



Original article

Huffmanella selachii n. sp. (Nematoda: Trichosomoididae: Huffmanellinae): A new species infecting the skin of the great hammerhead shark (*Sphyrna mokarran*) and the blacktip reef shark (*Carcharhinus melanopterus*) in the Arabian Gulf, off-shore Saudi Arabia

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ABSTRACT

From January 2017 - December 2019, 75 out of 850 (8.8 %) great hammerhead sharks from the Arabian Gulf had skin lesions of black irregular discolorations on the ventral surface of the head. The lesions consisted of pencil-like lineations often advancing forward by about 2 mm in back-and-forth looped scribbles often forming a relatively linear bands of about 5–7 cm wide. Similar lesions were also found in the blacktip reef shark from the same area within the same period, and consisted of straight to irregular black lines, extended indiscriminately across the skin of the sharks. Microscopic examination of the skin revealed the presence of dark-brown eggs exhibiting the spindle or ellipsoidal eggs characteristic of *Huffmanella* sp. The morphometrics of eggs from both hosts were similar (62.9–89.9 μm long and 29.3–56.1 μm wide). The eggshells were smooth with polar plugs protruding or not, with an abruptly truncated crown-like or shoulder-like collar surrounding the plug. The eggs were only found in the epidermal layer of the skin. Based on the unique morphometrics of the eggs, we report a new species, named: *Huffmanella selachii* n. sp.. This appears to be the first report of *Huffmanella* from either the great hammerhead shark or the blacktip reef shark, and the third reported *Huffmanella* in sharks from the Arabian Gulf. It is also one of few species reported from connecting waters of the greater Indian Ocean. This new finding contributes to our understanding of the diversity and ubiquity of *Huffmanella* sp. in marine creatures.

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1. Introduction

The shrimp fishing season in the Eastern Region of Saudi Arabia usually runs from first of August till the end of January each year. Unfortunately, the shrimp harvest generates a substantial bycatch of many fish species, including the capture of great hammerhead sharks (*Sphyrna mokarran* Rüppel, 1837) and the blacktip reef shark (*Carcharhinus melanopterus* (Quoy and Gaimard, 1824)) due to their abundance in the Arabian Gulf and predilection for bottom feeding. Since some bycaught fish will later be sold in the fish market, routine veterinary inspection of those fish is carried out to establish suitability for human consumption, and fine irregular black lines are occasionally observed in the skin of bycaught great hammerhead sharks during such inspections. Similar skin lesions have been described previously in other shark species and were

attributed to the presence of eggs of *Huffmanella* spp. (Ruiz and Bullard, 2013; Attia et al., 2021b).

Members of the genus *Huffmanella* (family Trichosomoididae) are histozoic nematodes that infect mainly marine fishes, with only one exception: *H. huffmanii*, which has only been reported from swimbladders of centrarchid fishes from the upper spring run of the San Marcos River in central Texas (Moravec, 1987; Huffman and Moravec, 1988). Approximately 21 species have been formerly described (Fig. 1), most of which had been recovered only in the egg and larval stages (Justine and Iwaki, 2014). The taxonomy of the genus is based mainly on the features and morphometrics of eggs, host affiliation, host organ, and geographic locality (Moravec and Garibaldi, 2000). *Huffmanella* spp. occur in many habitats of diverse geographic regions across the world's oceans (Ruiz and Bullard, 2013; Justine and Iwaki, 2014) and exhibit a high level of organ specificity (Moravec, 2001). They are reported to infect many different tissues of fishes including skin, oral cavity, gill openings, spinal column, muscles, swim bladder and even bones (Huffman and Moravec, 1988; Moravec et al., 1998; Moravec and Fajer-Avila, 2000; Justine, 2004, 2005, 2007, 2011; Ruiz and Bullard, 2013; Justine and Iwaki, 2014).

Recently, two in nominate species of *Huffmanella* were reported by Attia et al. (2021b) from the skin of the whitecheek shark (*Carcharhinus dussumieri* (Valenciennes, 1839)) and from muscular tissues of the orange spotted grouper (*Epinephelus coioides*) from the Arabian Gulf (Attia et al., 2021a). In the latter two reports, the identification of the eggs was conducted to the genus level, and to the best of the authors' knowledge, *Huffmanella* infections have never been reported from the great hammerhead sharks nor the blacktip reef sharks. Therefore, this study was conducted to investigate the causative agent of the black discoloration observed in skin of great

hammerhead sharks and the blacktip reef shark and to report the pathology and the morphological features of the recovered *Huffmanella* eggs infecting the sharks.

2. Materials and methods

2.1. Sample collection

The sampled fish were examined during routine veterinary inspection of health condition and quality of fish at boat landing stations in the harbor (27°0'52.186"N, 49°40'45.814"E, Fig. 2A), at the adjacent fish auction market, and at the fish market at Jubail province. The duration of this study was two years, starting January 2017 and ending December 2019. The collected sharks were all accidentally bycaught using shrimp bottom fishing nets during the shrimp-fishing season. During the study, 850 great hammerhead sharks were examined. Lengths of the sharks ranged from 2 to 2,5 m and weights from 40–45 kg. Out of the 850 sharks examined, 85 presented with unusual black irregular discoloration (Fig. 2B) in the form of linear tracks together with fine very small black spots on the skin at the ventral surfaces of the heads of the sharks with an incidence rate of 8,8%. In the same period and from the same area a number of bycaught blacktip reef sharks were also examined and exhibited the same lesions of black irregular lines in the skin of the dorsal side of the head and back and of the ventral side of the snout (Fig. 2C, D). Sharks with such changes were culled from the market and condemned due to aesthetic reasons. Skin lesions from one of each shark species were excised and fixed in 10 % neutral buffered formalin for histopathological examination and one sample from the infected skin was excised and preserved

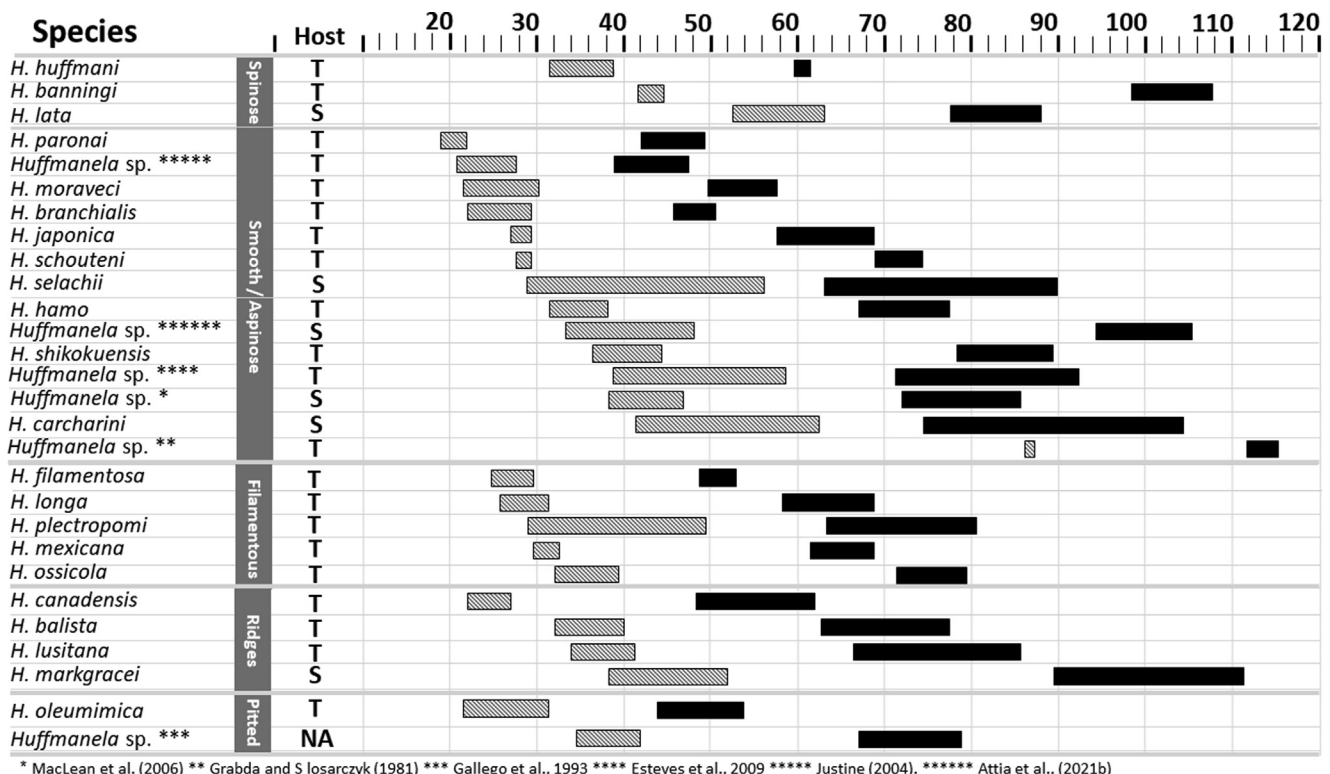


Fig. 1. A graphical chart showing differences in the egg dimension from published reports of *Huffmanella*. The eggs were first classified based on the eggshell morphology (vertical grey column): spinose, smooth/spinose, filamentous, contain ridges or pitted. The diagonal hatch pattern is the range of width of the eggs, and the black line is the range of length of the eggs. Scale on top is in micrometres. Letter abbreviations under (Host): T: Teleost; S: Shark; NA: Not identified, passage of eggs in fecal samples from a human. ** Grabda, J., Ślusarczyk, W. (1981): Parasites of marine fishes from New Zealand. Acta Ichthyol. Piscat. 11(2):85–103. <https://doi.org/10.3750/AIP1981.11.2.09>. *** Gallego, J., Riera, C., Portús, M. (1993): *Huffmanella* sp. eggs (Nematoda: Trichosomoididae), as a human spurious parasite in a child from Barcelona (Spain). Folia Parasitol. 40, 208–210.

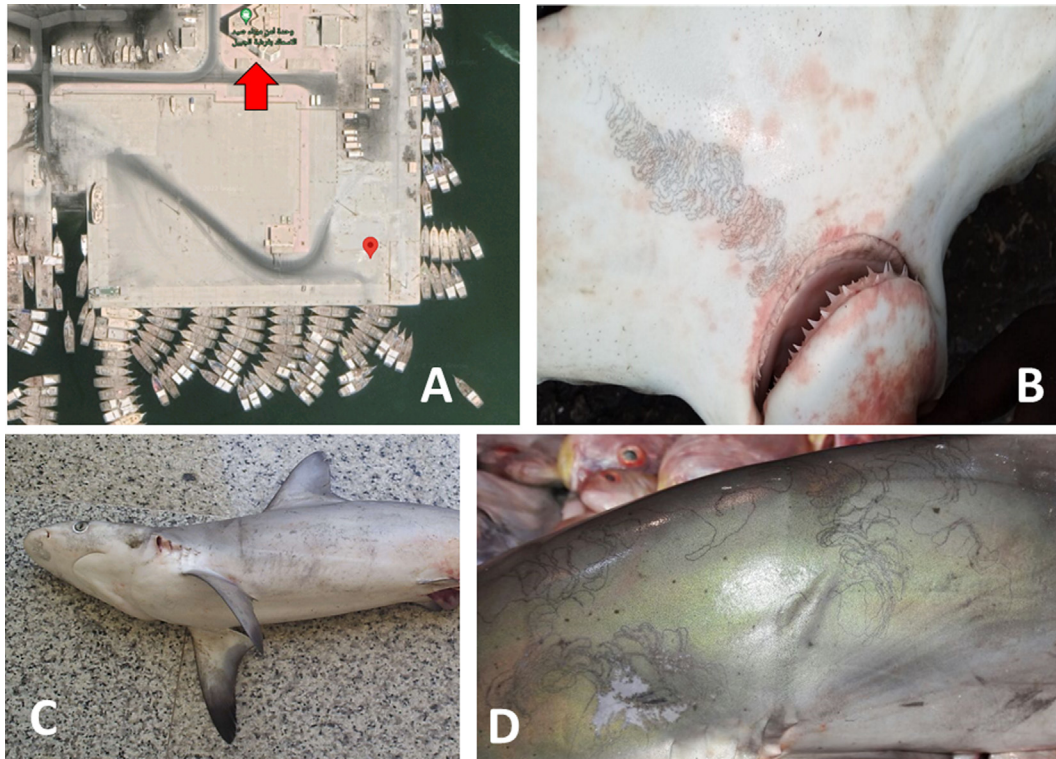


Fig. 2. A: Boat landing stations in the harbor of Jubail where fish are being inspected by the veterinary authority (Red arrow, office of the authorities). B: Ventral side of the great hammerhead shark (*Sphyrna mokarran*) showing infected skin with *Huffmanella selachii*, exhibited as a band of unusual black irregular discoloration in the form of thin scribbled tracks of eggs on the ventral side of the upper jaw and extends longitudinally. C: The blacktip reef shark (*Carcharhinus melanopterus*) showing infection with *H. selachii* on the ventral side of the snout. D: The gross appearance of the dorsolateral surface of an infected blacktip reef shark, revealing discoloration with irregular long dark black lines of egg tracks of *H. selachii* (about 1 mm in width). The lines were associated with very minute small black spots, forming either long chain-like or dense black lines in patches along the entire body of the infected shark on both sides of the body.

in 70 % ethanol in a glass flask in an ice box and submitted for parasitological examination at the College of Veterinary Medicine at King Faisal University, Saudi Arabia. The internal organs of all sharks were routinely examined for the presence of any pathological conditions.

2.2. Parasitological examination

Eggs recovered from the infected skins were obtained by collecting eggs that had been dislodged in the fluid containing the skin samples. Eggs were readily released from skin by manually shaking the vial containing the infected skin samples, by washing the infected skin with ethanol, or by scrapping using a scalpel blade. The fluid containing the eggs was then transferred to a glass petri dish and examined under inverted microscope (Labomed, TMC 400). Eggs were then collected with a glass pipette and mounted on a glass slide, then left to dry and DPX was then placed on the dried eggs and covered with a glass cover slip. In some samples, the skin was preserved in 10 % formalin, in which the fluid containing the eggs was sieved through 31 μm sieve (Scrynel, Lanz-Anliker, Rohrbach, Switzerland), and the trapped eggs were backwashed with distilled water and placed on a glass slide, then mounted as a wet smear for direct photography. Skin scrapings were mounted on a glass slide and left to dehydrate in air then mounted with one drop of DPX and covered with a glass cover slip. The prepared slides were then examined under light microscope (Olympus AX70) and photographed using an attached Nikon camera. The measurements of eggs (Fig. 3) are in micrometres (μm).

Eggs examined via scanning electron microscopy (SEM) were collected by placing the scraped eggs on metal stubs using two-sided sticky tape. The eggs were left on the stubs for 3 hrs to dry

and were then sputter-coated in gold (Spi-Module Sputter Coater, UK), then examined and photographed using a JSM 5200 electron probe microanalyzer (JEOL, Japan). Identification to *Huffmanella* sp. was based on described features reported previously (Bullard et al., 2012; Ruiz and Bullard, 2013; Justine and Iwaki, 2014).

2.3. Histopathological examination

Samples from the infected tissues were fixed in 10 % neutral buffered formalin and processed according to standard methods (Roberts et al., 2012). Tissue was sectioned to 3 μm thickness and stained with Hematoxylin and Eosin (H&E).

3. Results

3.1. Taxonomic summary

Huffmanella selachii n. sp.

Eggs: Color dark-brown; surface smooth, no evidence of uterine layer; shape truncated ellipsoid; size (μ) as min-max (n) 63–85 (72) long 29–46(72) wide.

Adults: No adult or juvenile worms found.

Type host: Great hammerhead, *Sphyrna mokarran* (Rüppell, 1837) (Carcharhiniformes: Sphyrnidae).

Additional hosts: Blacktip reef shark, *Carcharhinus melanopterus* (Quoy and Gaimard, 1824) (Carcharhiniformes: Carcharhinidae).

Site of infection: Eggs deposited in the epidermal layer between the placoid scales; gross appearance as narrow, black squiggly lines zig-zagging back and forth forming a band 5–7 cm wide.

Type locality: Arabian Gulf, off Saudi Arabia.

Prevalence: 10 % (n = 850).

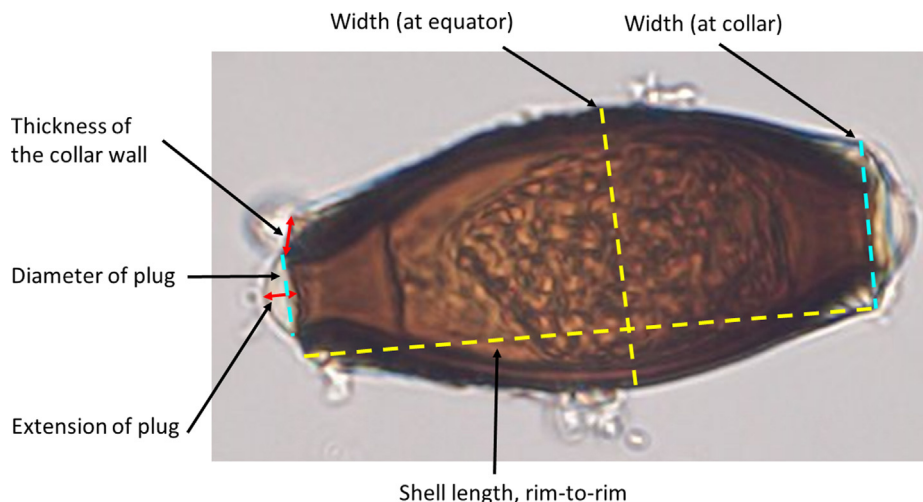


Fig. 3. An embryonated egg of *Huffmania selachii* showing the dimensions analyzed in this study.

Etymology: The clade within which modern sharks are classified.

Deposition of specimens: Syntype eggs from type host (*S. mokarran*) deposited in the Parasitology Collection repository, College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia (cat. no. 437 *Huffmania selachii* (437/1 [syntype]) ex *Sphyrna mokarran*, 437/2 [voucher] ex *Carcharhinus melanopterus*). Separate vouchers containing infected skins from individual sharks of both species deposited in the Helminthological Collection of the Czech Institute of Parasitology (IPCAS N-1271 *Huffmania selachii* (N-1271/1 [syntype]) ex *Sphyrna mokarran*, N-1271/2 [voucher] ex *Carcharhinus melanopterus*)).

3.2. Description

Adults: not observed.

Eggs: dark-brown, spindle or ellipsoidal in shape (Fig. 4A, B, E). Eggshell outer surface smooth (Fig. 4C, F) with no clear evidence of a uterine layer (Fig. 4B-C, E-G). Slightly protruding polar plugs in freshly isolated eggs (Fig. 4E). Tips of eggs appearing as though cut off square forming truncated crown-like structures (“shoulders”) surrounding the polar plugs at each end.

Measurements (Table 1) of larvated eggs from *S. mokarran* [and from *C. melanopterus*] given as μm (n): 62.9–85.3 (72) [66.2–89.9 (83)] long, 29.8–45.5 (72) [32.7–56.1 (83)] max width; the width of the collar around the polar plugs (termed in Fig. 2: width at collar) 15.4–22.2 (56) [13.9–28.1 (92)] across; diameter of emergent polar plug 6.8–16.0 (58) [4.5–15.8 (92)]. Polar plug extending beyond shoulders up to 4.8 (33) [n/a]. Thickness of the collar wall surrounding polar plug 3.1–5.5 (58) [2.5–8.6 (92)].

3.3. Gross pathological lesions

The skin lesions in the great hammerhead sharks appeared as black, irregular discolorations in the form of linear bands about 5–7 cm in width on the white surface of the ventral part of the head near the mouth in both shark species and in the dorsal surface of the blacktip reef shark. Closer inspection of the bands revealed back-and-forth tracings of scribble-like black lines about 1 mm wide progressing along the bands. The lines were composed of small grey to black spots. In the blacktip reef shark the lesions consisted of irregular black lines that could be seen in the whole body skin of the sharks, sometimes were exhibited as single lines especially at the dorsal surface of the skin (Fig. 2D). Parasitological

examination of skin scrapings by light microscope revealed the presence of the characteristic spindle or ellipsoidal shaped eggs of *Huffmania* sp. (Fig. 4A).

3.4. Histopathological examination

The histopathological examination of skin lesions of the infected sharks revealed spindle to oval eggs of *Huffmania* in the epidermal layer in between the placoid scales (Fig. 5A–B, D). The presence of eggs was associated with desquamation of the most superficial epithelium with some eosinophilic granulocytes (Fig. 5C). Tissues adjacent to infected epidermal tissues also exhibited evidence of hyperplasia, including eosinophilic granulocytes and aggregates of lymphocytes. The dermal layer beneath infected epidermis also showed aggregation of lymphocytic cells. No eggs were observed in the dermal or hypodermal layers, nor in muscle tissue and no obvious changes in the muscular layer were noted. No pathological lesions were found in the internal organs that could be associated with *Huffmanella* infection.

3.5. Differential diagnosis

The ranges of the egg dimensions from published reports of *Huffmania* are represented graphically in Fig. 5. Given reported localizations, morphologies, and dimensions of the eggshells of all *Huffmania* spp., only three nominate species and three innominate populations share characteristics similar to the eggs described herein (Fig. 1). The confined localization of the currently described eggs in the epidermis of the sharks makes these eggs differ from those found in the muscles, including *H. hamo* (Justine and Iwaki, 2014), *H. shikokuensis* (Moravec et al., 1998) and an unidentified *Huffmania* sp. (Esteves et al., 2009). The current eggs differ morphologically from those of *H. carcharhini* due to the presence of evident collar around the plugs, which *H. carcharhini* lacks (Bullard et al., 2012). The two other *Huffmania* sp. eggs that share the same egg dimensions with the those herein were reported without a formal taxonomic assignment to species. The first one published by MacLean et al. (2006) from sandbar shark, but those eggs have rugose outer eggshell surfaces. The remaining record of a recently reported *Huffmania* sp. in the skin of whitecheek sharks, also in the Arabian Gulf (Attia et al., 2021b). These worms may belong to *Huffmania selachii* n. sp. due to similarities in egg size, shape, and confined localisation on the skins of sharks caught from waters in the same region.

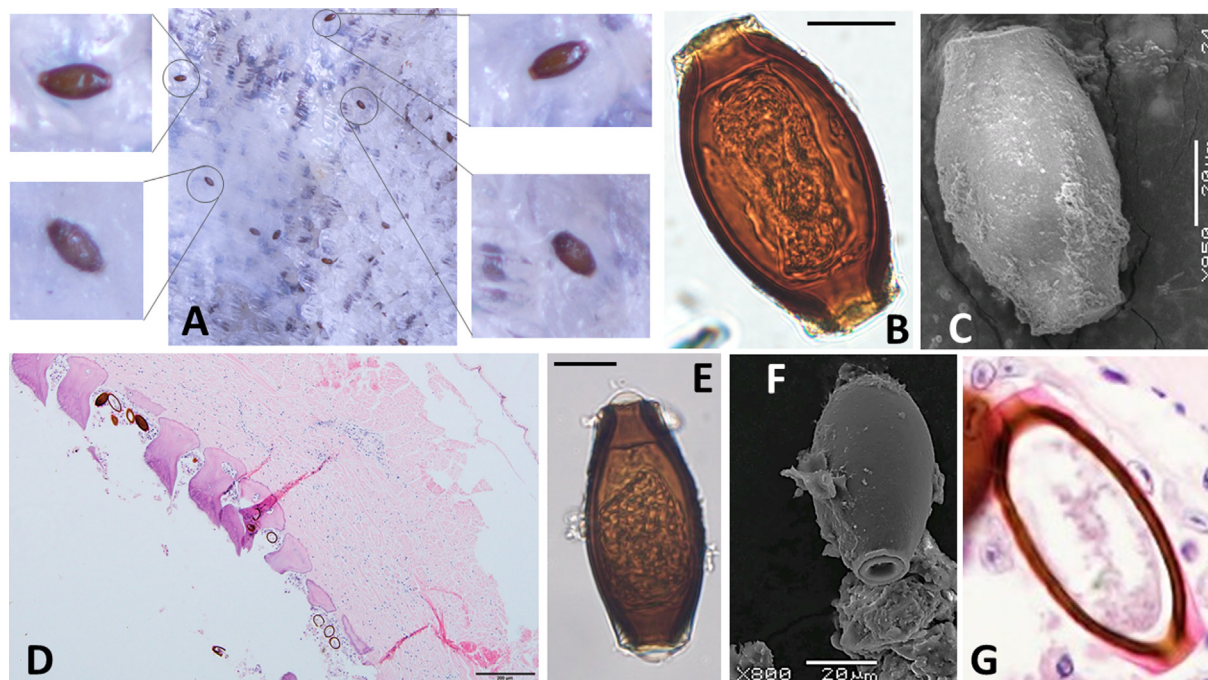


Fig. 4. Photomicrograph of eggs of *Huffmanella selachii* from skin of the blacktip reef shark (*Carcharhinus melanopterus*) (A–C), and the great hammerhead shark (*Sphyrna mokarran*) (D–G) from the Arabian Gulf. A: A photo of the skin of the shark showing the presence of a number of *H. selachii* eggs between and under the scales, four of which were magnified. B & E: *H. selachii* eggs as seen using a light microscope. Scale bar in B & D = 20 μ m. C & F: Scanning electron microscope photos showing the eggs of *H. selachii* collected from a skin scraping of the epidermis. In F: notice the smooth eggshell and the presence of a gap between two “rings” that forms the crown-like structure or “shoulders” that carries the polar plugs (vitelline layer normally covering gap destroyed during preparation). D: Histopathological preparations of the skin showing the confined superficial location of the eggs between the placoid scales. G: Histopathological preparations of the skin showing the smooth eggshell with presence of a developing larva inside. Scale bar in D = 100 μ m.

Table 1

Egg dimensions of *Huffmanella selachii* in μ m. * Readings were not taken due to small sample size.

	Sphyrna mokarran			Carcharhinus melanopterus		
	n	Mean	Range	n	Mean	Range
Shell length	72	75.2	62.86–85.26	83	77.57	66.17–89.85
Width (at equator)	72	38	29.8–45.5	83	40.86	32.74–56.12
Width at collar	56	19.7	15.39–22.24	92	20.6	13.92–28.14
Diameter of plug	58	10.1	6.78–15.98	92	8.94	4.52–15.81
Extension of plug	33	3.3	1.93–4.82	*	*	*
Thickness of the collar wall	58	4.8	3.09–5.47	92	5.95	2.46–8.62

4. Discussion

The parasitological examination of the unusual black discolorations of the skin of great hammerhead sharks and the blacktip reef shark revealed the presence of dark brown bipolar nematode eggs, typically oval or spindle in shape consistent with the genus *Huffmanella* (Bullard et al., 2012; Esteves et al., 2016). The occurrence of *Huffmanella* in the skin of great hammerhead shark and the blacktip reef shark in the Arabian Gulf indicated a new host species of sharks. Given the overlapping morphometrics between *H. selachii* n. sp. and the previous report of *Huffmanella* sp. by Attia et al. (2021b), the worms in the latter population should be considered conspecific with *H. selachii* n. sp.

The current clinical and histopathological examination on the host-parasite relationships revealed that *H. selachii* in the great hammerhead shark and the blacktip reef shark deposited eggs in epidermal layer of the skin only. This observation was also noted by MacLean et al. (2006) who found *Huffmanella* sp. infected skin only of the sandbar shark (*Carcharhinus plumbeus*). The epidermis of the infected great hammerhead shark had *Huffmanella* eggs at the base between placoid scales with desquamation of the most

superficial epithelia layer. This desquamation was associated with presence of inflammatory cells of eosinophilic granulocytes. No eggs were present in the underlying skin layers. Similar observations were also reported elsewhere in *Huffmanella* infections in sharks (Justine, 2005; MacLean et al., 2006; Bullard et al., 2012, and Attia et al., 2021b).

To our knowledge, *H. selachii* n. sp. is also the first species of the genus to be reported from fish of two different families (Sphyrnidae and Carcharhinidae), albeit of the same order. All other 30+ distinct populations are reported from fish in the same family. This may mean that the species represents two cryptic species or subspecies, but that determination awaits extensive DNA sequencing work.

The strong seasonality in the most recent Attia et al. (2021b) species is probably due to the sharks getting infected at one time of year, the worms laying eggs in another time of year, and the epidermis clearing the eggs in a third time of year. In contrast, the course of the life cycle of *H. huffmanii* (eggs locked in internal organs) showed prolonged durations between the onset of adult worm infection, commencement of egg shedding, and clinical appearance of lesions in infected organs (Worsham et al., 2016).

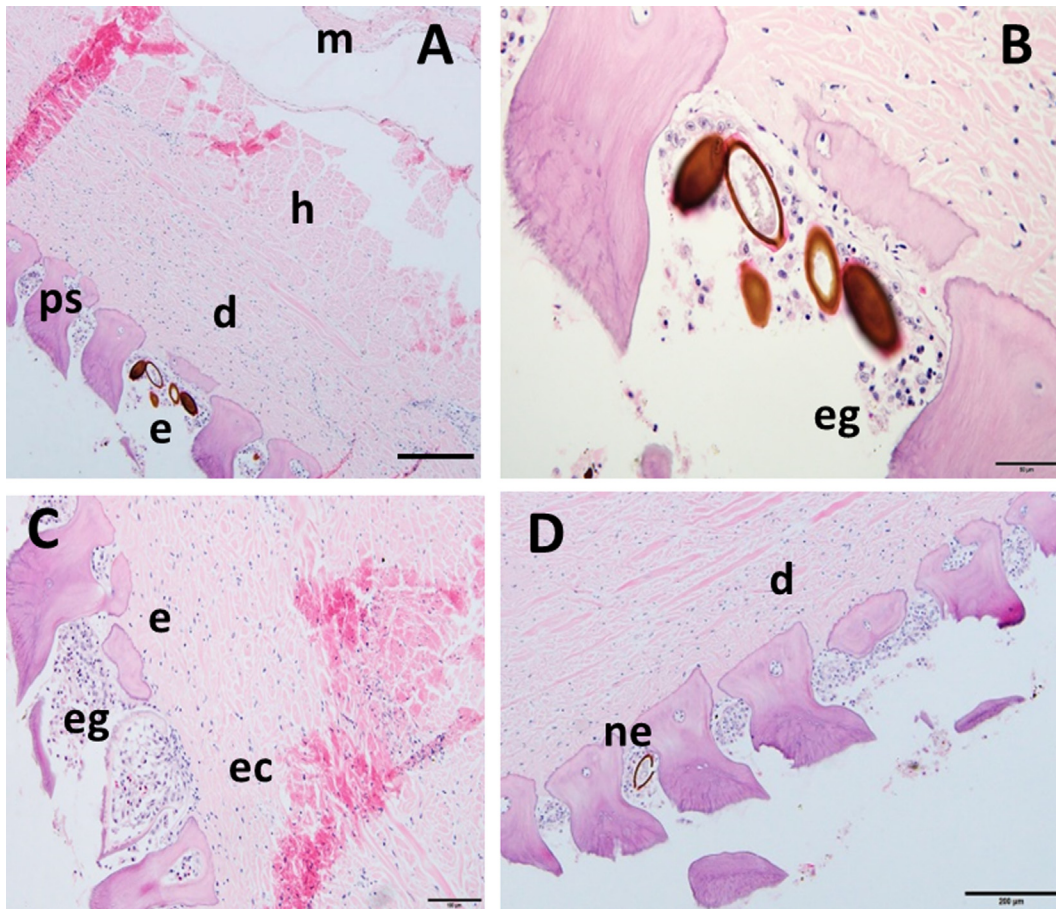


Fig. 5. Histopathological section of skin of *Sphyrna mokarran* infected with *Huffmanella selachii* n. sp., stained with hematoxylin and eosin. A: Epidermis with desquamated epithelial layer (e) between placoid scales (ps). Eggs were not detected in the dermal (d) hypodermal (h) or muscular (m) layers. B: Five eggs of *Huffmanella selachii* n. sp. (ne) with aggregates of eosinophilic granulocytes (eg). C: The epidermis (e) with hyperplasia of the epithelial cells (ep) associated with eosinophilic granulocytes (eg) adjacent to the infected epidermal layer. Dermis (d) showed aggregates of lymphocytes cells (l). D: one nematode egg (ne) in between placoid scales at its base (ps) with lymphocytes in the dermal layer (d). Scale bars = 100 μ m.

After the ingestion of an infected amphipod, the eggs were deposited in the infected fish organs about 8 + months later, and the adults were not detectable in the host about 2 weeks after commencement of ovipositing. At that time, the newly formed lesions caused by egg deposition were not visible at clinical inspection, and it took around one month before the eggs became dark enough for clinical discovery, during which time the adults disappear from the lesions. This may explain the inability of finding the adults in the current and most previously published reports of *Huffmanella* infections (Moravec and Garibaldi, 2000; Ruiz and Bullard, 2013).

The life cycles of all marine species of *Huffmanella* remain a mystery, but they probably require a crustacean first intermediate host; in the case of the very large bottom-oriented hosts of *H. selachii*, crabs would be immediately suspect. The fact that no adults or larvae have been discovered in the infected tissues of the sharks from the Arabian Gulf in this study or the Attia et al. (2021b) study suggests that these sharks are probably picking the parasites up in a part of their migratory range that is outside the Arabian Gulf, and that we should look elsewhere in their range for the hypothesized intermediate host (and adult worms in the shark hosts).

Interestingly, the habitat preferences of the three known hosts of *H. selachii* n. sp. (including *Carcharhinus dussumieri* of the Attia et al. (2021b) species which is herein assigned to *H. selachii*) are very different, with *S. mokarran* being oceanodromous and ranging widely from brackish to blue water throughout latitudes 30° N and S, with *Carcharhinus melanopterus* ranging south and east of the

Arabian Gulf all the way to mid-Pacific islands but tightly restricted to coastal waters, while *C. dussumieri* is restricted to the shallow coasts of the NW Indian Ocean. Hopefully, attentive inspectors of marine fisheries in these areas will be on the watch for the signs of *H. selachii* n. sp. and report such in the fisheries literature.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Contributions of authors

Mohammad Nafi Solaiman Al-Sabi: conceptualization, authoring the manuscript, preparing figures, and leading the research work, **Mustafa M. Ibrahim:** Conceptualization, field sampling, reading histopathology slides, authoring and preparing the manu-

script, **Fahad Al-Hizab**: conceptualization, preparing histopathology slides, reading and preparing the histopathology part, **Omar A Al-Jabr**: conceptualization and authoring, **Salem Al-Shubaythi**: isolating the parasites and preparing slides, taking measurements of the parasite eggs, preparing summary tables, and **David G. Huffman**: conceptualization and authoring the manuscript.

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