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PACIFIC OCEAN**

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HUFFMANELA CF. CARCHARHINI (NEMATODA: TRICHOSOMOIDIDAE: HUFFMANELINAE) FROM SKIN OF A SANDBAR SHARK, *CARCHARHINUS PLUMBEUS*, IN THE PACIFIC OCEAN

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ABSTRACT: Eggs of *Huffmanela* cf. *carcharhini* from the skin of an aquarium-held, juvenile sandbar shark, *Carcharhinus plumbeus*, from the Pacific Ocean were studied using light and scanning electron microscopy. Grossly, eggs imparted a scribble-like skin marking approximately 130 × 60 mm on the right side of the shark's snout adjacent to its eye and nostril. Fresh (unfixed) eggs were elliptical, 75–95 µm long (\bar{x} = 85 µm, SD = ±4.5; n = 75), 48–63 µm wide (53 ± 3.4; 75), 8–10 µm in shell thickness (9 ± 1.3; 27), 45–68 µm in vitelline mass length (52 ± 6.9; 8); had a smooth shell surface and nonprotruding polar plugs 8–13 µm wide (10 ± 1.5; 73); lacked thin filaments, superficial envelope, and shell spines; sank in 35 ppt artificial seawater; and did not spontaneously hatch after 12 hr in 35 ppt artificial seawater. Formalin-fixed eggs measured 193 days postfixation were 75–95 µm long (84 ± 3.9; 150), 45–60 µm wide (50 ± 2.2; 150), 5–10 µm in shell thickness (8 ± 1.2; 87), 45–60 µm in vitelline mass length (51 ± 3.0; 92), and 30–40 µm in vitelline mass width (33 ± 2.0; 84), and had nonprotruding polar plugs that were 10–15 µm long (11 ± 1.4; 93) and 8–10 µm wide (9 ± 1.1; 108). Forcibly hatched first-stage larvae (unfixed) were filiform, 188–273 µm long (212 ± 25.5; 13), 8–13 µm wide (10 ± 1.2; 13), and had fine transverse striations. Eggs infected the epidermis only. Histology revealed intra-epithelial inflammation with eosinophilic granulocytes and hyperplasia, plus dermal lymphofollicular hyperplasia associated with the infection. The eggs of *H. cf. carcharhini* likely undergo considerable ex utero development before being sloughed (unhatched) from the host, along with epidermal cells.

Huffmanela Moravec, 1987 (Nematoda, Trichosomoididae, Huffmanelinae) includes 17 nominal species (16 marine, 1 freshwater) and 6 unidentified marine species (Justine, 2011) that mature in fishes of 1 shark and 15 teleost families (Justine, 2007, 2011). An adult specimen has been described for only 6 of those species (Justine, 2011), and, accordingly, the taxonomy of *Huffmanela* spp. has largely been based on egg morphology, with considerations of host affiliation, egg infection site, and host geographic locality (Moravec, 1987, 2001; Justine, 2004, 2007, 2011; Esteves et al., 2009). *Huffmanela* spp. are histozoic, infecting a wide variety of sites, where they are intercellular (typically) or intracellular (Moravec et al., 1998; Žďárská et al., 2001), and they exhibit a high level of microhabitat site specificity (Moravec, 2001). No life cycle of a *Huffmanela* species has been demonstrated experimentally or elucidated with a molecular marker matching eggs, larvae, and adults; however, specialists have considered it likely that these worms use an intermediate host and possibly a paratenic host to reach adulthood (Moravec, 2001). Interestingly, the eggs of *Huffmanela schouteni* Moravec and Campbell, 1991 (see Schouten et al., 1968; Moravec and Campbell, 1991) and 1 unidentified congener (Gállego et al., 1993) were isolated from the stools of humans who presumably had eaten infected fishes.

Chondrichthyans (Chondrichthyes: Elasmobranchii) host *Huffmanela carcharhini* (MacCallum, 1925) Moravec, 1987 (type species), *Huffmanela lata* Justine, 2005, and an unidentified congener (MacCallum, 1925, 1926a; Moravec, 1987; Justine, 2005; MacLean et al., 2006). MacCallum (1925) established *Capillaria carcharhini* based on nematode eggs that he found in the skin of 2 sharks noted as *Carcharhinus commersoni*, captured

in the northwestern Atlantic Ocean, and landed at Woods Hole, Massachusetts. One year later, MacCallum (1926a) reported on similar eggs that he discovered in a shark identified as *Carcharhinus milberti*. He established *Capillaria spinosa* based on those samples because they infected a different host species, were observed in gill arch epithelium rather than in epidermis of the shark's head and fins, and exhibited characteristic eggshell spines that he had not observed in *C. carcharhini*. Moravec (1987) studied MacCallum's (1925, 1926a) type materials, comprising histological sections and whole-mounted eggs, and concluded that *C. spinosa* was a junior subjective synonym of *C. carcharhini* because his egg measurements of the 2 species overlapped and he saw no evidence of shell spines in *C. spinosa*. As a result, he designated *C. carcharhini* as the type species for a new genus, *Huffmanela*. Since then, Justine (2005) described *Huffmanela lata* from eggs infecting the gills and skin of a gill strap of a gray reef shark, *Carcharhinus amblyrhynchos* (Bleeker, 1856), captured in the southwest Pacific Ocean off New Caledonia. MacLean et al. (2006) reported eggs of *Huffmanela* sp. infecting the skin of the ventral surface of the snout and mouth of a sandbar shark, *Carcharhinus plumbeus* (Nardo, 1827), captured in the Cape Fear River (coastal North Carolina). No other species of *Huffmanela* has been reported from a shark, and few observations on the host-parasite relationships between these shark nematodes and their hosts have been published (MacCallum, 1925, 1926a; Moravec, 1987; Justine, 2005; MacLean et al., 2006).

Herein, we provide supplemental information on an infection of *Huffmanela* cf. *carcharhini* (MacCallum, 1925) Moravec, 1987, in the skin of a sandbar shark from the Pacific Ocean using light and scanning electron microscopy (SEM) of infected skin, eggs, and forcibly hatched larvae.

MATERIALS AND METHODS

An infected sandbar shark (female, 80.1 cm TL, 6 kg total weight) was captured off Hawaii (21°30.093'N, 157°46.37'W), central Pacific Ocean (19 October 2010), shipped to the Animal Research and Care Center, Monterey, California (11 November 2011), skin-biopsied (7 January 2011), and killed by immersion in a buffered solution of tricaine methanesulfonate (MS-222) at 200 ppm before shipment (25 January

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2011) on ice for subsequent necropsy at Auburn University, Auburn, Alabama. At necropsy, beginning nearest the infected area of shark skin and expanding outward, a thorough examination of the shark's body was conducted, including underlying musculature of gill arches, buccal chamber, branchial chamber, and general body surface, as well as the body cavity, serosae of visceral organs, and mesentery. Patches of skin from infected and grossly normal body surfaces of the shark were excised using a scalpel and hemostats. Skin samples for histopathology and SEM were fixed immediately in 10% neutral buffered formalin, while remaining samples were immersed in 35 ppt artificial seawater and subsequently examined for the presence of nematode adults, juveniles, larvae, and eggs. With the aid of a stereo-dissecting microscope, fresh (unfixed) eggs were gently scraped from the epidermis of skin patches using dowel-mounted steel insect pins before being transferred in glass pipettes to stendor dishes containing artificial seawater. Hundreds of eggs were gathered in this manner, and approximately 75 were wet-mounted in artificial seawater on a glass slide under a coverslip (without coverslip pressure) before being measured (ocular micrometer) with aid of a $\times 100$ oil immersion lens and differential interference contrast (DIC). To examine nematode larvae, unfixed eggs were removed similarly from skin, wet-mounted on glass slides with artificial seawater, coverslipped, subjected to slight coverslip pressure that forced larvae out of the eggs, rinsed into a stendor dish, and remounted on a clean glass slide with fresh, artificial seawater, and coverslipped before being measured and photographed. Formalin-fixed eggs were wet-mounted and measured similarly 193 days postfixation; morphometrics of amber, brown, and black eggs were gathered. An attempt was made to study the contents of black eggs, which were immersed for 6–48 hr in lactic acid, glacial acetic acid, clove oil, and xylene. Fixed skin patches for histology were processed routinely following demineralization in Bouin's solution (Sigma/Aldrich, St. Louis, Missouri), sectioning ($4\ \mu\text{m}$) in paraffin, and staining with hematoxylin and eosin. Separate samples, i.e., skin patches containing eggs, free eggs, and forcibly hatched larvae for SEM, were routinely processed by dehydration through a graded ethanol series and desiccation in hexamethyldisilazane for 30 min, followed by evaporation and mounting on metal stubs using 2-sided sticky tape. To identify eggs, we primarily relied on egg morphology and morphometrics, and herein the latter are reported in μm as a range followed by its mean \pm SD and sample size (n) in parentheses.

RESULTS

Morphometric comparisons (Table I) and the following morphological attributes of freshly mounted (unfixed) eggs, formalin-fixed eggs (removed from formalin-fixed skin patches and measured 193 days after initial fixation), and forcibly hatched larvae together indicated an infection by a species of *Huffmanella*, as *Huffmanella cf. carcharhini* (MacCallum, 1925) Moravec, 1987. Infected shark skin had thousands of eggs (Figs. 1–3) that together appeared as a dark pencil scribble or thin, chain-like tattoo measuring 130 mm in maximum length and 60 mm in maximum width on the right side of the shark's snout adjacent to the eye and nostril (Fig. 1A–C). Egg tracks in some regions paralleled one another or formed a chain-link-fence-like pattern, looping the necks of a single, or several, placoid scales (Fig. 1B), while elsewhere they exhibited no apparent pattern (Fig. 1C). Stereomicroscopy of breaks in egg tracks failed to reveal a hatched larva or adult nematode. Some eggs were easily dislodged from the skin by light scraping, while others detached during the gentle process of excision and relocation of skin patches to the seawater-filled examination dishes.

Fresh eggs (Fig. 1D–G) were oblong (elliptical in longitudinal profile; circular in polar profile), 75–95 long (85 ± 4.5 ; 75), 48–63 wide (53 ± 3.4 ; 75), 8–10 in shell thickness (9 ± 1.3 ; 27), 45–68 in vitelline mass length (52 ± 6.9 ; 8); had a smooth shell surface and nonprotruding polar plugs 8–13 wide (10 ± 1.5 ; 73); lacked associated thin filaments, superficial envelop or thin transparent

layer, and eggshell spines; were negatively buoyant in 35 ppt artificial seawater; and did not spontaneously hatch after 12 hr in 35 ppt artificial seawater. Formalin-fixed eggs (Fig. 3C), measured 193 days postfixation, had comparable attributes: 75–95 long (84 ± 3.9 ; 150), 45–60 wide (50 ± 2.2 ; 150), 5–10 in shell thickness (8 ± 1.2 ; 87), 45–60 in vitelline mass length (51 ± 3 ; 92), and 30–40 in vitelline mass width (33 ± 2.0 ; 84) and had nonprotruding polar plugs 10–15 long (11 ± 1.4 ; 93) and 8–10 wide (9 ± 1.1 ; 108). Shell color was not affected by formalin fixation and ranged from translucent amber (early-stage eggs containing vitelline-like material, but lacking cleaved contents or a recognizable larva; Fig. 1D), to brown (Fig. 1E), to black (advanced-stage eggs lacking vitelline-like material and containing a tightly in-folded, fully developed filiform larva; Fig. 1F, G). In situ, amber, brown, and black eggs were mixed together (Fig. 1B, C). The shell of early-stage eggs (Fig. 1D) had a translucent outer layer of uneven optical density overlaying a thin inner layer of even optical density. The shell of advanced-stage eggs (Fig. 1F, G) had those layers, but the inner layer was dark (Fig. 1G). No attempt to clear the shell of black eggs was successful. Stained histological sections of early-stage eggs (Fig. 2A–B) exhibited a shell composed of an outer eosinophilic layer and a black inner layer; polar plugs were eosinophilic; egg contents were basophilic. Segregated by shell color, measurements for egg length, egg width, and polar plug width for amber, brown, and black eggs (fixed) did not seem taxonomically different based on the overlap of standard deviations of various morphometric means (Table I). Likewise, morphometrics of fresh versus fixed eggs did not seem dissimilar.

Within the egg, the first-stage larva lacks a distinct gut or any other discernible structure evident with light microscopy and is tightly in-folded at least 4 times (Fig. 1G). Freshly prepared, forcibly hatched first-stage larvae were filiform, 188–273 long (212 ± 25.472 ; 13), 8–13 wide (10 ± 1.233 ; 13) or 16–28 \times longer than wide at midbody (Fig. 1H), and they had fine transverse striations clearly evident with SEM at $\times 15,000$ magnification.

Histology (Fig. 2) and SEM (Fig. 3) revealed that eggs infected within the epidermis (typically; Fig. 2A, B) or perforated the epidermis such that 1 polar plug was unobstructed (Fig. 3A, C–E). Spheroid, phagocyte-like epidermal cells (likely malpighian cells) were typically associated with perforating eggs and partially encased them in some instances (Fig. 3C–E); aggregates of these phagocyte-like cells were evident even at low magnification (Fig. 3A). Histology of infected skin revealed intra-epithelial inflammation with eosinophilic granulocytes and hyperplasia as well as lymphofollicular hyperplasia in the dermis (Fig. 2). An intensive search of the shark's general body surface, epidermis, dermis, underlying musculature of the gill arches, buccal chamber, branchial chamber, and head as well as the body cavity, serosae of visceral organs, and mesentery failed to locate any gross lesion or a separate group of nematode eggs, a larval nematode, an empty egg, or an adult nematode.

DISCUSSION

To identify the eggs, we compared them to published descriptions of 17 nominal species and 5 unidentified species for which laid eggs are described (Table I). We discounted the *Huffmanella* sp. reported by Moravec and Justine (2010) from further taxonomic consideration because its laid eggs were not

TABLE 1. *Huffmanela* spp. having eggs $\geq 40 \mu\text{m}$: egg width and length, select egg features, biological attributes, distribution, and references.

Species	Egg width*	Egg length*	Egg features	Host type	Tissue	Locality	Reference
<i>Huffmanela</i> cf. <i>carcharhini</i>	48–63 (53) (unfixed)†; 45–60 (50) (fixed)	75–95 (85) (unfixed); 75–95 (84) (fixed)	Ellipse-shaped egg, nonprotruding polar plugs, egg surface smooth, no shell envelope	Shark	Skin	Central North Pacific Ocean	Present study
<i>Huffmanela carcharhini</i>	42–54 (fixed)	90–105 (fixed)	Ellipse-shaped egg, nonprotruding polar plugs, egg surface smooth or possibly with tiny spines, shell envelope only near poles	Shark	Skin, mucosa of gill arches	Western North Atlantic Ocean	MacCallum (1925, 1926a); Moravec (1987)
<i>Huffmanela</i> sp.	39–47 (median = 40.6) (unfixed)	73–86 (median = 80.2) (unfixed)	Ellipse-shaped egg, slightly protruding polar plugs, egg surface smooth, thin shell envelope	Shark	Skin	Western North Atlantic Ocean‡	MacLean et al. (2006)
<i>Huffmanela lata</i>	52–63 (57) (fixed)	77–88 (84) (fixed)	Ellipse-shaped egg, slightly protruding polar plugs, egg surface spiny, no shell envelope	Shark	Skin	Eastern South Pacific Ocean	Justine (2005)
<i>Huffmanela ballista</i>	32–41 (fixed)	63–78 (fixed)	Ellipse-shaped egg, protruding polar plugs, surface of eggs with longitudinal ridges, shell envelope present	Teleost	External mucosa of swimbladder	Eastern South Pacific Ocean	Justine (2007)
<i>Huffmanela banningi</i>	42–45 (fixed)	99–108 (fixed)	Ellipse-oval shaped egg, protruding polar plugs, egg envelope with tiny canals, which may appear as superficial spines	Teleost	Muscle	Eastern North Atlantic Ocean	van Banning (1980); Moravec (1987)
<i>Huffmanela ossticola</i>	32–40 (fixed)	72–80 (fixed)	Ellipse-oval shaped egg, slightly protruding polar plugs, egg surface smooth, shell envelope contains numerous thin filaments	Teleost	Within bones	Eastern South Pacific Ocean	Justine (2004)
<i>Huffmanela plectropomi</i>	29–50 (fixed)§	64–82 (fixed)§	Ellipse-oval egg, slightly protruding polar plugs, egg shell smooth, shell surrounded by superficial filaments, no shell envelope	Teleost	Mesentery about swimbladder	Eastern South Pacific Ocean	Justine (2011)
<i>Huffmanela shikokuensis</i>	36–45 (fixed)	78–90 (fixed)	Ellipse-oval shaped egg, protruding polar plugs, egg surface smooth, thin shell envelope	Teleost	Muscle	Eastern North Pacific Ocean	Moravec et al. (1998)
<i>Huffmanela</i> sp.¶	86–87 (w/ envelope); 60 (w/o envelope)#	113–116*	Ellipse-shaped egg, protruding polar plugs, egg surface smooth, thick shell envelope present	Teleost	Muscle	Western South Pacific Ocean	Grabda and Słosarczyk (1981)
<i>Huffmanela</i> sp.	34–43 (fixed)	67–79 (fixed)	Oval-elongated egg, protruding polar plugs, shell surface with tiny bumps, shell envelope present	Fish (suspected host)¶¶	Unknown, but likely muscle¶¶	Reported from Barcelona, Spain¶¶	Gállego et al., 1993
<i>Huffmanela</i> sp.	39–59 (fixed)	73–94 (fixed)	Oval-shaped egg, protruding polar plugs, shell surface smooth, no shell envelope	Teleost	Muscle	Eastern North Atlantic Ocean	Esteves et al., 2009

* In some instances, mean or median values are reported in parentheses (median values explicitly indicated), and fixation status of study eggs is reported in parentheses as unfixed or fixed.
 † Measurements of amber, brown, and black eggs given as the range of egg length, egg width, and polar plug followed by the standard deviation of the mean and sample size in parentheses. Amber eggs = 80–92 (84 ± 3; 50); 45–60 (50 ± 2.6; 50); 8–10 (9 ± 1.2; 50). Brown eggs = 75–90 (82 ± 3.5; 50); 48–58 (50 ± 2.0; 50); 8–10 (9 ± 1.2; 48). Black eggs = 78–95 (87 ± 3.8; 50); 48–55 (50 ± 1.9; 50); 10 (10 ± 0; 10).
 ‡ Eggs were collected from an aquarium-held shark captured in a coastal portion of the Cape Fear River (North Carolina) contiguous with the western North Atlantic Ocean; the sandbar shark (*Carcharhinus plumbeus*) is a coastal species that routinely enters bays and river mouths from the sea (Compagno et al., 2005).
 § Measurements reported here include eggs reported as normal and aberrant; fixation status unknown.
 ¶ *Capillaria* sp. of Grabda and Słosarczyk (1981).
 # Egg measurements calculated from two eggs illustrated in Grabda and Słosarczyk (1981).
 ¶¶ Eggs of this species were collected from the stools of a child who presumably had eaten fish (Gállego et al., 1993).

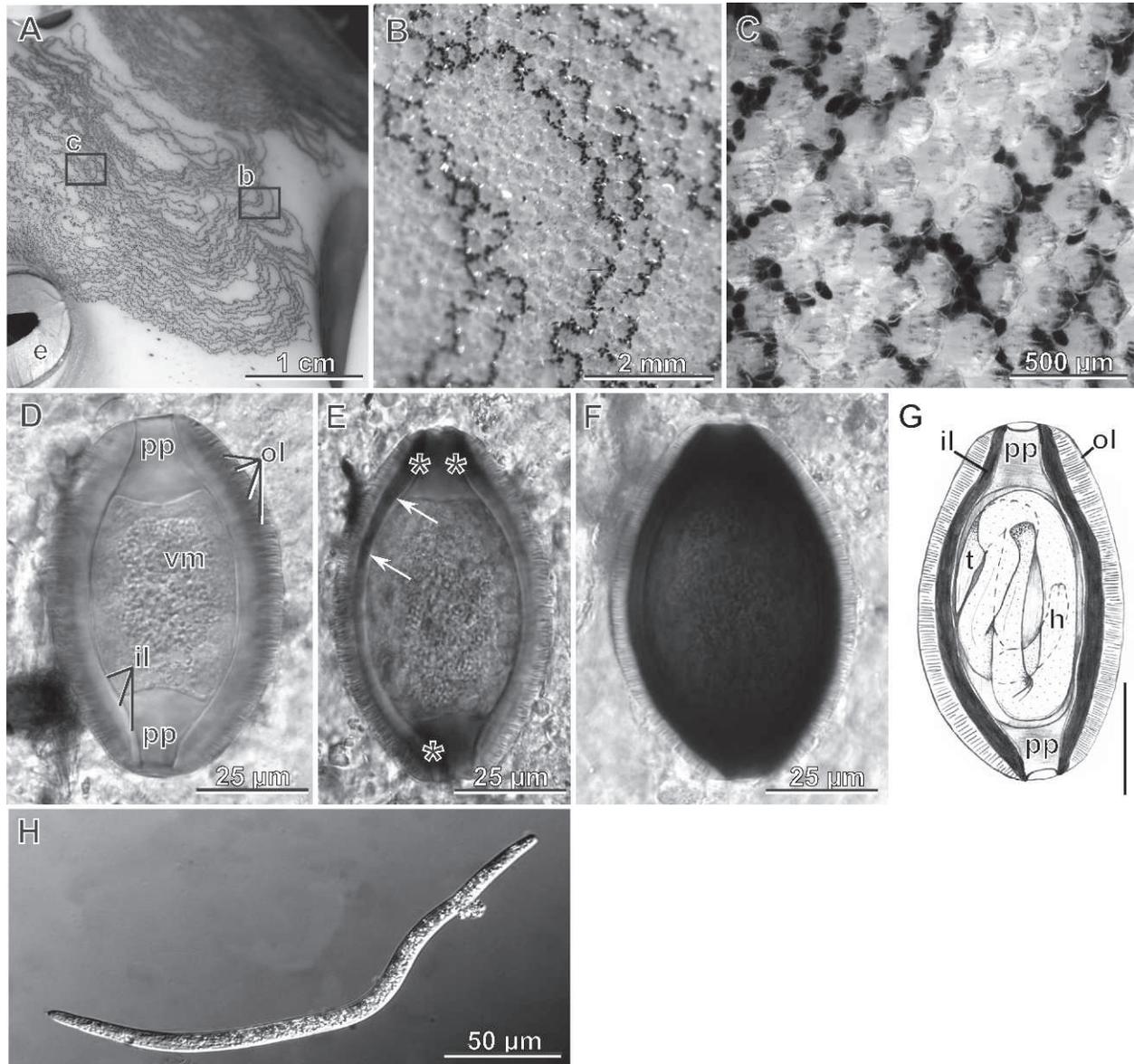


FIGURE 1. *Huffmanella* cf. *carcharhini* (MacCallum, 1925) Moravec, 1987 (Nematoda, Trichosomoididae, Huffmaniellinae) from the snout of a sandbar shark, *Carcharhinus plumbeus* (Nardo, 1827) (Carcharhiniformes, Carcharhinidae), captured in the central Pacific Ocean. (A) Gross, ventrolateral view of infected shark; eye (e), comparable areas to those enlarged in (B) and (C). (B) Skin showing black nematode eggs among placoid scales where loops in egg tracks occurred. (C) Skin showing black nematode eggs among placoid scales where egg tracks showed no apparent pattern. (D) Amber nematode egg (early stage of development) showing outer shell layer of uneven optical density (ol), inner shell layer of even optical density (il), polar plugs (pp), and vitelline mass (vm). (E) Brown egg (presumed intermediate stage of development) exhibiting darkened shell (*) at poles as well as within the inner shell layer (arrows). (F) Black egg (advanced stage of development) with dark inner layer and translucent outer layer. (G) Rendering of advanced stage egg showing inner layer (il), outer layer (ol), and polar plugs (pp) and containing a filiform first-stage larva: head (h) and tail (t). Bar = 25 μ m. (H) First-stage filiform larva forcibly hatched from egg of similar size and appearance as (F) and (G). Note that no gut is evident in the larva.

described, a teleost was the host, and it was from the western Pacific Ocean. Seven species of *Huffmanella*, i.e., *Huffmanella balista* Justine, 2007, *Huffmanella banningi* Moravec, 1987, *H. carcharhini*, *H. lata*, *Huffmanella ossicola* Justine, 2007, *Huffmanella plectropomi* Justine, 2011, *Huffmanella shikokuensis* Moravec, Koudela, Ogawa & Nagasawa, 1998, and 4 unidentified species (Table I) have eggs $\geq 40 \mu$ m wide, but the ranges for the majority of these species overlap or differ by only several μ m. Collectively, egg length among these species is 63–116 μ m, with extensive interspecific overlap (Table I). Several workers (Moravec, 1987;

Justine, 2011) have conducted detailed morphological studies of these eggs toward extracting characters useful in the taxonomy of the group, e.g., egg shape, polar plug features, eggshell surface features, and presence, absence, and character of shell envelope (Figs. 1G, 3B; Table I and references therein). Based on nonmorphometric criteria, the eggs we report herein most closely resemble *H. carcharhini* by having the combination of nonprotruding polar plugs, a smooth eggshell surface, and no obvious shell envelope. *Huffmanella lata*, the only other nominal species of *Huffmanella* reported from a shark (Justine, 2005), is most easily

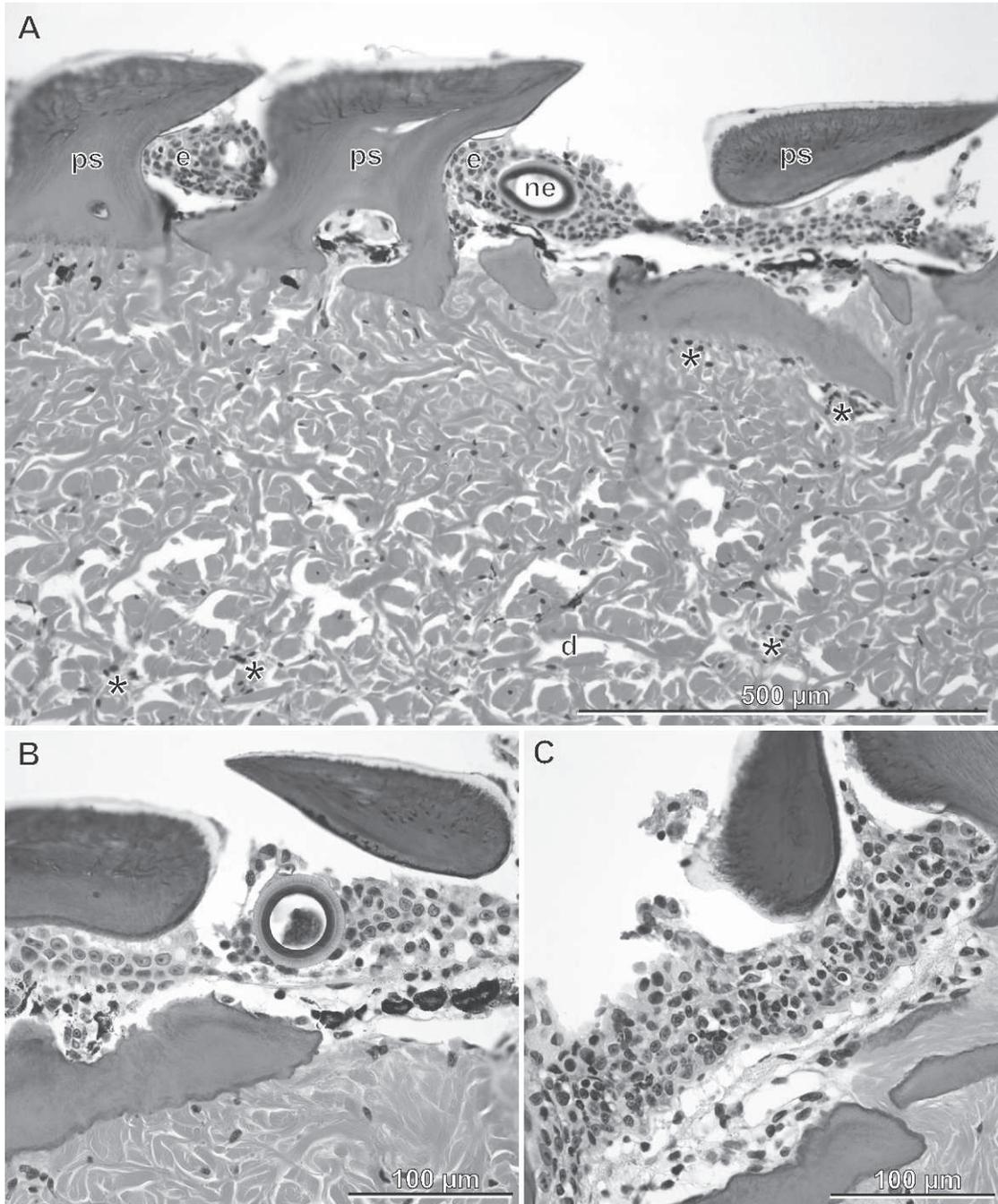


FIGURE 2. *Huffmanela* cf. *carcharhini* (MacCallum, 1925) Moravec, 1987 (Nematoda, Trichosomoididae, Huffmanelinae) from the snout of a sandbar shark, *Carcharhinus plumbeus* (Nardo, 1827) (Carcharhiniformes, Carcharhinidae), captured in the central Pacific Ocean; histological sections stained with hematoxylin and eosin. (A) Longitudinal section of skin showing placoid scales (ps), epidermis (e), nematode egg (ne), and lymphofollicular hyperplasia (*) in dermis (d). (B) Infected epidermis showing signs of inflammation. (C) Infected epidermis showing eosinophilic granulocytes and hyperplasia adjacent to nematode eggs (nearby eggs not in view).

differentiated from the eggs we report herein by having protruding polar plugs, a spinous shell, and a thin shell envelope. The aforementioned combination of features also differentiates *H. carcharhini* from the *Huffmanela* spp. that infect teleosts (Table I).

Despite the anatomical and morphometric similarities between the eggs we report herein and those of *H. carcharhini*, 3 items compelled us to question them as conspecifics. First, *H.*

carcharhini previously was known from the Atlantic Ocean only (MacCallum, 1925, 1926a; Moravec, 2001), but the eggs we report herein infected a shark in the Pacific Ocean. Second, the ranges for egg length and width certainly did not strongly indicate *H. carcharhini*, and, considering all species of *Huffmanela* having eggs ≥ 40 μm wide, 1, or both, of the ranges for egg length and width overlapped among all of them, i.e., regarding egg width: *H. carcharhini*, *H. sp.* of MacLean et al., *H. lata*, *H. plectropomi*, *H.*

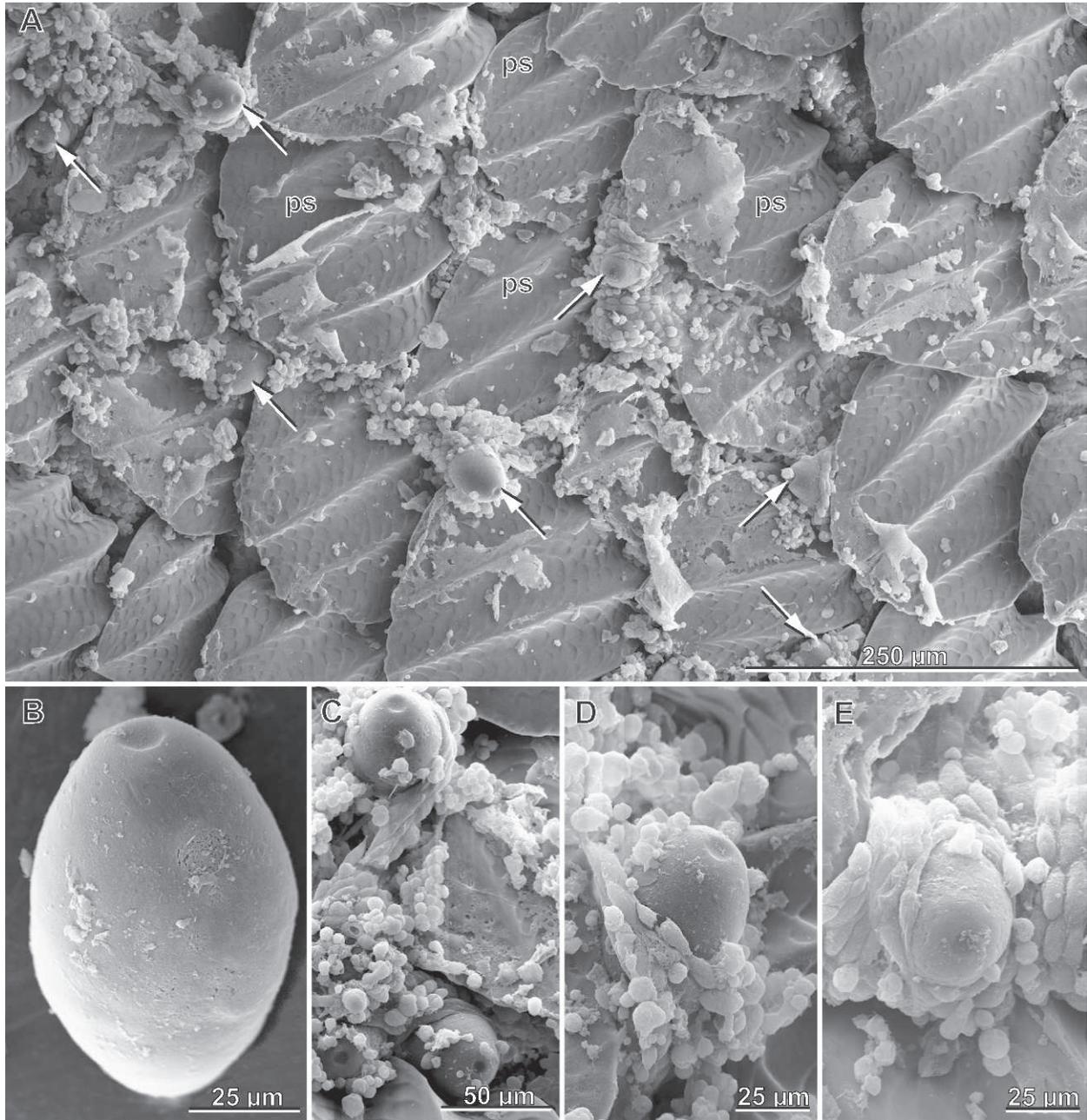


FIGURE 3. *Huffmanella* cf. *carcharhini* (MacCallum, 1925) Moravec, 1987 (Nematoda, Trichosomoididae, Huffmanelinae) from the snout of a sandbar shark, *Carcharhinus plumbeus* (Nardo, 1827) (Carcharhiniformes, Carcharhinidae), captured in the central Pacific Ocean; scanning electron micrographs. (A) Outer surface of infected skin showing crowns of placoid scales (ps) and nematode eggs (arrows), the latter each surrounded to some extent by spheroid epidermal cells. (B) Nematode egg thoroughly rinsed in seawater before SEM preparation. (C–E) Eggs associated with spheroid cells in epidermis.

sp. of Esteves et al.; regarding egg length: all species listed in Table I except *Huffmanella* sp. of Grabda and Ślósarczyk. Third, we were uncertain regarding the effects of various researchers having used different fixation methods to prepare their eggs for taxonomic study. MacLean et al. (2006) reported that fixed (ambient temperature, 10% formalin) and sectioned eggs were significantly smaller than unfixed eggs. Justine (2007) reported morphometric differences between unfixed and fixed (hot ethanol) eggs while studying the teleost parasites *H. balista* and *Huffmanella longa*, but concluded that even when the differences were

statistically significant, they were small, such that they would not likely be interpreted as being taxonomically meaningful. In the present study, among 225 eggs (75 unfixed, 150 fixed) measured, we detected no striking differences in egg length or width, eggshell thickness, vitelline mass length or width, or polar plug width among eggs (see Results). Based on this information, we do not believe that fixation artifact affected the anatomic features or morphometrics of our eggs in a taxonomically meaningful way. However, we acknowledge that the interspecific osmotic attributes of *Huffmanella* eggs are unknown, and different workers

have used fixatives other than 10% formalin in their studies without investigating possible fixation effects. Another factor challenging to the taxonomy of *Huffmanella* spp. is the scant information available on the development of *Huffmanella* eggs. Moreover, few adult forms are known, and, while substantial larval development occurs ex utero, the larva itself is minute, challenging to manipulate for microscopy, and seemingly lacks species-level taxonomic characteristics. To learn more about larval development, we attempted, but failed, to effectively “clear” the black eggs (advanced stage) after several, 6–48 hr immersions in a range of clearing agents, i.e., lactic acid, glacial acetic acid, clove oil, and xylene. Finally, regarding species-level identification of our study eggs, we did not sequence any specimen, and a search of all NCBI GenBank databases for “*Huffmanella*” on 3 August 2011 yielded no entry.

Huffmanella carcharhini purportedly infects several species of *Carcharhinus* (Carcharhinidae) (see MacCallum, 1925, 1926a; Moravec, 2001; Justine, 2005), but its host(s), including the type host, have yet to be confidently identified due to taxonomic confusion stemming from MacCallum’s (1925) and (1926a) reports. Specifically, the binomens “*Carcharhinus commersonii*” of MacCallum (1926a) and “*Carcharhinus commersoni*” of MacCallum (1925) could have specified at least 4 shark species in the western North Atlantic Ocean (Bigelow and Schroeder, 1948), i.e., dusky shark, *Carcharhinus obscurus* (Lesueur, 1818), bull shark, *Carcharhinus leucas* (Valenciennes, 1839), blacktip shark, *Carcharhinus limbatus* (Valenciennes, 1839), and oceanic whitetip shark, *Carcharhinus longimanus* (Poey, 1861). Although the first 3 species are littoral inhabitants and the last is an offshore species (Compagno et al., 2005), this information does not help to resolve the aforementioned host conundrum because MacCallum’s reports (1925, 1926a) were clear in specifying only the landing location of the sharks he examined rather than where the sharks were captured. That reporting convention, which has sometimes resulted in subsequent locality misinterpretations, stemmed from the fact that the U.S. Bureau of Fisheries routinely fished along the east coast of the United States and sometimes landed fishes at Woods Hole without specifying their capture location (L. R. Penner, pers. comm.). MacCallum’s (1925) description of his host as “large” likewise is equivocal since adult blacktip, dusky, bull, and oceanic whitetip sharks may all exceed 255 cm in total length (Compagno et al., 2005). Further confounding matters, *C. commersonii* is a junior synonym of the blacktip reef shark, *Carcharhinus melanopterus* (Quoy & Gaimard, 1824) (see Compagno, 1984). That fact has more recently resulted in *C. melanopterus*, which is a small littoral shark only known from the eastern Mediterranean Sea, Indian Ocean, and western Pacific Ocean (Compagno et al., 2005), being erroneously listed (Moravec, 2001; Justine, 2005) as a host of *H. carcharhini* based on specimens that MacCallum (1925) collected in the western North Atlantic Ocean. Caira and Healy (2004) referred to MacCallum’s type specimens of *H. carcharhini* as coming from *Carcharhinus plumbeus* (Nardo, 1827), which they considered, but did not justify, as a senior synonym of *C. commersoni*. MacLean et al. (2006) stated, without justification, that MacCallum’s collections of *H. carcharhini* were from sharks captured off the southern coast of the United States. Of possible importance here is the fact that MacCallum (1926b) reported several monogenoids (Monogenoidea) infecting another individual *C. commersonii* from Woods Hole, and, based on monogenoid

host-parasite records, Bullard et al. (2004) concluded that MacCallum’s (1926b) host was probably *C. obscurus*.

We tentatively consider MacCallum’s (1926a) report of *C. milberti* to refer to the sandbar shark, *C. plumbeus* (*C. milberti* = junior synonym; Compagno, 1984). Uncertainty regarding the matter stems from the fact that MacCallum (1926a) noted that his *C. milberti* specimen was sometimes referred to as the “great blue shark” or “dusky shark.” Certainly, those names have most commonly been linked to *Prionace glauca* (Linnaeus, 1758) and *C. obscurus*, respectively (Bigelow and Schroeder, 1948; Compagno, 1984; Froese and Pauly, 2011); however, the sandbar shark also has been referred to as “blue shark” and “small blue shark” (Castro, 2011). Still, based on their morphologic dissimilarities (Schulze-Haugen et al., 2003; Castro, 2011), it seems unlikely that MacCallum could have mistaken *C. plumbeus* for *P. glauca*, and, although *C. plumbeus* and *C. obscurus* are often confused (Castro, 2011), the large and forward-located first dorsal fin of a large *C. plumbeus* (as reported by MacCallum, 1926a) distinguishes it from *C. obscurus* (see Garrick, 1982; Castro, 2011). Certainly, the recent report by MacLean et al. (2006) validated that the sandbar shark is a host of an unidentified species of *Huffmanella* in the western North Atlantic Ocean. Considering all this, as well as the fact that 2 of us have observed infections of probable *Huffmanella* eggs in other carcharhinids (Carcharhinidae) in the western North Atlantic Ocean and Gulf of Mexico (S.A.B. and G.W.B., pers. obs.), we conclude that the species identity of a host for *H. carcharhini* remains unconfirmed.

In the present study, we observed eggs of *Huffmanella* cf. *carcharhini* in the epidermis only, corroborating previous reports suggesting a high level of microhabitat specificity among *Huffmanella* species infecting sharks (MacCallum, 1925, 1926a; Justine, 2005; MacLean et al., 2006). Epidermal cells of fishes remain mitotically active (Ferguson, 2006) and are sloughed as cell division proceeds. In considering infections by *Huffmanella* spp. in the skin of swordfish (*Xiphias gladius*) and sharks, Moravec and Garibaldi (2000) proposed that the ongoing process of epidermal sloughing resulted in the release of *Huffmanella* eggs from the definitive host such that another host might subsequently be infected. Based on our results herein, we concur that such a process is likely. The fact that we did not locate any life-history stage of *Huffmanella* cf. *carcharhini* other than the egg in a variety of developmental stages (with some containing a first-stage larva) suggested that egg hatching takes place away from the vertebrate definitive host.

The consolidated nature of the eggs we studied herein matched observations of others regarding infections by *Huffmanella* spp. in sharks (MacCallum, 1925, 1926a; Justine, 2005; MacLean et al., 2006) and suggested that a single female laid them or that the egg laying of multiple females had been localized. The mixture of amber (early stage) and dark (advanced stage) eggs that we observed perhaps indicated that (1) a single female or multiple females crisscrossed paths while depositing eggs, (2) eggs developed at varying rates, or (3) some eggs were unfertilized or rendered unviable. Regardless, the multitude of eggs we observed suggested that females of *Huffmanella* species that infect the skin of sharks are highly fecund and mobile when laying eggs within the epidermis. Direct observations of egg-laying females would be helpful, but, to date no adult specimens of a *Huffmanella* species have been reported from a shark. MacCallum (1925) postulated that females of *H. carcharhini* die soon after egg deposition

because he did not find one in his egg-infected sharks. Although we similarly failed to find an adult nematode, we cannot rule out the possibility that a “spent” female escaped detection by moving through, or beneath, the epidermis to another body region. That possibility might explain the presence of lymphofollicular hyperplasia in the deeper dermis beneath areas of skin harboring eggs of *Huffmanella* cf. *carcharhini*; however, that observation might simply indicate that ovigerous females had moved into the epidermis through the dermis. In addition, the presumed rapid “turnover” of the epidermis, along with the fact that fish epidermis is typically avascular, perhaps indicates a life-history adaptation that protects some adult *Huffmanella* spp. from an effective host immune response.

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