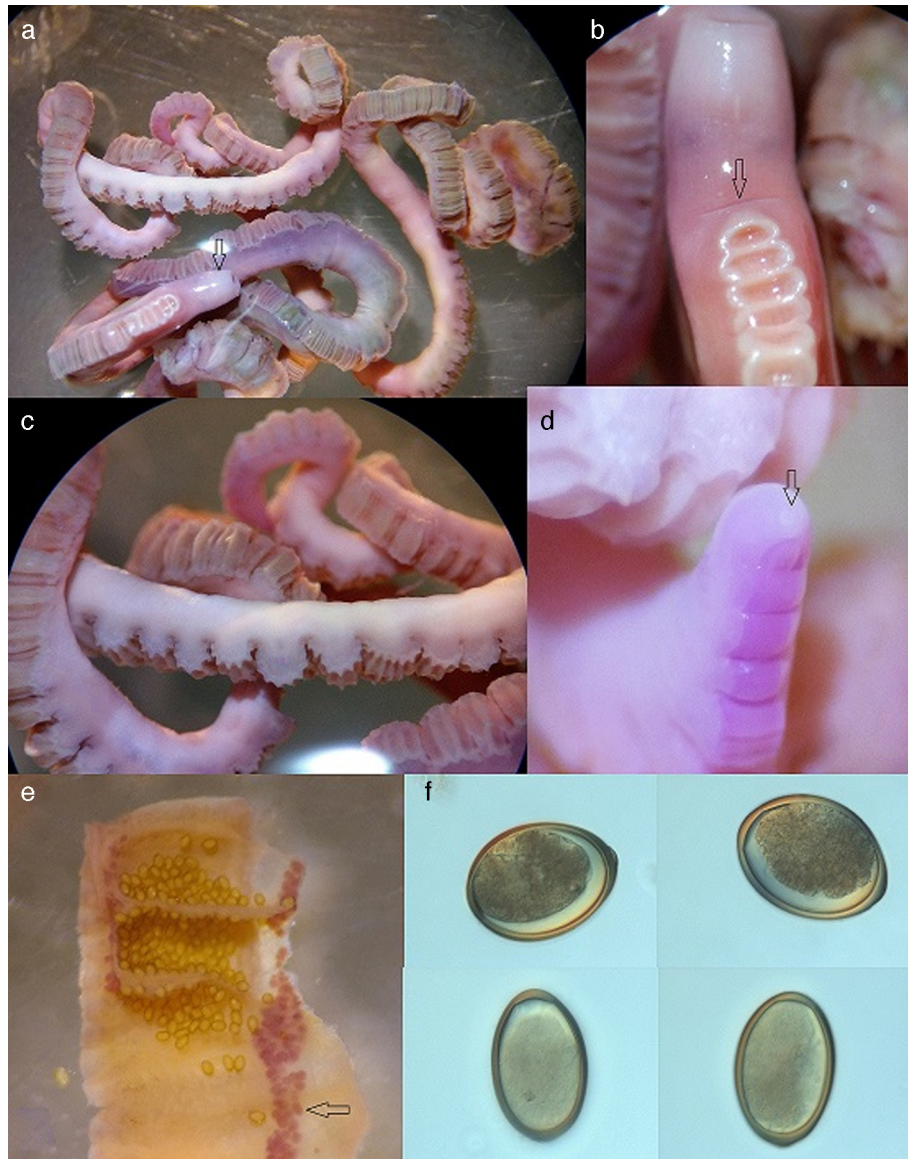


Figure 1. Morphology of *Multicalyx cristata*: (a) complete individual, the arrow shows the anterior portion (10×); (b) anterior view showing the mouth, genital pore (arrow), and alveoli (top) (40×); (c) side view showing the transverse ridges of the alveoli (40×); (d) posterior view showing the position of the excretory papilla (arrow) (40×); (e) mid-section showing part of the uterus with eggs and the vitellogen (arrow) in the lateral position (40×, and (f) embryonated eggs (40×). Images (a)–(e) were captured with a Motic SMZ-168 stereoscopic microscope, (f) was taken with an Olympus BX50 optical microscope.

the order (Stichocotylida) to which *M. cristata* belongs can use crustaceans as intermediate hosts.

Thoney and Bureson (1986) examined 16 species of sharks collected between Cape Fear, North Carolina, and Long Island, New York, USA, identifying specimens of *M. cristata* exclusively in *Myliobatis freminvillei* (Lesueur, 1824) (Bullnose eagle ray) and *Rhinoptera bonasus* (Mitchill, 1815). According to the authors, the absence of *M. cristata* in the elasmobranchs they examined was due to the small sample size. However, no *M. cristata* were reported in 42 individuals of the shark *Mustelus canis* (Mitchill, 1815) (Triakidae).

We report the presence of 3 individuals of *M. cristata* in the gallbladder of a male hammerhead shark, *S. lewini* (Griffith & Smith, 1834), caught by the artisanal fishery in Chachalacas, Veracruz, Mexico. The specimens were placed in a bottle with

7% saline solution, and cooled in ice to encourage their relaxation and to be able to separate them. Subsequently, they were fixed in warm formalin at 4%. In the laboratory the samples were transferred to 70% ethanol. Due to the large size of the specimens, it was not possible to prepare permanent slides; however, 1 individual was prepared for scanning electron microscopy (SEM). For SEM, the specimen was washed for 30 min with 0.1 M sodium cacodylate buffer, and post-fixed with 1% osmium tetroxide for 30 min. Subsequently the sample was washed again for 30 min in sodium cacodylate and then dehydrated in ethanol gradients from 30% to 100% for 10 min at each step; finally it was 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). Micrographs were obtained using a Philips XL-30 environmental SEM. One specimen was

Table 1
Reports of *Multicalyx cristata* in elasmobranch species.

Host	Location	Reference
<i>Mustelus canis</i>	Senegal	Dollfus (1958)
<i>Rhinobatos cemiculus</i>		
<i>Rhizoprionodon terraenovae</i>		
<i>Mustelus canis</i>	New Zealand	Manter (1954)
<i>Cephaloscyllium ventriosum</i>	Santa Barbara, California, USA	Hendrix and Overstreet (1977)
<i>Sphyrna lewini</i>	South Africa	Bray (1984)
<i>Odontaspis taurus</i>		
<i>Dasyatis centroura</i>	Woods Hole, Massachusetts, USA	Stunkard (1962)
<i>Pristis pectinata</i>	Gulf of Mexico	Hendrix and Overstreet (1977)
<i>Dasyatis sayi</i>		Thoney and Burreson (1986)
<i>Myliobatis freminvillei</i>	North Carolina and New York, USA	Thoney and Burreson (1986)
<i>Rhinoptera bonasus</i>	Veracruz, Gulf of Mexico	Present study
<i>Sphyrna lewini</i>		

deposited in the Colección Nacional de Helmintos (CNHE) at the Instituto de Biología, UNAM, as reference number CNHE-9994.

The 3 specimens were identified as *M. cristata* because their characteristics match those described by Faust and Tang (1936). The recovered specimens were adults with abundant

eggs in the uterus. The bodies were elongated and cylindrical, reaching 202–223 mm (211.6 ± 10.5) total length (TL). Despite their large size, these organisms did not obstruct the gallbladder as there were only 3 individuals. Thoney and Burreson (1988) mention that mature *M. cristata* can reach 60 cm TL. The individuals reported by Hendrix and Overstreet (1977) were juveniles measuring 28–101 mm TL. In contrast, *Multicalyx elegans* (Olsson, 1869) reach sizes of 23 mm and parasitizes the gallbladders of holocephals. *M. cristata* can occur individually or in groups of 2 or more organisms; however, there are records of a single individual with developing eggs within the uterus suggesting that self-fertilization may occur (Thoney & Burreson, 1986). Intraspecific competition may occur due to the large size attained by *M. cristata* in the gallbladder. Further research is required to confirm this; however, authors like Thoney and Burreson (1988) suggest that such competition would limit the intensity of infection to 1 or 2 individuals.

The ventral haptor extends almost the entire length of the body, consisting of a single row of 1668 alveoli subdivided by the transverse septum (Figs. 1a–d and 2a, c). This is similar to Bray's (1984) report of 1500 alveoli in a specimen of *M. cristata* obtained from a hammerhead shark, *S. lewini*, on the shores of South Africa. Our *M. cristata* specimen has a terminal mouth in the form of funnel; a ventral genital pore anterior to the haptor (Figs. 1b and 2a, b); oval, operculated eggs measuring

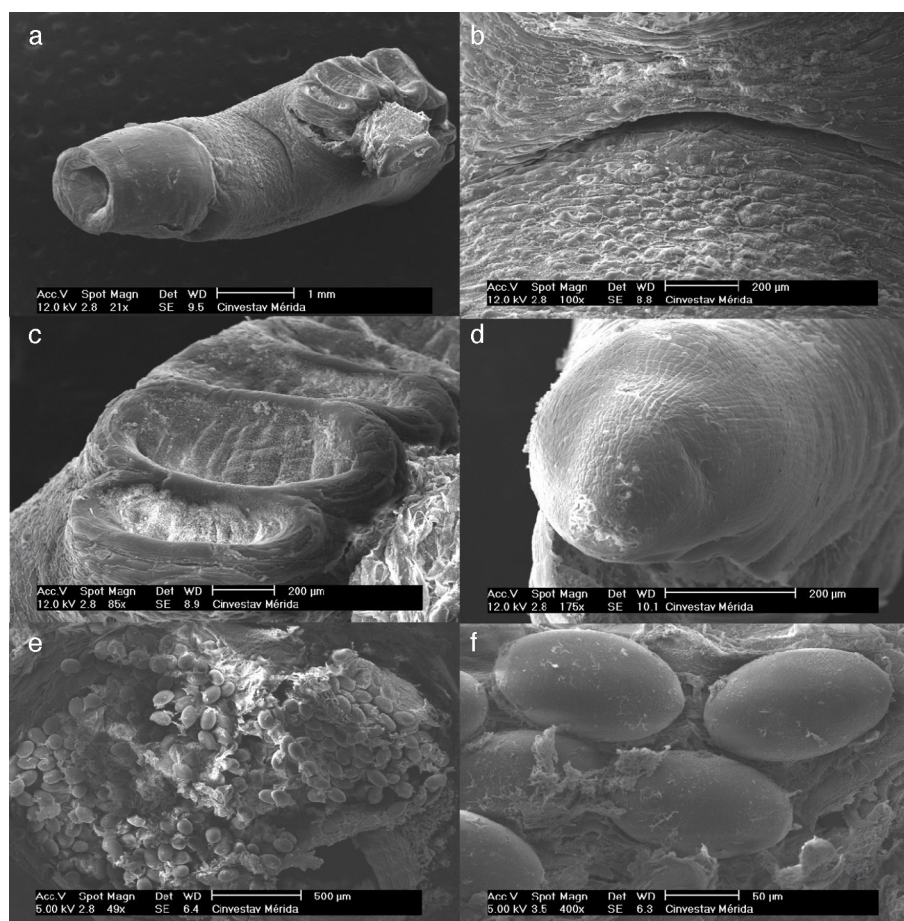


Figure 2. SEM images of *Multicalyx cristata*: (a) anterior view showing the mouth funnel, genital pore, and anterior alveoli; (b) genital pore; (c) alveoli; (d) excretory papilla; (e) uterus and eggs; and (f) embryonated eggs.

$0.12 \times 0.08 \mu\text{m}$ (Figs. 1e, f and 2e, f); and vitellaria formed by 2 bands of lateral (Fig. 1f).

The excretory papilla is located on the posterior end of the body (Figs. 1d and 2d).

This is the first report of *M. cristata* in *S. lewini* in Mexican waters and for the coast of Veracruz. Further research is necessary as we currently know very little about the life cycle of these organisms. Such studies should include a parasitological examination of the stomach contents of other elasmobranch species, particularly those that have already been identified as hosts (Table 1), as well as a pathology assessment of the damage caused to the gallbladder. Once this information has been collected, it will be possible to identify a potential intermediate host; it is unlikely that a single intermediate mollusk host occurs in such a broad range of diverse habitats.

Our report of *Multicalyx cristata* off the Veracruz coast is an important contribution to our understanding of the biogeography of the Aspidocotylea species that parasitize sharks. The presence of this parasite in the hammerhead shark *S. lewini* in the Mexican waters of the Gulf of Mexico expands its range in the Atlantic Ocean.

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